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Receptors involved in dexketoprofen analgesia in murine visceral pain

V Noriega[1,2], F Sierralta[3], P Poblete[4], N Aranda[5], R Sotomayor-Zárate[6], J C Prieto[1,3] and H F Miranda[5]*

[1]*Cardiovascular Department, Clinical Hospital, Universidad de Chile, Santiago, Chile*

[2]*Facultad de Medicina, Clinica Alemana, Universidad del Desarrollo, Santiago, Chile*

[3]*Pharmacology Program, ICBM, Faculty of Medicine, Universidad de Chile, Santiago, Chile*

[4]*Clínica Alemana, Santiago, Chile*

[5]*Neuroscience Department, Faculty of Medicine, Universidad de Chile, Santiago, Chile*

[6]*Laboratorio de Neuroquímica y Neurofarmacología, Centro de Neurobiología y Fisiopatología Integrativa, Instituto de Fisiología, Facultad de Ciencias, Universidad de Valparaíso, Valparaiso, Chile*

**Corresponding author (Email, hmiranda@med.uchile.cl)*

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Various animal models, especially rodents, are used to study pain, due to the difficulty of studying it in humans. Many drugs that produce analgesia have been studied and there is evidence among which NSAIDs deserve to be highlighted. Dexketoprofen (DEX) provides a broad antinociceptive profile in different types of pain; therefore, this study was designed to evaluate the profile of antinociceptive potency in mice. Analgesic activity was evaluated using the acetic acid abdominal constriction test (writhing test), a chemical model of visceral pain. Dose-response curves for i.p. DEX administration (1, 3, 10, 30 and 100 mg/kg), using at least six mice in each of at least five doses, was obtained before and 30 min after pre-treatment with different pharmacological agents. Pretreatment of the mice with opioid receptor antagonists was not effective; however, the serotonin receptor antagonist and nitric oxide synthase inhibitor produce a significant increase in DEX-induced antinociception. The data from the present study shows that DEX produces antinociception in the chemical twisting test of mice, which is explained with difficulty by the simple inhibition of COX. This effect appears to be mediated by other mechanisms in which the contribution of the NO and 5-HT pathways has an important effect on DEX-induced antinociception.

Keywords. Dexketoprofen; receptor antagonists; visceral; writhing test

1. Introduction

Experimental models in animals, especially in rodents, are frequently used to study pain due to the difficulty of carrying out these investigations in humans. Pain in animals is measured using quantifiable, sensitive and specific sensations. Animal signs of pain include limb or tail movements, or reduced vocalization or locomotion or agitation. There is substantial evidence that NSAIDs induce analgesia.

NSAIDs acts mainly as inhibitors of cyclooxygenase enzymes (COXs) limiting prostaglandin synthesis, however COXs inhibition not only modulates pain, also mediates in other biological functions such as: blood pressure, sleep, vasoconstriction, flushing, vasodilatation, ocular pressure and others. NSAIDs are weak organic acids with hydrophobic properties, which facilitate their binding to COXs. All of them are absorbed completely orally, have minor first-pass hepatic metabolism, and are strongly bound to albumin.

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Based on their chemical structure, they are classified into different groups, among which it is possible to mention the derivatives of salicylic acid (aspirin and diflunisal), the members of phenylacetic acid (diclofenac, ketorolac), the members of enolic acid (piroxicam and meloxicam), the derivatives of anthanilic acid (meclofenamic and mefenamic), the related ones propionic acid (ibuprofen, naproxen and ketoprofen), those of the diarylheterocyclic group (celecoxib and eterocoxib) and those related to COX-3 (metamizole and paracetamol) (Patrignani and Patrono 2015; Grosser *et al.* 2017). The diverse NSAIDs, especially the members of propionic acid, have a chiral center due to encloses a carbon in the α-position in their structure, converting NSAIDs into a racemic compound. In the case of ketoprofen is formed a dextrorotatory enantiomer of S (+) configuration that possesses high antinociceptive activity and called dexketoprofen and another enantiomer R (−) of slight analgesic activity (Hardikar 2008).

Numerous studies have shown that dexketoprofen is a drug widely used, both in man and in animals, for the treatment of pain. These studies show consistent and confirmatory results in the role of the drug in their use for the treatment of acute, chronic, inflammatory, neuropathic and other pains (Zippel and Wagenitz 2007; Moore and Barden 2008; Miranda *et al.* 2009; Walczak 2011; Miranda *et al.* 2012; Moore *et al.* 2016; Derry *et al.* 2016; Fornasari *et al.* 2017; Hanna and Moon 2019).

