

Adrenergic modulation of dexketoprofen antinociception in murine formalin orofacial pain

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in pain whose mechanism of action is the inhibition of cyclooxygenase enzymes (COXs), however, there are evidence of other mechanisms of action, such as the inhibition of substance P, interaction with systems NO, monoaminergic and others. The objective of the present work was to study the participation of α -1 (prazosin) and α -2 (yohimbine) adrenoceptors antagonists in the antinociception of dexketoprofen, the S (+) enantiomer of ketoprofen. The antinociception evaluation was thru the mice orofacial formalin assay. Dexketoprofen (DEX) induced a dose-related antinociception 3.40 times more potent in phase I than in phase II. Prazosin i.p. decreased of the antinociception of DEX, 2.01 times in phase I and 4.02 times in phase II. Administered i.t. reduced the antinociception 5.30 times in phase I and 6.20 times in phase II. Yohimbine i.p. induced a reduction of the ED₅₀ of 3.40 times in phase I and 4.50 times in phase II, after i.t. administration the reduction was 5.30 times in phase I and 6.20 times in phase II. The mechanism of antinociception induced by DEX is mediated by the activation of α -1 and α -2 adrenergic receptors at supraspinal and spinal levels.

Keywords: Adrenergic Modulation; Dexketoprofen; Antinociception; Orofacial Pain; Prazosin; Yohimbine

1. Introduction

Pain is a perception sensitive to changes that occur in the tissues and that are harmful to the individual. The current International Association for the Study of Pain (IASP) recommended that the definition of pain as "An unpleasant sensory and emotional experience associated or similar to that associated with actual or potential tissue damage" [1]

Two main types of pain are described, with defined characteristics, the nociceptive and neuropathic pain. The nociceptive is the most common, due to dangerous noxious or noxious stimuli that are selectively detected by nociceptors. Nociceptors are specific receptors for capturing harmful stimuli whether mechanical, chemical or thermal in nature. Nociceptors can respond not only to noxious stimuli, but also to conditions derived from injury, such as changes in pH or ionic concentration, or changes in factors that mediate pain such as histamine, glutamate, substance P, a peptide related to the gene of calcitonin (CGRP), nerve growth factor (NGF) and others. Nociceptors generally have a high threshold, but when activated they send electrical signals to the central nervous system and the brain to transmit

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pain perception [2]. With advanced molecular techniques it has been shown that there are many nerve structures at the peripheral, medullary, subcortical and cortical levels that intervene in the perception of pain, starting at the modular level and transforming into pain when it reaches the brain level.

Two types of agents are mainly used in the pharmacotherapy of pain: opioids and non-steroidal anti-inflammatory drugs (NSAIDs). The latter are characterized by their analgesic, anti-inflammatory and antipyretic properties. The main mechanism of action of NSAIDs is the inhibition of cyclooxygenase enzymes (COX-1, COX-2 and COX-3), thus limiting the biosynthesis of prostanoids (prostaglandins and thromboxanes). However, there are numerous evidences of the existence of others mechanisms of action of NSAIDs, such as: inhibition of substance P, of N-methyl-D-aspartate (NMDA), of pro-inflammatory cytokines, interaction with different systems, such as NO, monoaminergic, cholinergic, opioidergic and others [3,4].

NSAIDs are weak organic acids, including the propionic acid group (ibuprofen, naproxen, ketoprofen) that have a chiral centre due to a carbon at the α -position that converts NSAIDs into a racemic unit. Ketoprofen is made up of a dextrorotatory enantiomer of S (+) configuration with high antinociceptive activity called dexketoprofen and another R (-) enantiomer with slight analgesic activity [5].

The adrenergic system modulates nociceptive information at both the spinal and supraspinal levels through afferent information and the activity of descending inhibitory pathways. The role of adrenergic pathways in pain has been confirmed by different evidences, both pronociceptive and antinociceptive, for example, is known that norepinephrine induces strong antinociceptive activity in the CNS. Activation of neuronal α_2 adrenergic spinal receptors at both the presynaptic and postsynaptic levels, induced a significant reduction of nociception. Also, in acute, inflammatory, and neuropathic pain models noradrenergic pathways producing an inhibitory effect on pain transmission [6-10].

