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(RESEARCH ARTICLE)

NSAIDs and opioids antinociception in a thermal murine phasic pain

Miranda Hugo F [1,*], Noriega Viviana [2], Sierralta Fernando [3], Sotomayor-Zárate Ramón [4] and Prieto Juan Carlos [2,3]

[1] *Neuroscience Departament, Faculty of Medicine, Universidad de Chile, Santiago, Chile.*
[2] *Cardiovascular Department; Clinical Hospital, Universidad de Chile, Santiago, Chile.*
[3] *Pharmacology Program, ICBM, Faculty of Medicine, Universidad de Chile, Santiago, Chile.*
[4] *Laboratorio de Neuroquímica y Neurofarmacología, Centro de Neurobiología y Fisiopatología Integrativa, Instituto de Fisiología,Facultad de Ciencias, Universidad de Valparaíso. Chile.*

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Abstract

Pain is an unpleasant sensation that causes mild or severe physical discomfort, by which induces the use of analgesics which are basically of two types: opioids or NSAIDs. The objectives of the present study was to evaluate the antinociception activity induced by NSAIDs and opioids in a thermal model of animal pain, the tail flick assay, and to determine the effect of the MOR antagonist, naltrexone. Antinociception was assessed by the tail flick test using an digital algesiometer. The rank orden of potency was fentanyl > morphine > ibuprofen > tramadol > codeine > meloxicam > paracetamol and the antinociceptive ratio, compared with paracetamol, was between 460 for fentanyl and 1 for meloxicam. Pretreatment with the opioid receptor antagonist, naltrexone, 1 mg/kg i.p., significant reversed the antinociceptive effect of NSAIDs and opioids. The results obtained with naltrexone in this assay confirm the antagonism of this agent on opioids. However, the antagonism over NSAIDs is a new finding since that has not been previously reported. This study test that NSAIDs and opioids induce antinociceptive activity in a thermal murine phasic pain with the following rank orden of potency fentanyl > morphine > ibuprofen > tramadol > codeine > meloxicam > paracetamol. Although the mechanisms of action of these drugs are different, naltrexone, a MOR antagonist, blocked the effects of both agents, suggesting that inhibition of pain seem partially mediated by MOR with association to central mechanisms.

Keywords: Antinociception; Naltrexone; Opoids; NSAIDs; Tail flick

1. Introduction

Pain is an unpleasant sensation that causes mild or severe physical discomfort, by which induces the use of analgesics, these agents are basically of two types: opioids or NSAIDs. Furthermore, there are different models of animals to assess pain, among which is the tail flick, one of the oldest nociceptive test [1]. In this phasic pain model, the tail is exposing to a controlled infrared heat beam and the measured parameter is the latency, in seconds, for tail flick reflex following tail exposure to a heat stimulus. The tail flick is a spinal reflex, but it is subject to supraspinal influences that can affect this reflex [2].

NSAIDs are drugs widely used in the treatment of pain and inflammation. The main mechanism assigned to these drugs is the inhibition of cyclooxygenase enzymes (COXs), compromised in the biosynthesis of prostaglandins (PGs) and thromboxanes (TXs). These agents are important mediators in the processes of pain, inflammation and fever. 3 isoforms of COXs are recognized: COX-1, COX-2 and COX-3, with peculiar characteristics each. Thus, COX-1 is found in platelets, stomach, blood vessels, kidney and other tissues. COX-2 is found in inflamed tissues and other tissues such as kidney. COX-3 is expressed in spinal cord, cerebral cortex, endothelial cells, monocytes and heart. Various evidences have shown

* Corresponding author: Miranda Hugo F

that NSAIDs in addition to their inhibitory effect on COXs there are other mechanisms that help explain the actions of NSAIDs. These include interactions with nitric oxide, cholinergic, monoaminergic, serotonergic, endocannabinoid and others systems at molecular level [3 - 5].