According to the previous background, dexketoprofen provide a broad antinociceptive profile in different types of pain. The objective of this work was to continue studying the mechanism of pharmacological profile of dexketoprofen and determine whether opioidergic, nitridergic and serotonergic receptors are involved in antinociception induced by dexketoprofen in a murine visceral pain model.

2. Materials and methods

2.1 *Animals*

CF-1 male mice, 28–30 g, housed in a 12 h light/dark at 22°±1° C, with free access to food and water and acclimatized to laboratory condition for at least 2 h, were used. Experimental procedures were carried out in agreement with the Institutional Animal Care and Use Committee. Each animal was used only once and injected only one dose of the drugs tested and the behavior assay was performed by investigators blinded to the treatment. Control saline animals, two mice by group, were run interspersed concurrently with the drug treated mice, which prevented run as a simple group.

2.2 *Drugs*

Drugs were freshly dissolved in sterile physiological salt solution of 10 ml/kg and administered intraperitoneally (i.p.) included deketoprofen trometamol (DEX) provided by Menarini, Spain, risperidone (RISPER) by Royal Pharma S.A. naltrexone hydrochloride (NTX), naltrindole hydrochloride (NTI), Nor-binaltorphimine (NOR-BNI) dihydrochloride, Nw-nitro-L-arginine methyl ester (L-NAME) and tropisetron hydrochloride (TROPI) were purchased from Sigma-Aldrich Chemical Co, St. Louis, MO, USA.

2.3 *Nocifensive assay*

Analgesic activity was assessed by the acetic acid abdominal constriction test (writhing test), a chemical visceral pain model, as previously described Miranda *et al.* (2006). The abdominal constriction is defined as an exaggerated extension of muscle abdomen accompanied with the outstretching of the hind limbs.

Antinociceptive activity, expressed as ED_{50}, was identified as percent inhibition of the usual number of writhes observed in saline control animals in this study (21.80 ± 1.40, N= 35).

2.4 *Protocol*

Dose-response curves for i.p. administration of DEX (1,3,10,30 and 100 mg/kg), using at least six mice at each of at least five doses, were obtained before and 30 min after pretreatment with 1 mg/kg of NTX, NT or NOR-BNI, or 3 mg/kg of L-NAME, or 0.01 mg/k of RISPER or 0.1 mg/kg of TROPI. The doses of antagonists used in this study were taken from those reported in the literature (González *et al.* 2019). The log dose-response curves permitted the calculation of the dose that produced 50 % of antinociception (ED50).

2.5 *Statistical*

All results are expressed as means ± standard error of mean (SEM) and were analyzed using a one-way ANOVA followed by Tukey's *post hoc* test. All

calculations were performed with the program Pharm Tools Pro, version 1.27, Mc Cary Group Inc., PA, USA. P values under 0.05 ($p < 0.05$) were considered significant.

3. Results

All doses of each drug used in this study did not cause any detectable variation in gross behavior, motor coordination or spontaneous motility activity of the mice

3.1 *Antinociception induced by DEX in the writhing assay*

Administration via i.p. of DEX at doses of 1, 3, 10, 30 and 100 mg/kg, produced a dose-dependent antinociception in the writhing test of mice, as can be seen in figure 1. The inhibition of the writhing assay response was expressed as the maximal possible effect in per cent (% MPE). The ED_{50} for DEX was 12.60 ± 0.72 mg/kg (table 1) and with a number of writhes of 13.05 ± 0.95, see figure 2.

3.2 *Effect of NTX, NTI and NOR-BNI on DEX antinociception*

When the mice were pretreated i.p. with 1 mg/kg of NTX, or NTI or NOR-BNI, 30 min previous to DEX administration, did not induced a significant modification of antinociceptive ED_{50} of DEX since the values were 14.23 ± 1.08 mg/kg for NTX, 13.90 ± 1.01 mg/kg for NTI and 12.34 ± 1.06 for NOR-BNI mg/kg, with 13.80 ± 1.25, 14.25 ± 1.22 and 14.91 24 ± 1.17 writhes, respectively (figures 2 and 3; table 1).

