Pharmacological Reports 67 (2015) 399–403

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

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

Hugo F. Miranda [a,b,*], Fernando Sierralta [c], Sebastian Lux [b], Rocío Troncoso [d], Natalia Ciudad [b], Ramiro Zepeda [a], Pilar Zanetta [b], Viviana Noriega [a,e], Juan Carlos Prieto [b,e]

[a] Facultad de Medicina, Escuela de Química y Farmacia, Universidad Andrés Bello, Santiago, Chile
[b] Facultad de Medicina, ICBM, Universidad de Chile, Santiago, Chile
[c] Dental School, Universidad Finis Terrae, Santiago, Chile
[d] Facultad de Odontología, Universidad de Chile, Santiago, Chile
[e] Hospital Clínico, Universidad de Chile, Santiago, Chile

ARTICLE INFO

Article history:
Received 7 April 2014
Received in revised form 22 October 2014
Accepted 23 October 2014
Available online 7 November 2014

Keywords:
Tonic inflammatory pain
Gabapentin
L-NAME
Naltrexone
Orofacial formalin test

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 algesiometer assay was performed by the administration of 20 μl 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_{50}) 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.

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^G-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 Aδ and C nociceptors as well as trigeminal and spinal nociceptive neurons and produces a painful sensation in humans [16].

* Corresponding author.
E-mail address: hmiranda@med.uchile.cl (H.F. Miranda).

http://dx.doi.org/10.1016/j.pharep.2014.10.018
1734-1140/

Materials and methods

Male CF-1 mice (28–30 g), housed in a 12 h light–dark cycle at 22 ± 1° 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 μl 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 (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 ED_{50} values ± 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 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

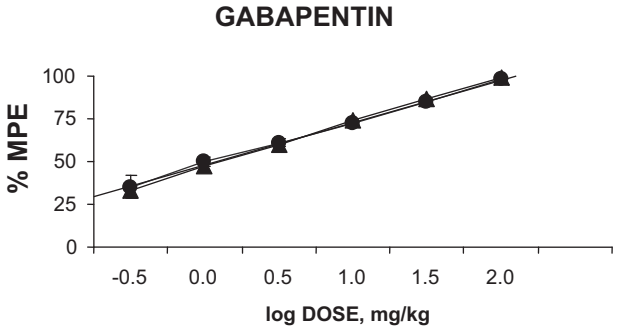

Fig. 1. Curve dose–response for gabapentin on the phase I (●) and in phase II (▲) of the formalin orofacial test. Each point represents the mean values ± SEM of eight animals.

Table 1
ED_{50} values (mg/kg ± SEM, $n = 24$) of the antinociceptive activity of gabapentin on the orofacial formalin test in mice.

Drugs	Phase I	Phase II
Gabapentin	1.13 ± 0.06	1.28 ± 0.04

dose of naltrexone (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. 3A–D.

Discussion

The results obtained in this study demonstrated the antinociceptive activity of gabapentin in the orofacial formalin test, either in phases I and II. This antinociception was dose-dependent with similar efficacy and potency. The antinociception induced by gabapentin is either partially or fully concordant with this effect in other types of pain in animals, depending of the type of nociceptive stimulus used, the type of pain, the specie, single or repeated administration, or the protocols. Thus, gabapentin resulted antinociceptive in phase II of the hind paw formalin test, but did not affect phase I, and the tail-flick response [20,21]. Gabapentin moderately affected neuropathic hyperalgesia following a single administration, but produced significant reversal following daily administration for 5 days. In addition, gabapentin was only weakly active against inflammatory hyperalgesia following single or repeated administration [22]. Also, gabapentin produce dose-dependent reduction in the rubbing behavior of phase II of the orofacial formalin test in the rat [23]. Gabapentin attenuates pain behavior in model of cancer induced bone pain [24]. It has been reported that the antinociceptive profile of gabapentin included acute and persistent model of pain, i.e., hind paw formalin, carrageenan injection, warm water tail withdrawal and nerve ligation [25].

