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Pharmacological profile of dexketoprofen in orofacial pain

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ABSTRACT

Background: Non-steroidal anti-inflammatory drugs (NSAIDs) may act through others mechanisms, in addition to inhibition of prostaglandin synthesis. These includes cholinergic, NO, serotonergic and opioids pathways.
Methods: The aim of this work was to evaluate the effect of systemic action of (S)-+-ketoprofen (dexketoprofen, DEX) on pain behaviors using the orofacial formalin test in mice and the potential involvement of cholinergic, NO, serotonergic and opioids pathways.
Results: The pretreatment of the mice with 1 mg/kg *ip* of atropine or opoid antagonists: 1 mg/kg, *ip* of NTX or 1 mg/kg *ip* of NTI or 1 mg/kg of NOR-BNI *ip*, did not produce significant change in the ED_{50} values of the antinociception to orofacial test induced by DEX. The pretreatment of the mice with 0.5 mg/kg *ip* tropisetron, increased in a significant fashion the values of ED_{50} of DEX. When the mice were treated with 5 mg/kg *ip* of L-NAME or 25 mg/kg *ip* of aminoguanidine or 50 mg/kg *ip* of 7-nitroindazole reversed the antinociception of DEX.
Conclusion: The findings of this study demonstrate activation of NO and 5-HTpathways play important roles in the systemic antinociceptive effect of DEX in a murine model of inflammatory pain.

Introduction

In the treatment of pain, either neuropathic or nociceptive, different agents are used, including nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs acts primarily by inhibiting prostaglandin synthesis restraining prostaglandin synthesis acting as inhibitors of cyclooxygenase enzymes (COXs), however its effects can not be explained exclusively by this enzyme inhibition. The effect of NSAIDs on the inhibition of COX, an enzyme that converts arachidonic acid to prostaglandins (PGs), provides an explanation for most of their pharmacological actions, including their anti-inflammatory, analgesic, antipyretic and platelet anti-aggregant effects, as well as for their deleterious side effects, such as stomach ulcer and renal insufficiency. However, several lines of evidence suggest that NSAIDs have additional mechanisms between them appear the ability of NSAIDs to penetrate biological membranes where they disrupt important processes of cellular function [1–5]

The different members of the NSAID family, include among them the derivatives of propionic acid, such as ibuprofen, naproxen, ketoprofen, etc. The most of them have a chiral center where the dextrorotatory enantiomer with S(+) configuration possesses high COX inhibitory activity, in contrast, R(−) enantiomer have poor inhibitory activity. (S)-+-ketoprofen, named, dexketoprofen (DEX), the active dextrorotatory enantiomer of the NSAID ketoprofen has been proved to be effective for relief pain either man and animal models [6–13]. Likewise other NSAIDs, antipyretic, anti-inflammatory, and analgesic mechanisms of action of DEX are based on the inhibition of prostaglandin synthesis. However, the pharmacological profile of DEX suggests that this drug may act through by others mechanisms, which it has been are scarcely investigated.

The present study was designed to evaluate the involvement of the cholinergic, NO, serotonergic and opioids pathways in the antinociceptive effect of DEX in the orofacial formalin inflammatory assay

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Materials and methods

CF-1 male mice, weighing 28–30 g, housed in a 12-h light-dark at 22° ± 1° C, with free acces to food and water, were used. The animals were acclimatized to the laboratory environment for at least 2 h before use. Experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals issued by the National Institute of Health, and experimental procedures were approved by the Institutional Animal Care and Use Committee. Each animal was used only once, and received only one dose of the drugs tested. All drugs were freshly prepared by dissolving them in normal saline, and administered intraperitoneally (*ip*) and the behavior test was performed by investigators blinded to the treatment groups. Control saline animals were run interspersed concurrently with the drug-treated animals (at least two mice per group), which prevented all the controls being run on a single group of mice at one time during the course of the research.

