Original Paper

Pharmacology

Pharmacology 2015;95:59–64
DOI: 10.1159/000370244

Received: September 26, 2014
Accepted after revision: December 1, 2014
Published online: January 15, 2015

Antinociceptive Synergism of Gabapentin and Nortriptyline in Mice with Partial Sciatic Nerve Ligation

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Key Words
Neuropathic pain · Hot plate test · Hyperalgesia · Isobolographic analysis · Synergy

Abstract
Background and Methods: Neuropathic pain results from nerve injury, and gabapentin, an antiepileptic drug, has been approved for the treatment of several types of neuropathic pain. On the other hand, nortriptyline, an antidepressant drug, has been suggested as an alternative treatment. In partial sciatic nerve ligation (PSNL) mice, the interaction of gabapentin with nortriptyline was evaluated by the hot plate assay using isobolographic analysis. ***Results:*** Gabapentin (3–100 mg/kg, i.p.) or nortriptyline (1–30 mg/kg, i.p.) induced dose-dependent antinociception, with an ED_{50} of 11.60 ± 0.54 mg/kg for gabapentin and of 5.16 ± 0.21 mg/kg for nortriptyline. The potency of gabapentin and nortriptyline in PSNL mice at 7 and 14 days after ligation was significantly increased ($p < 0.05$). Coadministration of gabapentin with nortriptyline, at a 1:1 ratio of their ED_{50}, had a synergistic effect, with an interaction index of 0.311 and 0.348 for these mice at 7 and 14 days, respectively. ***Conclusion:*** The data showed a synergy in antinociception at a gabapentin-to-nortriptyline ratio of 1:1 in PSNL mice. This finding suggests that this combination could provide a therapeutic alternative that can be used for neuropathic pain management.

Introduction

Neuropathic pain is a type of pain that is distinguishable from other types because it results from nerve damage or dysfunction in either the peripheral or the central nervous system. Neuropathic pain can be induced by autoimmune disease, metabolic diseases, infection, postherpetic neuralgia, vascular disease, stroke, trauma, and cancer [1]. Distinct neurophysiological and neurochemical mechanisms contribute to neuropathic pain. Tissue injury can trigger the release of pronociceptive mediators that induce a sensitization of the peripheral nerve terminals. Modifications of the sensory neurons can also occur

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0031–7012/15/0952–0059$39.50/0

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in the spinal cord, increasing excitability in the neurons of the dorsal horn. Moreover, the response of the nervous system to pain is not static and is modulated by descending pathways originating in the brainstem that can reduce pain thresholds [2, 3]. In recent years, the characterization of neuropathic pain and the lack of its satisfactory treatment have led to an increased interest in this research. With a growing number of animal models, the rat neuropathic pain models have been adapted to mice as well. Other techniques are also being developed from the classic model of peripheral nerve ligation that more closely imitates clinical pain syndromes [4].

Antiepileptic drugs, such as gabapentin, have been used in patients with neuropathic pain. However, the analgesic mechanisms of gabapentin are still not well known. Recently, the $\alpha_2\delta$ subunits of voltage-gated calcium channels have been hypothesized as the targets of gabapentin [5]. Additionally, it has been proposed that the inhibition of calcium influx induced by gabapentin is coupled to a subsequent release of excitatory neurotransmitters [6]. Tricyclic antidepressants may be classified as secondary or tertiary amines. Secondary amines, such as nortriptyline and desipramine, are relatively selective in their inhibition of norepinephrine reuptake; tertiary amines, such as amitriptyline and imipramine, induce a more balanced inhibition of norepinephrine and serotonin, but they also have greater anticholinergic side effects [7]. Thus, antidepressant drugs have been widely used in the management of chronic pain. It is reported that the analgesic effect of antidepressants is independent of their antidepressive effects [8].