The participation of the adrenergic system in nociceptive processes constitutes a fundamental phase in pain modulation. The antinociceptive effect of dexketoprofen appears to be due to the stimulation of pain-related central nervous system structures; However, the precise mechanism of action that mediates its analgesic effects has not yet been fully defined. On the other hand, the racemic molecule, ketoprofen, of which the S (+) isomer dexketoprofen (DEX) is part, administered either by i.p. or via i.t. is antagonized by yohimbine (YOHIM) and prazosin (PRAZ) has no effect [11,12].

The objective of the present work was to evaluate whether adrenergic pain modulation pathways participate in antinociception induced by the S (+) enantiomer of ketoprofen, called dexketoprofen (DEX), an effective and well-tolerated option for the management of pain. The evaluation was carried out using the orofacial formalin assay in mice and was mediated by the adrenoceptor antagonists α -1 PRAZ and α -2, YOHIM.

2. Material and methods

2.1. Animals

Male CF-1 mice weighing 28-32 g were tested divided randomly into groups of 6-8 mice. Animals were housed on a 12/12 h light-dark cycle at $22 \pm 1^\circ \text{C}$ with free access to food and water and acclimatized to the laboratory environment for at least 1 h. Each animal was used in one experiment only and euthanized by overdose of anesthetic, pentobarbital intraperitoneally (i.p.) 60 mg/kg, immediately after the test. Protocols were approved by the Animal Care and Use Committee at the Faculty of Medicine, University of Chile (Protocol CBA 0852/FMUCH/2018). The experiments were performed by researches blind to drug treatment.

2.2. Antinociception

The nociceptive test used was the formalin orofacial as previously described [13]. To perform the test subcutaneous (s.c.) 20 μL of 2 % formalin solution were injected into the upper right lip pad of each mouse. The intensity of pain was determined by the time, in sec, that the animal spent rubbing the injected area with the ipsilateral fore or hind paw, during each phase of the assay. The assay presents two different phases separated by a period of relative inactivity. An early short-lasting response of 0-5 minutes (phase I) is a tonic acute pain and a continuous prolonged response of 20-30 minutes (phase II) represents inflammatory pain. Total rubbing time in each phase was converted to a percentage of maximum possible effect (% MPE) as follow:

$$\% \text{ MPE} = 100 - (\text{post drug rubbing time} / \text{control rubbing time}) \times 100$$

2.3. Protocol

Dose response curves, i.p. for dexketoprofen (DEX) 3, 10, 30, 100, 300 mg / kg for the orofacial formalin test were obtained before and after pretreatment of mice with prazosin (PRAZ), (0.5 mg / kg i.p. or 10 µg / mouse i.t.) or with yohimbine (YOHIM), (1 mg/kg i.p. or 20 µg/mouse i.t.) in six or eight animals with at least four doses. A linear squared regression analysis of the logarithmic dose response curve allowed calculating the doses that produced 50% antinociception of DEX (ED50).

2.4. Drugs

Drugs were freshly dissolved in 10 mL/kg of sterile physiological saline solution. Dexketoprofen trometamol was provided by Menarini Laboratory, Spain. Prazosin hydrochloride and yohimbine hydrochloride were purchased from Sigma-Aldrich Chemical Co, St. Louis, MO, USA.

2.5. Statistical analysis

Results are presented as means \pm SEM. The statistical difference between the control rubbing and experimental rubbing was performed by one-way ANOVA followed by Tukey post hoc test. P values less than 0.05 ($p < 0.05$) were considered statistically significant. Results analyzed used Pharm Tools Pro, version 1.27, McCary Group Inc, PA, USA.

3. Results

The i.p. or i.t. pretreatment with PRAZ or YOHIM did not induce variation in rubbing control time nor did it induce significant motor or behavioral dysfunction in the mice.

3.1. Rubbing activity by 2% formalin

The nociceptive score was determined for each phase of the assay by measuring the number of seconds that the mice spent rubbing the area injected with 2% formalin. Phase I corresponds to the period between 1 and 5 minutes with 103.70 ± 4.68 seconds of rubbing and phase II corresponds to the period of 20 to 30 minutes with 133.40 ± 4.36 seconds of rubbing. These results are shown in Fig. 1.