The antagonist interaction obtained in this work, in both phases of the orofacial formalin test in the mice by the administration of L-NAME, an inhibitor of nitric oxide (NO) synthase, could be due to the reduction of the production of NO by the injection of formalin [26]. NO has been implicated at various levels of the nociception pathways. However, the effects of NO vary depending on the pain stimuli and the dose used, inhibition of NO has antinociceptive effects, in contrast, blockade of NO synthesis increase pain [11,26–28]. The findings of the present work with L-NAME, they are concordant with those reported by Ortiz et al. [28], however, the different protocol used (animal; doses, *via* of administration, time of administration, concentration of formalin solution, type of assay, response of phase I and phase II). Therefore, the L-NAME administration increases pain and in consequence it was obtained a significant non-parallel shift to the left of the dose–response curve of the combination L-NAME plus gabapentin.

In the case of the antagonist interaction between naltrexone with gabapentin in both phases of the orofacial formalin test in the mice, it has been suggested that the effect of gabapentin may be partially mediated through the NO/cyclic GMP signaling pathway [29]. On the other hand, changes in central NOS activity by morphine, appear to be mediated by opioid receptors because they were blocked by concurrent treatment with naltrexone [30]. Naltrexone is able to attenuate the rise in plasma NO levels, which are commonly observed after heat stress [31]. In addition, naltrexone inhibited morphine-induced sensitization of pentylenetetrazole clonic seizures in mice, probably as a result of the interaction with MOR receptors and the NO seizure threshold [32]. Therefore, the administration of naltrexone could block the antinociceptive effect of gabapentin increasing pain, and in consequence induces a significant non-parallel shift to the left of the dose–response curve of the combination naltrexone with gabapentin. This finding is not concordant with previous report [28], the discrepance could be due to the different opioid antagonist used: naltrexone *vs.* naloxone,