The effectiveness of opioids in relieving pain has been proven in several clinical and animal trials. Opioid-induced analgesia is due to the activation of receptors located in the central and peripheral nervous system. Currently, five types of opioid receptors have been described: mu receptor (MOR), kappa receptor (KOR), delta receptor (DOR), nociception receptor (NOR) and zeta receptor (ZOR). Within these different types, the following subtypes are described: mu1, mu2, mu3, kappa1, kappa2, kappa3, delta1 and delta2 [6]. Each of the different opioid receptors have been confirmed by studies of their crystalline structures derived with the use of antagonists [7]. Very different roles have been assigned to different receptor subtypes, so the mu-1 receptor is responsible for analgesia and dependence, the mu-2 receptor is responsible for euphoria, dependence, respiratory depression, miosis, constipation and the mu-3 receptor induces vasodilation. KOR receptors play a part in analgesia, diuresis and dysphoria. DOR receptors have a role in analgesia and the reduction of gastric motility. NOR receptors cause analgesia and hyperalgesia, depending on the concentration and ZOR receptors regulate the development of normal and tumorigenic cells and tissues [8].

The study of antinociceptive activity of NSAIDs and opioids has been recognized in animal and clinical studies, nevertheless comparative readings of such drugs in animal pain methods are few. On the other hand, the effect of MOR opioids antagonists in the antinociception induced by NSAIDs and opioids are still less. The objectives of the present study was to evaluate the antinociception activity induced by NSAIDs and opioids in a thermal model of animal pain, the tail flick assay, and to determine the effect of the MOR antagonist, naltrexone.

2. Material and methods

2.1. Animals

Male CF-1 mice (28–30 g), were used and housed on a 12 h light–dark cycle at 22° ± 2°C with access to food and water ad libitum. Experiments were performed in accordance with current Guidelines for The Care of Laboratory Animals and Ethical Guidelines for investigation of experimental pain approved by the Animal Care and Use Committee of the Faculty of Medicine, University of Chile. Animals were acclimatized to the laboratory for at least 1 h before testing, used only once in the protocol and were euthanized by intraperitoneal (i.p.) injection of 60 mg/kg of pentobarbital. In each protocol was used a minimum mice (6-8) to reach definitive results of the drug treatments.

2.2. Nociceptive test

Antinociception was assessed by the tail flick test as previously described [9], using an digital algesiometer (U. Basile, Comerio, Italy). The animal withdraws its tail in response to the heat applied, the reaction time of this movement is known as tail flick latency. A prolongation of the reaction time is accepted as antinociceptive activity. A cut-off time of 8 seconds was set as the threshold to avoid damage to the tail of the animal. Control reaction time (latency of the response) was recorded twice, with an interval of 10 min between readings. Only animals with baseline reaction times between 2 and 3 s were used in the experiments. For each mouse the tail flick latency was recorded prior to drug administration (control latency or baseline value) and at 30 minutes after i.p. drug administration. The antinociceptive response was calculated as percent of maximum possible effect (% MPE), where % MPE =[(test – control)/(8 – control)] × 100. Groups of six to eight animals were used for each dose and for each treatment.

2.3. Protocol

Dose response curves, i.p. for fentanyl (0.003 – 0.100mg/kg), morphine (1 – 30 mg/kg), tramadol (1-30 mg/kg), codeine (1-30 mg/kg), ibuprofen (1-30 mg/kg), meloxicam (1-30 mg/kg) and paracetamol (10 – 600 mg/kg) were obtained using at least six animals at each of at least four doses. A squares linear regression analysis of the log dose response curve allowed the calculation of the doses that produced 25 % of antinociception when each drug was administered alone (ED25). ED25 was used in the tail flick test as the equieffective dose for each NSAIDs or opioids before and after i.p. administration of 1mg/kg of naltrexone, dose from previously published studies [9].

2.4. Drugs

The drugs were freshly dissolved in a physiological salt solution of 10 mg/kg for i.p. administration. Ibuprofen by Laboratory Chile and meloxicam by Saval Laboratories Chile, Naltrexone hydrochloride, morphine hydrochloride, c odeine phosphate, fentanyl hydrochloride and tramadol hydrochloride were purchased from Sigma-Aldrich Chemical Co, St.- Louis, MO, USA.

2.5. Statistical analysis

Results are presented as means ± SEM. Statistical difference between before and after the treatment with naltrexone was assessed by Student's test for independent means and p values less than 0.05 ($p < 0.05$) were considered statistically significant. Statistical analyses were performed using the program Pharm Tools Pro, version 1.27, McCary Group Inc., PA, USA.

