



## Involvement of nitridergic and opioidergic pathways in the antinociception of gabapentin in the orofacial formalin test in mice



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### ABSTRACT

**Background:** Pain is one of the most common problems in clinical medicine. There is considerable evidence that pharmacologic approaches are the most widely used therapeutic options to ameliorate persistent or chronic pain. In this study it was evaluated the effect of L-NAME and naltrexone in the antinociception induced by administration of gabapentin in the orofacial formalin test of mice.

**Methods:** The algometer assay was performed by the administration of 20  $\mu$ l of 2% formalin solution injected into the upper right lip of each mouse.

**Results:** The dose of gabapentin that produces the 50% of the maximum possible effect (ED<sub>50</sub>) was significantly increased by the pretreatment with L-NAME or naltrexone.

**Conclusions:** These results suggest that gabapentin produce antinociception partly *via* the activation nitridergic pathways and opioid system.

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### Introduction

Pain is one of the most common problems in clinical medicine. It can be categorized as either nociceptive or neuropathic, the latter of which may be due to abnormal neural activity secondary to disease, injury, or dysfunction of the nervous system. There is considerable evidence that the pharmacologic approaches are the most widely used therapeutic options to ameliorate persistent or chronic pain. Studies have shown that the following drugs are effective in these types of pain; the non-steroidal anti-inflammatory drugs, the opioids and adjuncts or co-analgesic, such as: gabapentin, nortriptyline, amitriptyline, sertraline, citalopram, topiramate, valproic acid, tiagabine, lamotrigine [1–3].

Gabapentin, was originally developed as anticonvulsant to treat various seizure models for many years [4]. Recently it is widely used to alleviate neuropathic pain [5–8]. Gabapentin is a structural analog of gamma aminobutyric acid (GABA), but it does not bind to GABA A or B receptors to achieve the antinociceptive effect. Recently, the  $\alpha_2\delta$

subunit of voltage-gated calcium channel has been hypothesized as the target of gabapentin and inhibit the release of neurotransmitters, thereby modulating the influx of calcium [9].

L-NAME (N<sup>G</sup>-nitro-L-arginine methyl ester) is a non selective nitric oxide-synthase inhibitor (NOS) that induces controversial antinociception by stimulating the arginine/NO/cGMP pathway, and it has been suggested the gabapentin-induced antinociception [10–12].

Naltrexone, is a non selective opioid, which is competitive antagonist with MOR, DOR and KOR receptors, with a high affinity for MOR receptor but with a low efficacy. Though this property, it limits the possibility to precise the opioid receptor implicated in the antinociception, it could be used to differentiate activation of subtypes of opioid receptors [13–15].

This work was aimed to study the nitridergic and opioidergic mechanisms implicated in the antinociceptive response, induced by gabapentin on a model of acute and inflammatory pain in mice: the orofacial formalin test.

The stimulus produced by formalin can be considered noxious because it induces tissue injury, activates A $\delta$  and C nociceptors as well as trigeminal and spinal nociceptive neurons and produces a painful sensation in humans [16].

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## Materials and methods

Male CF-1 mice (28–30 g), housed in a 12 h light–dark cycle at  $22 \pm 1^\circ\text{C}$ , with access to food and water *ad libitum*, were used. Experiments were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and Ethical Guidelines for Investigation of Experimental Pain. All the experimental procedures were approved by the Institutional Animal Care and Use Committee at the Faculty of Medicine, University of Chile, and Santiago, Chile. Animals were acclimatized to the laboratory for at least 2 h before testing, were used only once during the protocol, and were killed, under anesthesia, by cervical dislocation immediately after algesiometric assay. The number of animals was kept at a minimum, compatible with consistent effects of the drug treatments. All observations during the assay were performed by the authors in a randomized and blind manner. 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

The antinociception was assessed by a modification of the orofacial formalin test described by Luccarini et al. [17]. To perform the test, 20  $\mu\text{l}$  of 2% formalin solution was injected into the upper right lip of each mouse, with a 27-gauge needle. The formalin solution induced a consistent rubbing behavior and the possibility to produce less tissue damage. The mice were immediately returned to the observation chamber. The degree of pain was determined by the total time the animal spent rubbing its lip with one of its extremities. The orofacial formalin induced two different phases that were separated by a period the relative inactivity: an early short lasting response, phase I (0–5 min) corresponding to the 5 min period starting immediately after formalin injection, and representing a tonic acute pain due to peripheral nociceptor sensitization, and a continuous prolonged response, phase II (20–30 min) the 10 min period starting 20 min after formalin injection which represents inflammatory pain. The effect of each drug was assessed after the administration of at least five doses in logarithmic increments. Maximum possible effect (MPE), which represents antinociception, was calculated as follows:

$$\% \text{MPE} = 100 - \frac{\text{post-drug rubbing time}}{\text{control rubbing time}} \times 100.$$

The dose that produced 50% of MPE ( $\text{ED}_{50}$ ) was calculated from the linear regression analysis of the dose–response curve obtained by plotting log dose vs. % MPE.