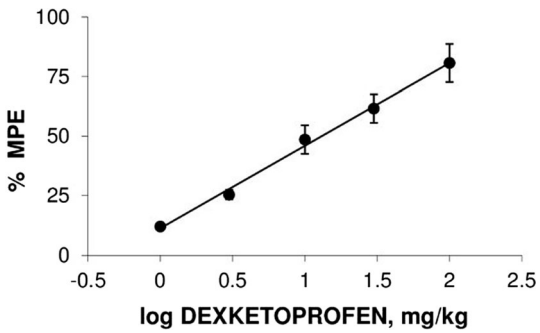

Figure 1. Dose-response curve for the antinociceptive activity induced by dexketoprofen (1,3,10,30,100 mg/kg, i.p.) in the visceral acetic acid writhing test of mice. Each point is the mean with SEM of 6–8 animals. % MPE antinociception represented as percentage of maximum possible effect.

Table 1. ED_{50} values (means ± SEM) for the antinociceptive activity of dexketoprofen administered i.p. in the acetic acid writhing test after pretreatment with 1 mg/kg of naltrexone (NTX), or naltrindole (NTI) or nor-binaltorphimine (NOR-BNI), 3 mg/kg of L-NAME, 0.1 mg/kg of tropisetron (TROPI) or 0.01 mg/kg of risperidone (RISPER)

Drug	ED_{50} (mg/kg)
Dexketoprofen	12.60 ± 0.72
DEX + NOR-BNI	13.80 ± 1.08
DEX + NTI	13.90 ± 1.01
DEX + NTX	14.23 ± 1.08
DEX+ RISPER	20.59 ± 1.78*
DEX + L-NAME	21.10 ± 1.95*
DEX + TROPI	23.44 ± 1.94*

*p < 0.05 compared to dexketoprofen.

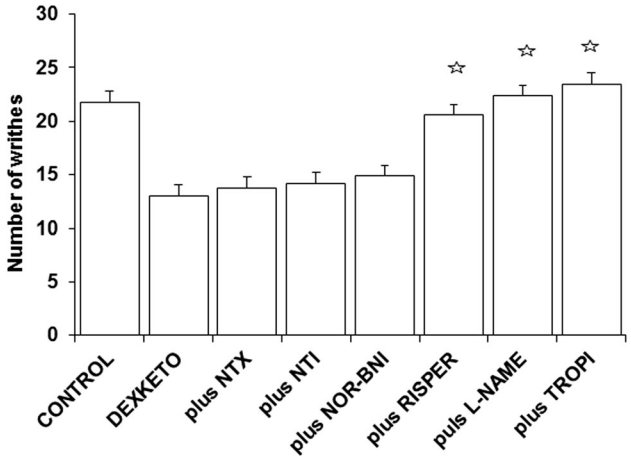

Figure 2. Effect of intraperitoneal administration of 12.60 mg/kg of dexketoprofen (DEXKETO) plus 1 mg/kg of naltrexone (NTX), 1 mg/kg of naltrindole (NTI), 1 mg/kg of nor-binaltorphimine (NOR-BNI), 3 mg/kg of L-NAME, 0.01 mg/kg of risperidone (RISPER) and 0.1 mg/kg of tropisetron (TROPI) on the number of writhes (mean ± SEM) induced by 10 ml/kg of 0.6 acetic acid solution. ☆Indicates significant difference versus DEXKETO ($p<0.05$).

3.3 *Effect of L-NAME on DEX antinociception*

The i.p pretreatment of the mice with 3 mg/kg of L-NAME preceding to DEX administration resulted in a significant increase of the antinociceptive ED_{50} of DEX to 21.10 ± 1. 95 mg/kg and 22.40 ± 1.80 writhes (figure 2 and 4; table 1).