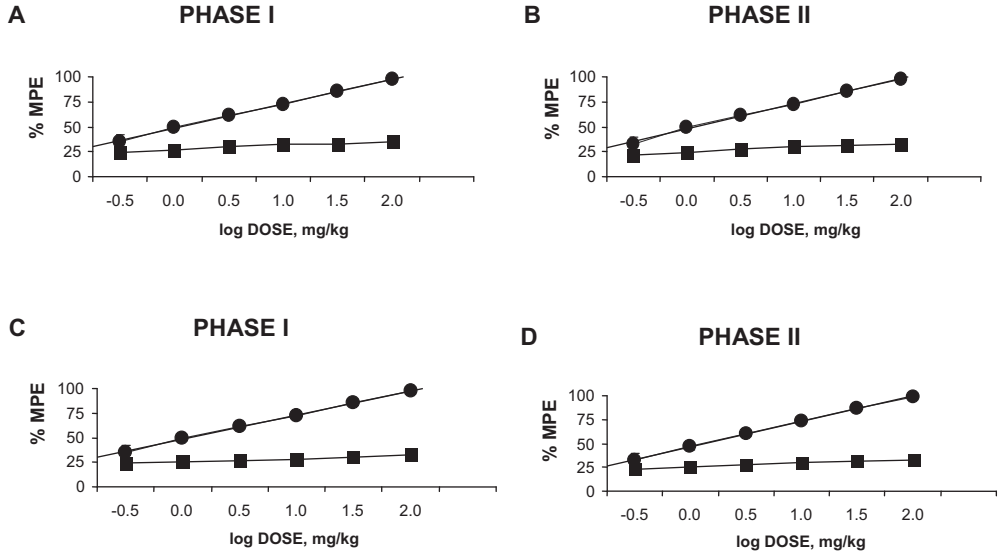

Fig. 2. (Panel 2A) Curve dose–response for gabapentin (●) alone and combined with L-NAME, 1 mg/kg, *ip* (■) on the phase I of the formalin orofacial test. Each point represents the mean values ± SEM of eight animals. All points of gabapentin alone are significantly different ($p < 0.05$) compared with the same dose of the combination gabapentin plus L-NAME, except for the dose of −0.5 log. (Panel 2B) Curve dose–response for gabapentin (●) alone and combined with L-NAME, 1 mg/kg, *ip* (■) on the phase II of the formalin orofacial test. Each point represents the mean values ± SEM of eight animals. All points of gabapentin alone are significantly different ($p < 0.05$) compared with the same dose of the combination gabapentin plus L-NAME, except for the dose of −0.5 log. (Panel 2C) Curve dose–response for gabapentin (●) alone and combined with L-NAME, 5 mg/kg, *ip* (■) on the phase II of the formalin orofacial test. Each point represents the mean values ± SEM of eight animals. All points of gabapentin alone are significantly different ($p < 0.05$) compared with the same dose of the combination gabapentin plus L-NAME, except for the dose of −0.5 log. (Panel 2D) Curve dose–response for gabapentin (●) alone and combined with L-NAME, 5 mg/kg, *ip* (■) on the phase II of the formalin orofacial test. Each point represents the mean values ± SEM of eight animals. All points of gabapentin alone are significantly different ($p < 0.05$) compared with the same dose of the combination gabapentin plus L-NAME, except for the dose of −0.5 log.