Partial sciatic nerve ligation (PSNL) is a model in mice which mimics important characteristics of chronic neuropathic pain as found in patients following peripheral nerve injury. The aim of this study was to assess the type of interaction between gabapentin and nortriptyline in PSNL mice by tonic algesiometric testing (hot plate assay) using isobolographic analysis.

Materials and Methods

Animals

In all experiments, male CF-1 mice 35–40 days of age, weighing 29 ± 1.0 g, housed in a 12-hour light-dark cycle at 22 ± 1°C, with free access to food and water, were used. The 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 the experimental procedures were approved by the Institutional Animal Care and Use Committee at the Universidad de Chile, Santiago, Chile. All drugs were freshly prepared by dissolving them in normal saline and were administered intraperitoneally (i.p.). The authors performed all observations during the assay in a randomized and blinded manner.

PSNL as developed by Malmberg and Basbaum [9] was used. In this study, the mice were anesthetized with 7% chloral hydrate, the left thigh was shaved, and the sciatic nerve was exposed. Then, the dorsal one third to one half of the nerve was loosely ligated with a 7-0 silk suture, and the wound was closed. The control mice underwent the exact same procedure without nerve ligation.

Algesiometric Assay

After previous acclimatization of the mice for 2 h, the hot plate assay was performed using the method described by Miranda et al. [10]. The latency period for the saline- and sham-treated animals was 22.90 ± 0.87 s (n = 12) and 22.75 ± 1.54 s (n = 12), respectively.

Isobolographic Analysis

Isobolographic analysis was used to characterize the interaction between gabapentin and nortriptyline in the hot plate assay. This analysis has been described by Tallarida [11, 12] and adapted by Miranda et al. [13].

Protocol

Dose-response curves for the antinociceptive effect of gabapentin and nortriptyline were obtained using at least 8 animals for each of the 4 doses administered i.p. A least-squares linear regression analysis allowed the calculation of the log that produced 50% antinociception (ED_{50}) for each drug, expressed as a maximum possible effect. A dose-response curve was also obtained by intraperitoneal coadministration of fractions of the drugs' respective ED_{50} values: 1/2, 1/4, 1/8, and 1/16. An isobolographic analysis was used to determine the drugs' interactions. The method has been described in detail by Miranda et al. [13]. The interaction index was also calculated.

Drugs

The drugs were freshly dissolved in a saline solution. Gabapentin and nortriptyline hydrochloride were purchased from Sigma Chemical Co. (St. Louis, Mo., USA).

Statistical Analysis

Results are presented as mean values ± SEM or as ED_{50} values with a 95% confidence level (95% CL). Statistical analyses of the results were conducted by t tests for independent means, and calculations were performed with the program Pharm Tools Pro (version 1.27; The McCary Group Inc., Elkins Park, Pa., USA) based on Tallarida [12]. $p < 0.05$ was considered significant.

Results

The different doses of gabapentin and nortriptyline used did not produce visuomotor dysfunction.

Partial Sciatic Nerve Ligation

This model, developed first in rats, mimics important characteristics of chronic neuropathic pain in patients

Table 1. ED_{50} values ± SEM (in mg/kg) for the antinociceptive effect of nortriptyline and gabapentin administered i.p. to control and PSNL mice in the hot plate assay

Drug	Control mice	PSNL mice	
		7 days	14 days
Nortriptyline	5.16±0.21	4.34±0.24*	4.06±0.32*
Gabapentin	11.60±0.54	9.02±0.40	8.98±0.23*

* $p < 0.05$ compared with the control mice.

following peripheral nerve injury [14]. The control value for saline licking time in the PSNL mice in the hot plate assay was 22.90 ± 0.87 s (n = 12). PSNL significantly decreased this value on day 7 (to 18.61 ± 1.13 s; n = 12) and on day 14 (to 17.00 ± 1.08 s; n = 12). The sham-operated mice had a value of 22.75 ± 1.54 s (n = 12) in the hot plate assay. All these results can be seen in figure 1a.