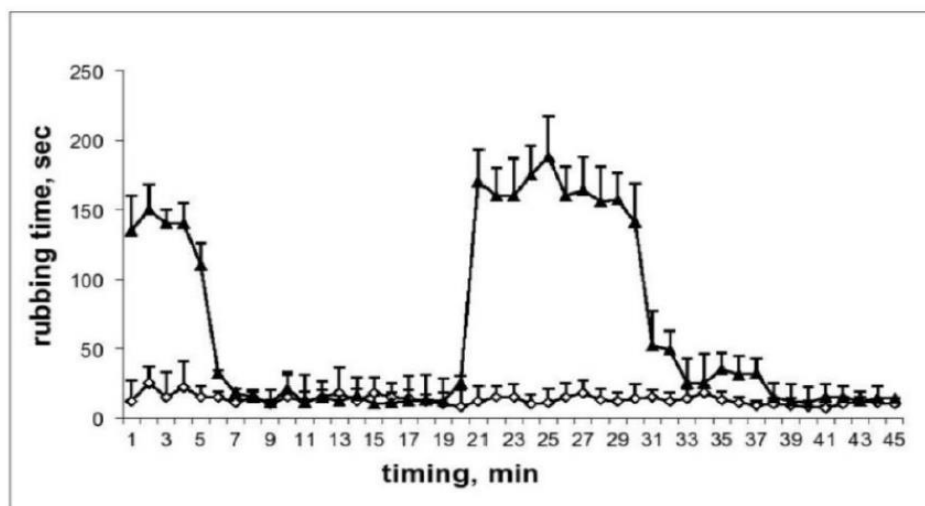


Figure 1 Time course of rubbing activity in the formalin orofacial in mice. Each point is then mean \pm SEM of 6-8 mice. Control saline (\square), formalin 2% (\blacktriangle).

3.2. Antinociception by DEX

Administration of 3, 10, 30, 100 and 300 mg/kg, i.p. of DEX induced a dose-related antinociceptive activity in phase I and phase II of the formalin orofacial test of mice. DEX was 3.40 times more potent in phase I than in phase II, see Fig. 2. The corresponding ED50 values were 16.10 ± 3.40 mg/kg for phase I and 54.70 ± 8.30 mg/kg for phase II (see Fig.3).

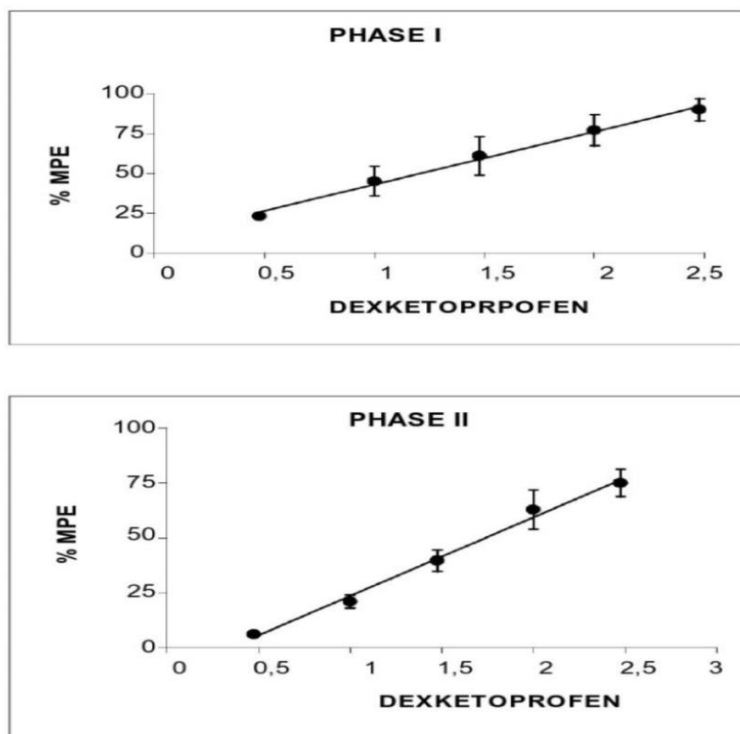


Figure 2 Dose-response curves for the antinociceptive activity induced by the i.p. administration of dexketoprofen in phase I and phase II of the orofacial formalin assay of mice. Each point is the mean \pm SEM of 6-8 mice. %MPE: antinociception represented as a percentage of maximum possible effect.