Administration of gabapentin (or saline solution for control group) and *L*-NAME or naltrexone occurred 30 min and 1 h, respectively, before formalin administration.

### Analysis of the interaction gabapentin with *L*-NAME or naltrexone

In order to assess the nature of interaction between gabapentin with *L*-NAME or with naltrexone, the fixed dose–method was used [18,19]. This type of analysis has been validated to establish the presence and type of interaction between two drugs, when one of them is inactive or does not generate a dose–response curve.

### Drugs

Gabapentin (0.3–100 mg/kg) or gabapentin plus *L*-NAME (1 or 5 mg/kg) or naltrexone (1 or 5 mg/kg) were administered intraperitoneally (*ip*) before the test, dissolved in physiologic saline

(0.9% (w/v) NaCl) in a constant volume of 10 ml/kg Gabapentin, *L*-NAME hydrochloride, naltrexone hydrochloride were purchased from Sigma, USA.

### Statistical analysis

Results are presented as  $\text{ED}_{50}$  values  $\pm$  SEM. The program used to perform statistical parameters was Pharm Tools Pro version 1.27, The McCary Group Inc., Elkin Park, PA, USA. Results were analyzed by Student's *t*-test for independent means or by two way ANOVA followed by the Student–Newman–Keuls test; *p* values lower than 0.05 ( $p < 0.05$ ) were considered statistically significant.

## Results

Mice tested with different doses of gabapentin, *L*-NAME or naltrexone did not exhibit significant behavioral impairment nor overt motor dysfunctions.

### Effect of gabapentin on the antinociception in the orofacial formalin test in mice

The *ip* administration of gabapentin in phases I and II of the orofacial formalin test induced statistically parallel dose–response curves with similar efficacy, but the relative potency was almost equal (see Fig. 1). The  $\text{ED}_{50}$  values and SEM for the antinociceptive effect of *ip* gabapentin are shown in Table 1.

### Effect of *L*-NAME on the antinociception proprieties of gabapentin in the orofacial formalin test in mice model

As it could not possible obtain a consistent dose response curve with *L*-NAME, the fixed dose–method was used to assess the presence of an interaction between gabapentin and *L*-NAME. The results demonstrated a rightward, non-parallel shift of the dose–response curve of the combination of gabapentin plus a fixed dose of *L*-NAME (1 or 5 mg/kg, *ip*) when compared with the dose–response curve of gabapentin alone. Two-way analysis of variance (ANOVA) demonstrate significant differences for the dose ( $p < 0.05$ ), the drug ( $p < 0.05$ ) and their interaction ( $p < 0.05$ ), demonstrating antagonism, see Fig. 2A–D.

### Effect of naltrexone on the antinociception proprieties of gabapentin in the orofacial formalin test in mice model

Since it was not possible to obtain a consistent dose response curve with naltrexone, the fixed dose–method was used to assess the presence of an interaction between gabapentin and naltrexone. The results demonstrated a rightward, non-parallel shift of the dose–response curve of the combination of gabapentin plus a fixed

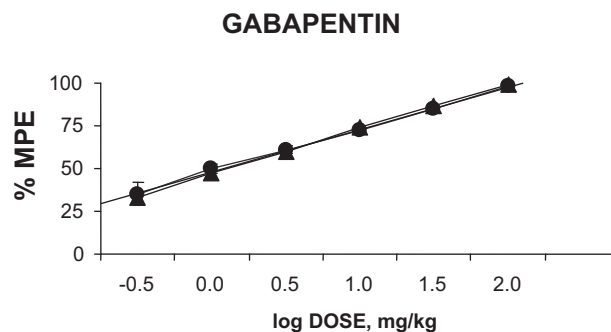


Fig. 1. Curve dose–response for gabapentin on the phase I (●) and in phase II (▲) of the formalin orofacial test. Each point represents the mean values  $\pm$  SEM of eight animals.

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