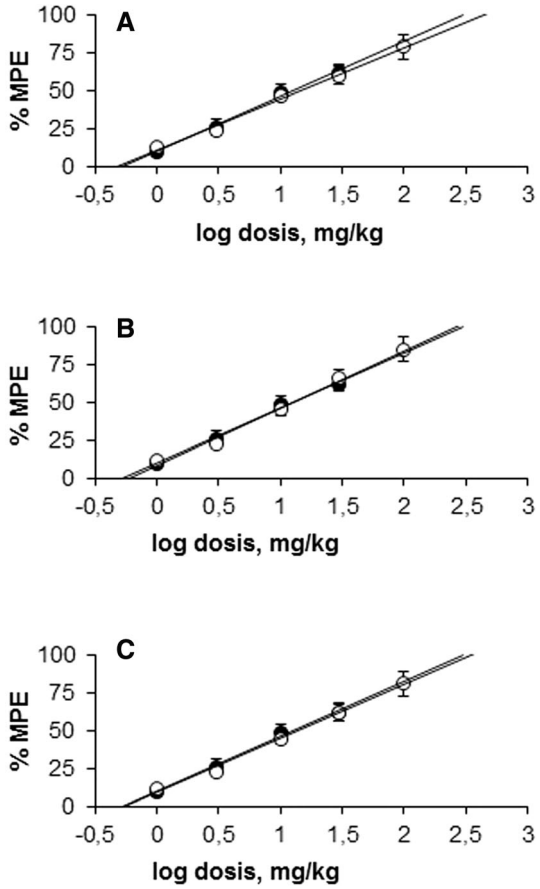

Figure 3. Dose-response curves for the antinociceptive activity in the writhing test of mice. (**A**) 1,3,10,30,100 mg/kg, i.p. of dexketoprofen (o) plus 1 mg/kg of naltrexone (●), (**B**) plus 1 mg/kg i.p. of naltrindole (●) and (**C**) plus 1 mg/kg i.p. of nor-binaltorphimine (●). Each point is the mean with SEM of 6–8 animals. % MPE antinociception represented as percentage of maximum possible effect.

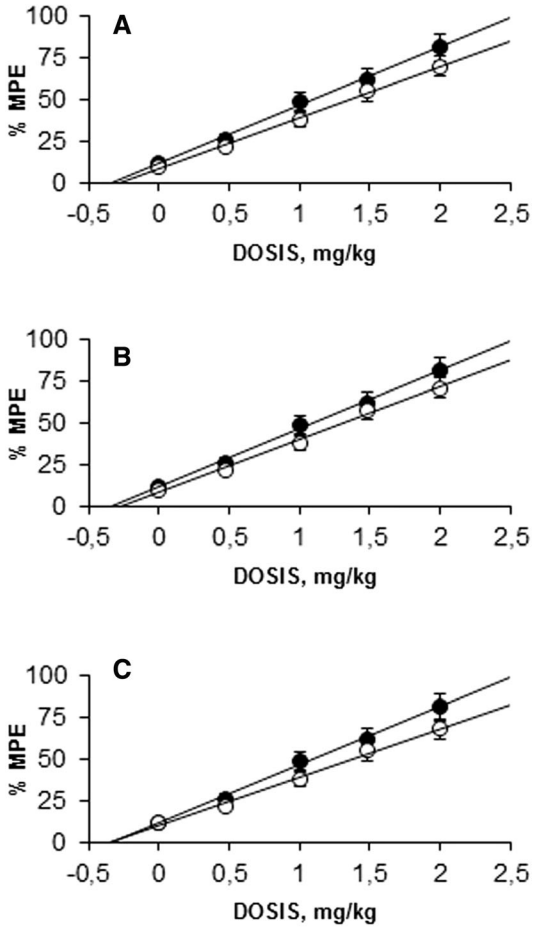

Figure 4. Dose-response curves for the antinociceptive activity in the writhing test of mice. (**A**) 1,3,10,30,100 mg/kg, i.p. of dexketoprofen (o) plus 0.01 mg/kg of risperidone (●), (**B**) plus 3 mg/kg of L-NAME (●) and (**C**) plus 0.1 mg/kg of tropisetron (●). Each point is the mean with SEM of 6–8 animals. % MPE antinociception represented as percentage of maximum possible effect.

3.4 *Effect of RISPER on DEX antinociception*

The i.p. administration of 0.01 mg/kg of RISPER, to the mice, prior to DEX treatment, caused a significant enhance of the antinociceptive ED_{50} of DEX to 20.59 ± 1.78 mg/kg and 20.10 ± 0.85 writhes, as can be seen in figures 2 and 4 and table 1.

3.5 *Effect of TROPI on DEX antinociception*

The i.p pretreatment of the mice with 0.1 mg/kg of TROPI, before DEX treatment, induced a significant augment of ED_{50} value of DEX antinociception to 23.44 ± 1.94 mg/kg and 23.50 ± 1.91 writhes, as shown in figures 2 and 4 and table 1.