3. Results

3.1. Antinociception induced by NSAIDs and opioids in the tail flick assay

The i.p. administration in the tail flick test, of the following NSAIDs: ibuprofen, meloxicam or paracetamol produced a dose-related antinociception accompanied by an increase the control time latency with an ED_{25} of 1.81 ± 0.37 mg/kg, 12.33 ± 1.92, and 35.40 ± 3.92 respectively. In addition, the i.p. administration in the tail flick assay, of the following opioids: fentanyl, morphine, tramadol or codeine increase the control latency time with an ED_{25} of 0.077 ± 0.01, 1.31 ± 0.54, 2.46 ± 0.15 and 11.85 ± 0.90, respectively. All these results are shown in table 1 and figure 1. The rank orden of potency of the mice latency time, demonstrated by the ED_{25} , was the following: fentanyl > morphine > ibuprofen > tramadol > codeine > meloxicam > paracetamol, see table 1. The antinociceptive ratio, compared with paracetamol, was between 460 for fentanyl and 1 for meloxicam, as shown in table 1.

Table 1 ED_{25} values with SEM in mg/kg, for the antinociceptive activity of opioids and NSAIDs, administered i.p., in the tail flick assay of mice

Drug	ED_{25} ± SEM (mg/kg)	Antinociceptive ratioa
Fentanyl	0.077 ± 0.01	460
Morphine	1.31 ± 0.54	27
Ibuprofen	1.81 ± 0.37	19
Tramadol	2.46 ± 0.15	14
Codeine	11.85 ± 0.90	3
Meloxicam	12.33 ± 1.92	3
Paracetamol	35.40 ± 3.92	1

a compared to paracetamol

3.2. Effect of naltrexone on antinociception of NSAIDs and opioids

The pretreatment of mice with 1 mg/kg of naltrexone i.p., dose which does not induce change in the basal antinociception or spontaneous behavior of the tail flick mice. Table 2 show that pretreatment with the opioid receptor antagonist, naltrexone, 1 mg/kg i.p., significant reversed the antinociceptive effect of fentanyl, morphine, ibuprofen, tramadol, codeine and meloxicam. The effect of naltrexone is demonstrated in an increased the ED_{25} values of latency time of NSAIDs and opioids. The ratio of change of the ED25 values was 1.29 for fentanyl, 1.94 for morphine, 2.07 for ibuprofen, 2.17 for tramadol, 2.54 for codeine, 2.90 for meloxicam and 3.11 for paracetamol.