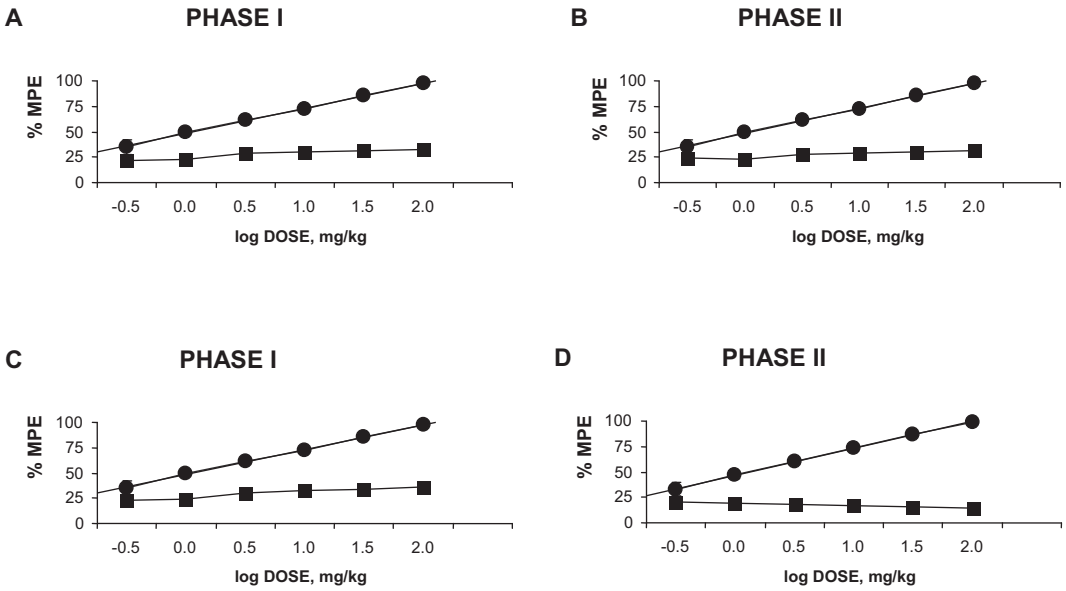

Fig. 3. (Panel 3A) Curve dose–response for gabapentin (●) alone and combined with naltrexone, 1 mg/kg, *ip* (■) on the phase I of the formalin orofacial test. Each point represents the mean values ± SEM of eight animals. All points of gabapentin alone are significantly different ($p < 0.05$) compared with the same dose of the combination gabapentin plus naltrexone, except for the dose of −0.5 log. (Panel 3B) Curve dose–response for gabapentin (●) alone and combined with naltrexone, 1 mg/kg, *ip* (■) on the phase II of the formalin orofacial test. Each point represents the mean values ± SEM of eight animals. All points of gabapentin alone are significantly different ($p < 0.05$) compared with the same dose of the combination gabapentin plus naltrexone, except for the dose of −0.5 log. (Panel 3C) Curve dose–response for gabapentin (●) alone and combined with naltrexone, 5 mg/kg, *ip* (■) on the phase II of the formalin orofacial test. Each point represents the mean values ± SEM of eight animals. All points of gabapentin alone are significantly different ($p < 0.05$) compared with the same dose of the combination gabapentin plus naltrexone, except for the dose of −0.5 log. (Panel 3D) Curve dose–response for gabapentin (●) alone and combined with naltrexone, 5 mg/kg, *ip* (■) on the phase II of the formalin orofacial test. Each point represents the mean values ± SEM of eight animals. All points of gabapentin alone are significantly different ($p < 0.05$) compared with the same dose of the combination gabapentin plus naltrexone, except for the dose of −0.5 log.

animal; doses, *via* of administration, time of administration, concentration of formalin solution, type of assay, response of phase I and phase II.

In conclusion, the study demonstrated the antinociceptive and anti-inflammatory activities of gabapentin at clinically relevant doses. These results suggest that gabapentin produce antinociception partly *via* the activation nitridergic pathways and the opioid system. This model of orofacial formalin test could be useful to estimate antinociceptive activity of drugs that could have clinical applications.

Conflict of interest

The authors declare that they have no conflicts of interest to reveal.

Funding

This work was supported by project DI-N° 76-12/CB from Universidad Andres Bello.

Acknowledgements

The expert technical assistance from José López and Alejandro Correa is gratefully acknowledged.

References

[1] Peniston JH. A review of pharmacotherapy for chronic low back pain with considerations for sports medicine. Phys Sports Med 2012;40(1):21–32.
[2] White AP, Arnold PM, Norvell DC, Ecker E, Fehlings MG. Pharmacologic management of chronic low back pain: synthesis of the evidence. Spine 2011;36(21 Suppl.):S131–43.
[3] Fine PG. Treatment guidelines for the pharmacological management of pain in older persons. Pain Med 2012;13(2 Suppl.):S57–66.
[4] McLean MJ. Gabapentin in the management of convulsive disorders. Epilepsia 1990;40(6 Suppl.):539–50.
[5] Wiffen PJ, McQuay HJ, Edwards J, Moore RA. Withdrawn: gabapentin for acute and chronic pain. Cochrane Database Syst Rev 2011;3:CD005452.
[6] Moore RA, Wiffen PJ, Derry S, McQuay HJ. Gabapentin for chronic neuropathic pain and fibromyalgia in adults. Cochrane Database Syst Rev 2011;3:CD007938. http://dx.doi.org/10.1002/14651858.CD007938.
[7] Peter C, Watson N, Gilron I, Sawynok J. .A qualitative systematic review of head-to-head randomized controlled trials of oral analgesics in neuropathic pain. Pain Res Manag 2010;15(1):147–57.
[8] Gilron I, Bailey JM, Tu D, Holden RR, Weaver DF, Houlden RL. Morphine, gabapentin, or their combination for neuropathic pain. N Engl J Med 2005;352(13):1324–34.
[9] Cheng JK, Chiou L-CH. Mechanisms of the antinociceptive action of gabapentin. J Pharmacol Sci 2006;100(5):471–86.
[10] Duarte ID, Ferreira SH. L-NAME causes antinociception by stimulation of the arginine-NO-cGMP pathway. Mediators Inflamm 2000;9(1):25–30.
[11] Sousa AM, Prado WA. The dual effect of a nitric oxide donor in nociception. Brain Res 2001;897(1):9–19.
[12] Kilic FS, Sirmagul B, Yildirim E, Oner S, Erol K. Antinociceptive effects of gabapentin & its mechanism of action in experimental animal studies. Indian J Med Res 2012;135(5):630–5.
[13] Waldhoer M, Bartlett SE, Whistler JL. Opioid receptors. Annu Rev Biochem 2004;73:953–90.
[14] Trescot AM, Datta S, Lee M, Hansen H. Opioid pharmacology. Pain Physician 2008;11(2 Suppl.). S133-S153.
[15] Pasternak GW. Preclinical pharmacology and opioid combinations. Pain Med 2012;13(1 Suppl.):S4–11.
[16] Raboison P, Dallel R. The orofacial formalin test. Neurosci Biobehav Res 2004;28(2):219–26.
[17] Luccarini P, Childeric A, Gayder AM, Voisin D, Dallel R. The orofacial formalin test in the mice: a behavioral model for studying physiology and modulation of trigeminal nociception. J Pain 2006;7(12):908–14.
[18] Berenbaum MC. What is synergism? Pharmacol Rev 1989;41(2):93–141.
[19] Miranda HF, Puig MM, Romero MA, Prieto JC. Effects of tramadol and dexketoprofen on analgesia and gastrointestinal transit in mice. Fund Clin Pharmacol 2009;23(1):81–8.
[20] Shimoyama N, Shimoyama M, Davis AM, Inturrisi CE, Elliott KJ. Spinal gabapentin is antinociceptive in the rat formalin test. Neurosci Lett 1997;222(1):65–7.
[21] Yoon MH, Yaksh TL. The effect of intrathecal gabapentin on pain behavior and hemodynamics on the formalin test in the rat. Anesth Analg 1999;89(2):434–9.
[22] Patel S, Naeem S, Kesingland A, Froestl W, Capogna M, Urban L, Fox A. The effects of $GABA_B$ agonists and gabapentin on mechanical hyperalgesia in models of neuropathic and inflammatory pain in the rat. Pain 2001;90(3):217–26.
[23] Grabow TS, Dougherty PM. Gabapentin produces dose-dependent antinociception in the orofacial formalin test in the rat. Reg Anesth Pain Med 2002;27(3):277–83.