3.3. Effect of PRAZ on the antinociception of DEX

The pretreatment of mice with the adrenergic antagonist α -1 prazosin (0.5 mg/kg, i.p.) resulted in an important change of the antinociception induced by DEX, reflected in a significant increase of the ED₅₀ of 2.01 times in phase I and 8.05 times in phase II. The i.t. administration of 10 μ g / mouse of PRAZ induced a significant enhance of ED₅₀ of 5.30 times in phase I and 6.20 times in phase II. These results denote a relative higher power of PRAZ by via of i.t. that via i.p. All results can be seen in Fig. 3.

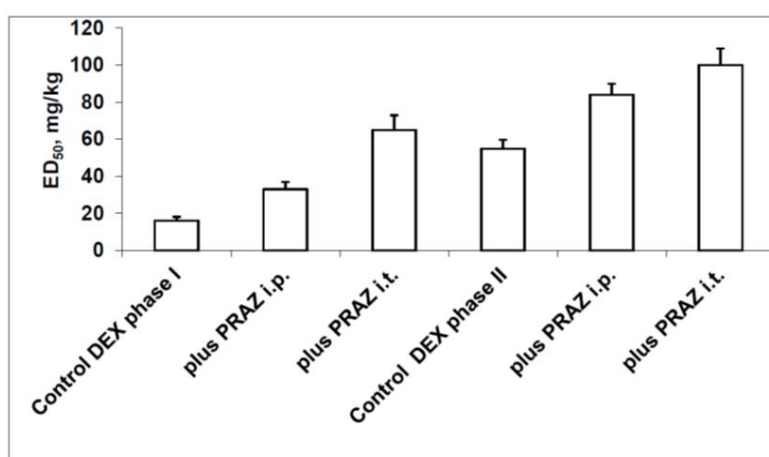


Figure 3 ED₅₀ of dexketoprofen (DEX) in phase I and in phase II of the orofacial formalin of mice before and after the pretreatment i.p. of 0.5 mg/kg i.p. and 10 μ g/mice of PRAZ. Each bar is the mean \pm SEM of 12 mice. PRAZ significantly increased the ED₅₀ value ($P < 0.05$) and all results are significantly compared with the respective DEX control.

3.4. Effect of YOHIM on the antinociception of dexketoprofen

In the case of the pretreatment of mice with the adrenergic antagonist α -2 yohimbine (1.0 mg/kg i.p.) caused in a significant increase of the antinociception induced by DEX, reflected in a significant augment of the ED₅₀ of 3.40 times in phase I and 4.50 times in phase II. After the administration 20 μ g / mouse i.t of YOHIM induce an important enhance of DEX ED₅₀ of 5.30 times in phase I and 6.20 times in phase II. These findings indicate that YOHIM was more potent via i.t. than via i.p. For results, see Fig. 4.

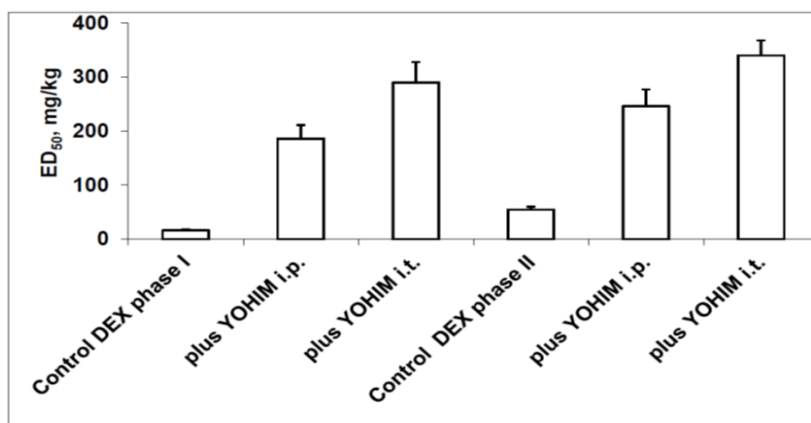


Figure 4 ED₅₀ of dexketoprofen (DEX) in phase I and in phase II of the orofacial formalin of mice before and after the pretreatment of 1 mg/kg i.p. and 20 μ g/mice i.t. of YOHIM. Each bar is the mean \pm SEM of 12 mice. YOHIM significantly increased the ED₅₀ value ($P < 0.05$) and all results are significantly compared with the respective DEX control.