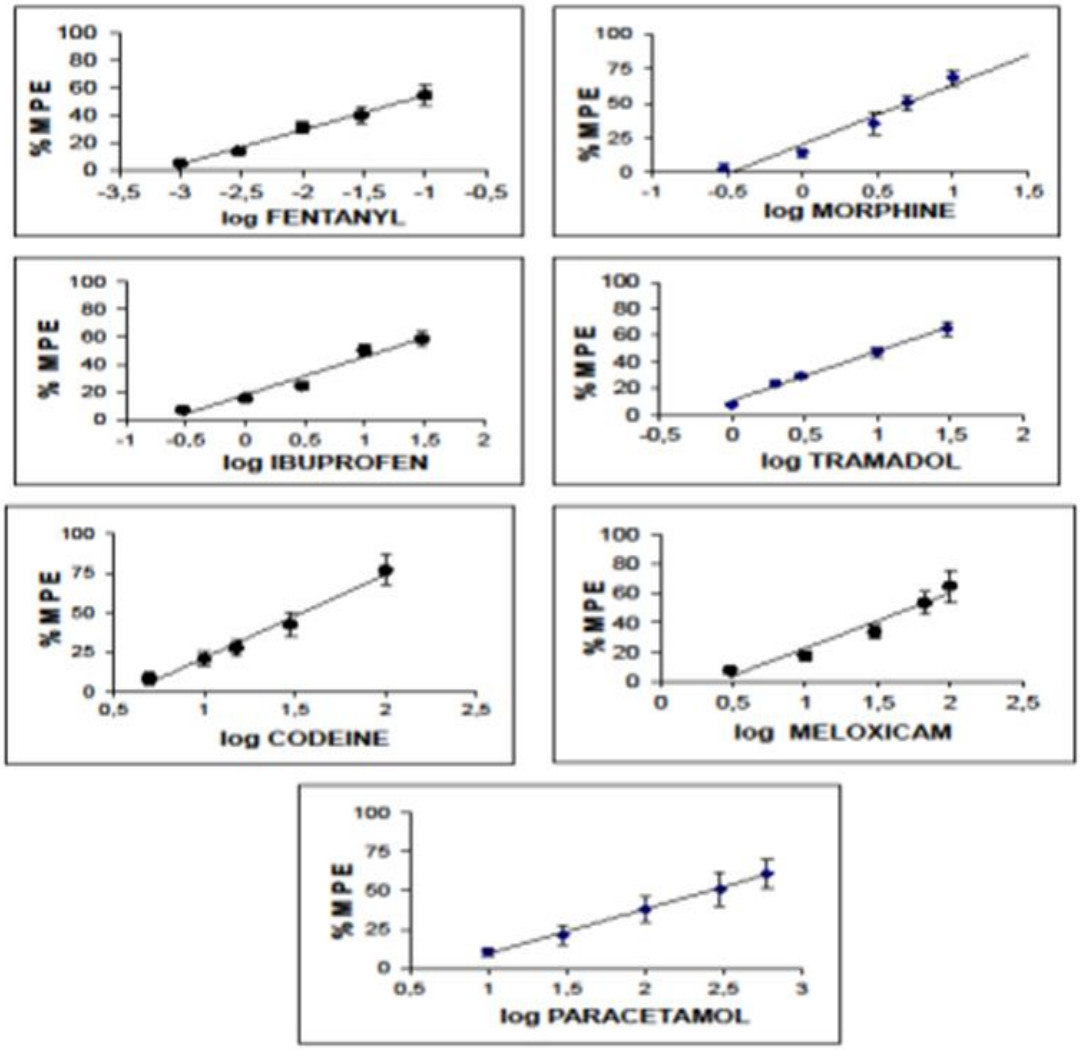

Figure 1 Dose-response curves for the antinociceptive activity induced by fentanyl, morphine, ibuprofen, tramadol, codeine, meloxicam and parcetamol in the tail flick test of mice. Each point is the mean with SEM of 6-8 mice. % MPE represented as percent of maximum possible effect.

Table 2 Effect of 1 mg/kg i.p. of naltrexone on the ED_{25} values with SEM in mg/kg, for the antinociceptive activity of opioids and NSAIDs, administered i.p., in the tail flick assay of mice.

Drug	Before naltrexone ED_{25} ± SEM (mg/kg)	After naltrexone ED_{25} ± SEM (mg/kg)	Ratio After/Pre
Fentanyl	0.077 ± 0.01	0.10 ± 0.09*	1.29
Morphine	1.31 ± 0.54	2.55 ± 0.51*	1.94
Ibuprofen	1.81 ± 0.37	3.75 ± 1.27*	2.07
Tramadol	2.46 ± 0.15	5.36 ± 1.37*	2.17
Codeine	11.85 ± 0.90	30.10 ± 5.81*	2.54
Meloxicam	12.33 ± 1.92	35.84 ± 2.52*	2.90
Paracetamol	35.40 ± 3.92	110.20 ± 8.65*	3.11

* Indicates significant difference (P> 0.05) than before naltrexone

4. Discussion

The aim of the present study was to examine the nocifensive activity of NSAIDs and opioids in a model of moderate to severe pain, the tail flick assay and in addition the effect of naltrexone, a known MOR antagonist. The findings confirm the effect dose-dependent with specific potency of fentanyl, morphine, ibuprofen, tramadol, codeine, meloxicam and paracetamol. In greement with previous studies it was obtained a marked antinociception of NSAIDs and opioids in the tail flick assay [9 - 14]. However, the present findings do not agree with those reported by Zelcer et al., [15] that point out that NSAIDs are inactive in the tail flick trial. Furthermore, Kolesnikow et al [16] have reported that ibuprofen does not produce antinociception in the tail flick test. The difference between these results could be due to the species of animal used (rat versus mice), the doses administered (mg / kg versus ug / kg), the routes of administration (i.t. versus i.p.), the assay used (visceral pain vs thermal pain).

The main finding of the present study was the inhibition of the antinociception through the antagonist MOR, naltrexone, on the antinociception induced by NSAIDs and opioids in the acute thermal assay of the tail flick. The results obtained with naltrexone in this assay confirm the antagonism of this agent on opioids [17-19]. However, the antagonism over NSAIDs is a new finding since that has not been previously reported.