Antinociception Induced by Gabapentin and Nortriptyline

In the control animals, intraperitoneal administration of gabapentin (3–100 mg/kg) or nortriptyline (1–30 mg/kg) induced a dose-dependent antinociceptive activity with different potencies in the hot plate assay, with an ED_{50} for gabapentin of 11.60 ± 0.54 mg/kg and for nortriptyline of 5.16 ± 0.21 mg/kg (table 1); thus, nortriptyline was 2.2 times more potent than gabapentin. The corresponding dose-response curves for the drugs are shown in figure 1b and c.

Dose-response curves were also obtained for gabapentin and nortriptyline individually at 7 and 14 days after PSNL. The ED_{50} values obtained for gabapentin were 9.02 ± 0.40 and 8.18 ± 0.23 mg/kg on days 7 and 14, respectively, and the ED_{50} values for nortriptyline were 4.34 ± 0.24 and 4.06 ± 0.31 mg/kg on days 7 and 14, respectively (table 1). These results demonstrate that the potency of gabapentin and nortriptyline at 7 and 14 days after PSNL was significantly increased ($p < 0.05$) when compared to the sham-treated control mice.

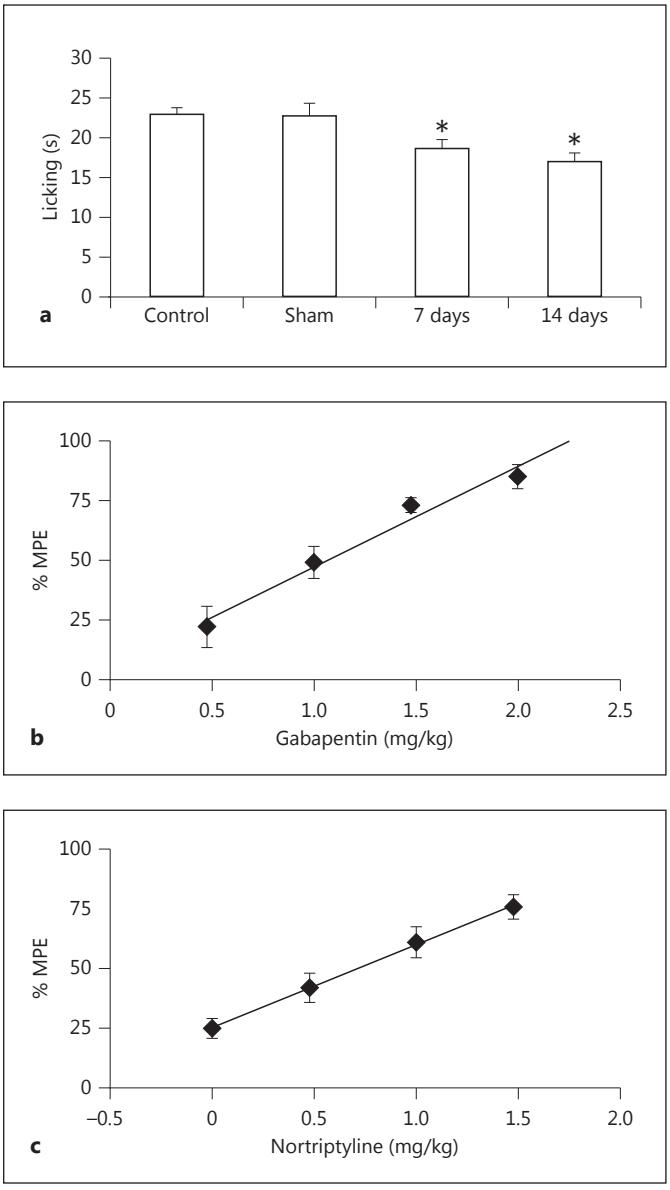

Fig. 1. a Loose ligation of the sciatic nerve in mice. Each bar is the mean ± SEM of 12 animals. * $p < 0.05$ with respect to day 0. **b**, **c** Dose-response curves for the antinociceptive activity induced by gabapentin and nortriptyline (i.p.) in the hot plate assay. Each point is the mean ± SEM of 8 animals. MPE (%) = Antinociception as a percentage of the maximum possible effect.