4. Discussion

In this study, the formalin orofacial model was used to evaluate the modulation of adrenoceptor antagonist α -1 and α -2 on biphasic pain behavior after subcutaneous administration of diluted formalin solution into lip pad. The first phase reflects direct stimulation of C-nociceptors whereas the second phase is related with spinal and brainstem. Adrenergic monoamines are involved in modulation of nociception using various pain models. In this work, the antinociceptive activity induced by DEX, in the orofacial formalin assay in mice, was significantly decreased by the adrenoceptor antagonist's PRAZ (α -1) or YOHIM (α -2). These findings are partially consistent with the reported effects of the same adrenergic blockers on other NSAIDs. Thus YOHIM, blocked the antinociception induced by ibuprofen [14]. Also, PRAZ and YOHIM, attenuated dipyrrone antinociception [15]. PRAZ and YOHIM antagonized the antinociception induced by diclofenac and dipyrrone [16]. Besides, YOHIM antagonized antinociception induced by ketoprofen, diclofenac and piroxicam, however did not affect antinociception induced by paracetamol. Nevertheless, PRAZ antagonized only the effect of paracetamol, without affecting the antinociception of the other drugs [11]. YOHIM, did not modified antinociception of paracetamol [16]. Dipyrrone-induced antinociception was not affected by YOHIM and PRAZ [17]. YOHIM, but not PRAZ reduced partially, but significantly, the antinociceptive effect of nefopam during phase 1, but not during phase 2 of the formalin test [18]. PRAZ and YOHIM had little effect on the antinociception of DUP-697 (a new COX-2) during both phases of the formalin test [19].

The involvement of the adrenergic system in pain modulation is expressed not only in NSAIDs, but also in opioids, for example, tapentadol is an analgesic drug with a dual mode of action: MOR agonist and noradrenaline reuptake inhibitor [20]. Similarly, tramadol, another opioid used in pain, shares an adrenergic pathway in its mechanism of action [21]. Additionally, it has been hypothesized that drugs capable of inducing the activation of the adrenergic system could exert their analgesic efficacy by inhibiting the activation of microglia [22].

This study demonstrated that the antinociceptive activity of DEX is modulated by the adrenergic antagonist PRAZ (α -1) and YOHIM (α -2). This modulatory action of adrenoceptors antagonists, could be assumed to a pharmacodynamic interaction of the S (+) isomer of DEX with the antagonists used. These findings are partially consistent with those previously reported, suggesting that adrenergic modulation of pain induced by s.c. formalin administration seems controversial. The differences might be assumed to the diversity of pain tests used and to the different doses of adrenergic agents used [11,12].

Another explanation for the present study could be assumed with the modulation of pain mediators exerted on the central transmission routes by adrenergic receptors, one of the most abundant groups in the CNS. [7-23-25]. It has been postulated, similar to murine models of neuropathic pain, in the orofacial formalin assay, proinflammatory cytokines including interleukins IL-1 β IL-6, IL-12, TNF- α , glutamate and others could be released. Therefore, the administration of alpha-adrenergic antagonists would limit the release of pro-inflammatory mediators and the antinociceptive effect of DEX could be significantly altered.

The results obtained in this work would allow us to generalize that the antinociception induced by NSAIDs, including racemic ketoprofen, in various pain models, involves the activation of adrenergic receptors α -2 at the spinal and supraspinal level and of adrenergic receptors α -1 of the descending noradrenergic inhibitor system. However, the contribution of cyclooxygenases and other mechanisms cannot be excluded.

5. Conclusion

It is proposed that the mechanism of antinociception induced by DEX in the orofacial formalin assay is mediated by the activation of α -1 and α -2 adrenergic receptors at supraspinal and spinal levels. Nevertheless, the results show that the i.t. administration both of PRAZ and YOHIM produce a greater effect, compared to the i.p. route, which suggests that DEX has important adrenergic compromise at the spinal level.

Compliance with ethical standards

Disclosure of conflict of interest

All the authors declare that they have no conflicts of interest.

Statement of ethical approval

All procedures with animals were approved by the Animal Care and Use Committee of the Faculty of Medicine of Universidad de Chile. (Protocol CBA 0852/FMUCH/2018).

Authors' contributions

All the authors participate in planning research and performing experiments. Also were responsible for data interpretation and collaborate in writing process of the manuscript. All the authors read and approved the final manuscript.

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