NSAIDs are a heterogeneous group of drugs widely used for the treatment of pain. It is widely accepted that the main mechanism of action of these compounds, also involved for the side effect of gastric mucosal damage, is inhibition of cyclooxygenases (COXs), a key enzyme in prostanoids synthesis. The results obtained in this study demonstrate greater potency of COX-1 selective inhibitor NSAIDs (ibuprofen) than COX-2 selective (meloxicam) and both of greater potency than COX-3 associated inhibitors (paracetamol) [20]. COXs inhibition is not the only antinociceptive mechanism of action of NSAIDs. Additionally, preclinical studies have shown that there are other mechanisms that are involved in this antinociceptive effect, including their interaction with endocannabinoids CB1 and CB2, monoaminergic, and cholinergic systems. Interaction with nitric oxide (NO) through the inhibition of expression/activity of iNOS. Furthermore, antinociceptive effects of NSAIDs have been related with an endogenous opioid system possibly involving the descending pain modulatory circuit. Furthermore, NSAID-induced antinociception is also associated with decreased levels of dinorphins A in the frontal cortex and is prevented by blocking the κ-opioid receptors. Additionally, NSAIDs have been shown to have other alternative mechanisms for their antinociceptive function, among which are mentioned, down-regulation of L-selectin, inhibition of β_2 integrin activation, inhibition of NF-kappaβ, modulation of IL-6, IL-1β, matrix metalloproteinases, lactoferrin, phospholipase and others [3, 4, 10, 21 - 27].

In the present study the antinociceptive efficacy of fentanyl in the mouse tail flick assay was greater compared to morphine, tramadol or codeine. This finding agrees with previous work [28-29] and shows the higher potency of fentanyl. This finding confirms the functional selectivity [30-31] of fentanyl, an MOR agonist capable of producing antinociception and receptor regulation, in contrast to morphine, which by activating the same receptor, only produces antinociception. Furthermore, despite the fact that the opioids used are MOR agonists and antagonized by a MOR blocker (naltrexone), the difference in their activity could lie in the multiplicity of variants of the MOR-1 subtype, of which have been described around 20 in mice, with variable intrinsic activity and efficacy [15].

Opioids such as fentanyl, morphine, codeine, and tramadol are an important type of pain reliever and produce their effects by activating defined opioid receptors, although prolonged use induces serious side effects such as tolerance, dependence, and addiction. There are selective opioid receptor antagonists, including naloxone, naltrexone, naltrindole, and norbinaltorphimine that block the effects of opioids. In addition to activating the corresponding opioid receptors, there are alternative mechanisms that could help explain the antinociceptive activity of opioids. It has been reported that opioid receptor agonists induce antinociception by interaction through cannabinoid (CB1) receptors [8]. Also opioids activity have been reported by inhibition of both NA and 5-HT reuptake [24]. However, an important contribution to opioid antinociception is the interaction capacity to open K^+ channels and inhibit the opening of Ca^{+2} channels, ions that play a very important role in the transmission of the pain signal to through Substance P, neurokinin1, calcitonine gene related peptide, glutamate [28].

The administration of NSAIDs and opioids induced an antinociceptive effect, but with different potency, in the tail flick test. The mechanism of action of these drugs is different since one of them is related to the inhibition of COXs and the other to the activation of specific receptors. However, the antagonism produced by the MOR blocker, naltrexone, suggests that the inhibition of pain produced by these drugs is partially mediated by MOR and associated to central mechanisms.

This study provides new information related to the activity of antinociceptive drugs in a test that is highly correlated with the improvement of human pain.

5. Conclusion

This study test that NSAIDs and opioids induce antinociceptive activity in a thermal murine phasic pain with the following rank orden of potency fentanyl > morphine > ibuprofen > tramadol > codeine > meloxicam > paracetamol. Although the mechanisms of action of these drugs are different, naltrexone, a MOR antagonist, blocked the effects of both agents, suggesting that inhibition of pain seem partially mediated by MOR with association to central mechanisms.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

Experiments were performed in accordance with current Guidelines for The Care of Laboratory Animals and Ethical Guidelines for investigation of experimental pain approved by the Animal Care and Use Committee of the Faculty of Medicine, University of Chile, protocol CBA N° 852/2018.

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