[24] Donovan-Rodriguez T, Dickenson AH, Urch CE. Gabapetin normalizes spinal neuronal responses that correlate with behaviour in a rat model of cancer-induced bone pain. Anesthesiology 2005;102(1):132–40.
[25] Urban MO, Ren K, Park KT, Campbell B, Anker N, Stearns B, et al. Comparison of the antinociceptive profiles of gabapentin and 3-methylgabapentin in rat models of acute and persistent pain: implications for mechanism of action. J Pharmacol Exp Ther 2005;313(3):1209–16.
[26] Kawabata A, Manabe S, Manabe Y, Takagi H. Effect of topical administration of L-arginine on formalin induced nociception in the mouse: a dual role. Br J Pharmacol 1994;112(2):547–50.
[27] Espluges JV. NO as a signalling molecule in the nervous system. Br J Pharmacol 2002;135(5):1079–95.
[28] Ortiz MI, Medina-Tato DA, Sarmiento-Heredia D, PalmaMartínez J, Granados-Soto V. Possible activation of the NO-cyclic GMP-protein kinase G-K^+ channels pathway by gabapentin on the formalin test. Pharmacol Biochem Behav 2006;83(3):420–7.
[29] Dixit RK, Bhargava VK. Neurotransmitter mechanisms in gabapentin antinociception. Pharmacology 2002;65(4):198–203.
[30] Kumar S, Bhargava HN. Time course of the changes in central nitric oxide synthase activity following chronic treatment with morphine in the mouse: reversal by naltrexone. Gen Pharmacol 1997;29(2):223–7.
[31] Sachidhanandam SB, Low KS, Moochhala SM. Naltrexone attenuates plasma nitric oxide release following acute heat stress. Eur J Pharmacol 2002;450(2):163–7.
[32] Shafaroodi H, Baradaran N, Moezi L, Dehpour S, Kabiri T, Dehpour AR. Morphine sensitization in the pentylenetetrazole-induced clonic seizure threshold in mice: role of nitric oxide and μ receptors. Epilepsy Behav 2011;20(4):602–6.