Orofacial formalin test

To perform the test, the method described previously was used [14]. 20 μL of 2% formalin solution was injected into the upper lip, just lateral to the nose with a 27-gage needle attached to a 50-μL Hamilton syringe. The applied chemical stimulus (formalin) can be considered noxious since it produces tissue injury, activates Aδ and C nociceptors as well as trigeminal and spinal nociceptive neurons. Each mouse was immediately returned to a plexiglass observation chamber. The test shows two clear-cut phases: Phase I corresponds to the 5-min period starting immediately after the formalin injection and represents a tonic acute pain due to peripheral nociceptor sensitization. Phase II was recorded as the 10-min period starting 20 min after the formalin injection and represents inflammatory pain. The nociceptive score was determined for each phase by measuring the total number of seconds that the animals spent grooming the injected area with the ipsilateral extremity. Grooming time for saline control was 103.07 ± 4.68 (n = 20) and 133.20 ± 4.36 (n = 20) seconds, respectively. Drugs or saline were administered to animals 30 min before formalin injection, a time at which preliminary experiments showed occurrence of the maximum effect. Total grooming time in each period was converted to per cent MPE as follows:

%MPE = 100-(postdrug grooming time/control grooming time saline) × 100

The dose that produced 50% of MPE (ED_{50}) was calculated from the linear regression analysis of a dose–response curve obtained by plotting log doses versus % MPE.

Protocol

Dose–response curves for *ip* administration of DEX, before and after pretreatment with different antagonists, were obtained using at least six to eight animals at each of at least four doses. A least-squares linear regression analysis of the log dose–response curve allowed the calculation of the dose that produced 50% of antinociception for DEX.

Drugs

Drugs used in this study were disolved in normal saline (0.9% w/v NaCl) in a constant volumen of 10 ml/kg and *ip* administered as mg/kg on the basis of the salts, included dexketoprofen DEX trometamol was provided by Menarini, Spain. 7-Nitroindazole sodium salt, aminoguanidine hydrochloride, atropine, sulfate, L-NAME (Nω-nitro-L-arginine methyl ester), naltrexone (NTX) hydrochloride, naltrindole (NTI) hydrochloride, nor-binaltorphimine dihydrochloride (NOR-BNI), and tropisetron hydrochloride were purchased from Sigma-Aldrich Chemical Co, St. Louis, MO, USA.

Statistical analysis

Results are presented as mean ± standard error of the mean (SEM) of 6–8 animals per group. All calculations were performed with the program Pharm Tools Pro (version 1.27; McCary Group Inc., PA, USA). p Values under 0.05 ($p < 0.05$) were considered significant.

Results

All experiments performed were carried out using dose of each drug that did not cause any detectable modification in gross behavior, motor coordination, or spontaneous motility activity.

Antinociception induced by DEX orofacial formalin inflammatory pain

The *ip* administration of DEX at doses of 3,10, 30, 100 and 300 mg/kg displayed a dose dependent antinociception in the phase I and phase II of the orofacial formalin test, as can be seen in Fig. 1. The ED_{50} of DEX in phase I resulted to be 16.66 ± 1.71 mg/kg and in phase II was 50.73 ± 4.04 mg/kg, see Table 1. DEX was 3.04 fold more potent at phase I than phase II.

Interaction of atropine in DEX induced antinociception

The pretreatment of the mice with 1 mg/kg *ip* of atropine, 30 minutes prior to the administration of DEX, lack of effect, in both phases, of the orofacial formalin test, in the antinociception produced by all the doses of the DEX tested. The corresponding ED_{50}'s values are not significant different, compared with the control value, see Table 1.

Interaction of opioidergic agent in DEX induced antinociception

After pretreatment of the mice with 1 mg/kg, *ip* of NTX did not produce significant change in the ED_{50} values of the antinociception to orofacial test induced by DEX, comparing with the mice in the control group. Pretreatment with the opioid antagonists NTI (1 mg/kg *ip*) or NOR-BNI (1 mg/kg *ip*) showed no change in the antinociceptive effect of DEX (see Table 1).

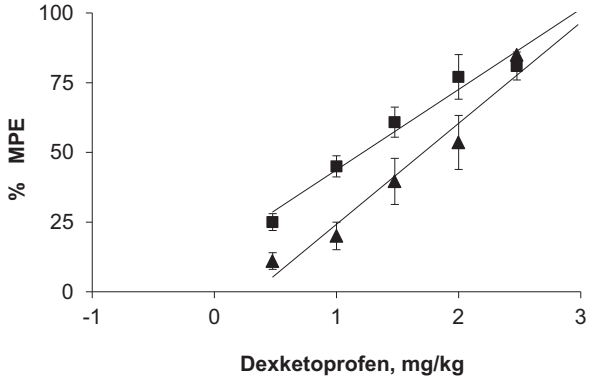

Fig. 1. Dose-response curves for the antinociceptive activity induced by deexketoprofen *via ip* in the fomalin orofacial assay in mice. ▲ = Phase I, . ■ = Phase II. Each point is the mean ± SEM of 8 animals. % MPE = antinociception as a percentage of the maximun possible effect.