4. Discussion

The acetic acid writhing test in mice was used to evaluate the effect of dexketoprofen on visceral antinociception. This assay has been described as having good sensitivity to analgesics, its induction is easy and reproducible, it allows screening of multiple compounds in short periods and can be considered as a model for preclinical assessment of inflammatory pain (Muley *et al.* 2016). On the other hand, result with DEX, confirm that the two enantiomers of ketoprofen (S+) and (S−) have differences in their pharmacological effects, so (S+), called DEX, has high COX inhibitory activity, which was the enantiomer used in this study.

The test used in this work has allowed the screening of the antinociceptive activity of multiple compounds that has been associated with a wide variety of mediators among which can be mentioned eicosanoids, neuropeptides, tackykinins, 5-HT, histamine, glutamate, NE, NO, DA, nerve grow factors (NGF) dynorphins, calcitonin gene-related peptide (CGRP), GABA, glycine, cannabinoids (CB1 and CB2), opioid peptides, prostanoids, neurokinin, adenosine and others (Argoff 2011; Baptista-de-Souza *et al.* 2018; Yam *et al.* 2018). The findings of the present study suggest that in this visceral trial, DEX produces antinociception, therefore, the drugs that modify or modulate the mediators of the nociception processes could modify the antinociception induced by DEX.

To describe the possible mechanism of antinociceptive action of DEX, the role of the opioidergic receptors in the visceral test of abdominal contortions induced by administration of acetic acid solution was investigated. Although, opioids activation induce analgesia and antinociception, the pretreatment of mice with NTX, NTI or NOR-BNI were not able to modify the antinociceptive activity of DEX in this visceral assay. According to this result, it seems that opioid receptors are not involved in the antinociception of DEX in this type of pain test, finding that is concordant with previous finding but in another algesiometric test (Zegpi *et al.* 2009).

The participation of 5-HT_1, 5-HT2, 5-HT_3 and 5-HT_7 receptors in central and peripheral areas related to pain modulation has been reported, however, this effect is dependent on several factors, among which the dose of agonists or antagonists, pain type and duration are included (Díaz-Reval *et al.* 2004; Tiippana *et al.* 2013; Cortes-Altamirano *et al.* 2018). The results of this study revealed that pretreatment of the mice with TROPI, a selective $5HT_3$ antagonist reversed the antinociception induced by DEX in the writhing assay of mice. A similar increase in the number of writhes induced by DEX was obtained by the pretreatment of mice with RISPER, an antagonist of 5-HT_2 and D2 receptor (Corena-McLeod 2015). The results obtained with TROPI and RISPER are in agreement to reported previously (Miranda *et al.* 2016; González *et al.* 2019) and support the conclusion that inhibition of writhes induced by DEX, following TROPI and RISPER were mediated by 5-HT.

Several studies have shown that nitric oxide (NO) is an important neurotransmitter involved in nociceptive processes by modulating multiple and complex mechanisms. It has been shown that NO exerts dual effects by inhibiting nociception and on the other hand mediating the analgesic effects of opioids and other drugs. From experimental data, information has been obtained that inhibitors of NO synthesis, such as L-NAME, have analgesic effects (Luo and Cizkova 2000; Miclescu and Gordh 2009; Cury et al. 2011) [36-38]. The increased number of writhes obtained in the current visceral test after pretreatment of animals with L-NAME, inhibitor of nitric oxide synthase, suggest that NO is involving in DEX antinociception.

The data of the present study show that DEX produces antinociception in the chemical writhing test of mice which it hard explains by the simple COX inhibition. This effect appears to be mediated by others mechanisms in which the contribution of NO and 5-HT pathways have an important effect in the antinociception induced by DEX.

5. Conclusions

Administration of DEX produces antinociception in mice. Pretreatment of mice with opioid antagonists (NTX, NI, NOR-BNI) does not change the antinociceptive activity of DEX. However, the administration of serotonin and dopamine antagonists (TROPI and RISPER) as the nitric oxide synthase inhibitor (L-NAME) significantly modifies DEX activity. These findings cannot be explained only by COX inhibition, it is possible to suggest that other mechanisms related to nitridergic and serotonergic pathways could be involved.

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