Interaction between Gabapentin and Nortriptyline

The combination of gabapentin with nortriptyline, at 1:1 ratios of their ED_{50}, also generated isobolograms at 7 and 14 days. The analysis of the interaction was synergistic with the following ED_{50}: for the theoretical control 8.30 (9.70–7.23), and for the sham-treated group 3.80 (6.15–2.10). For PSNL mice, the experimental values were 2.27 (2.63–1.95) at 7 days and 2.78 (2.91–1.65) at 14 days. The isobolograms are displayed in figure 2a–c. The interaction index for the control animals was 0.457, and for the PSNL animals, it was 0.311 at 7 days and 0.348 at 14 days (table 2).

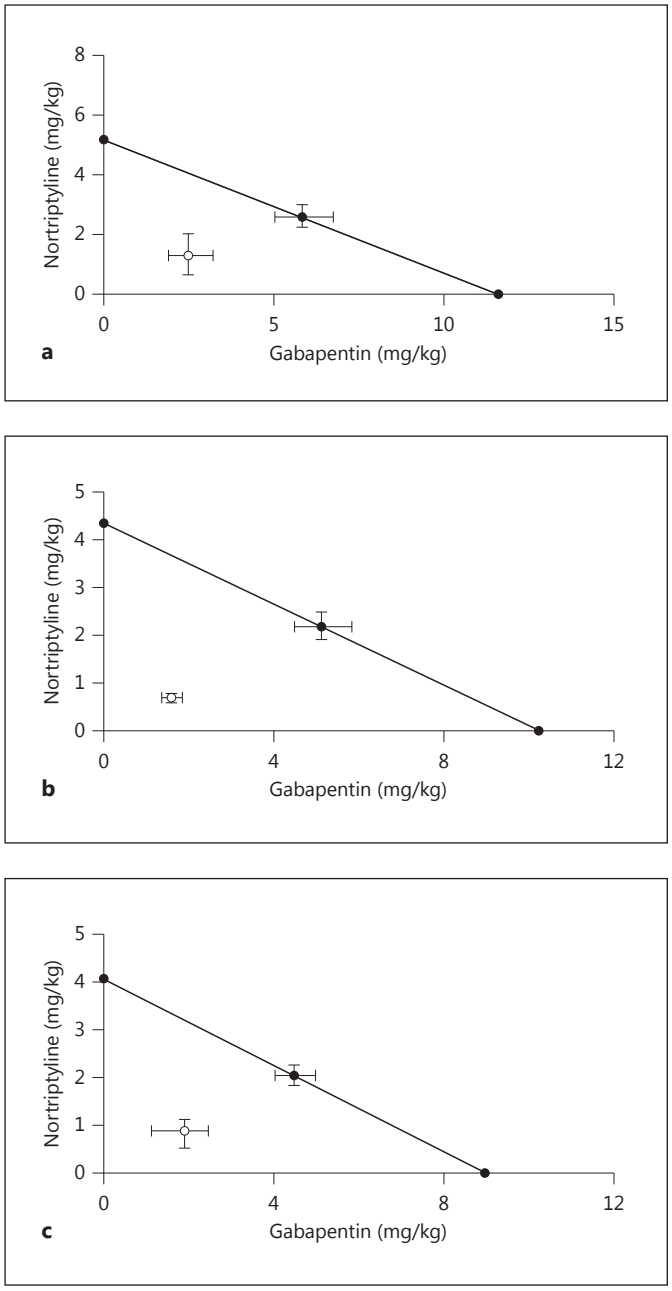

Fig. 2. Isobolograms for the coadministration of gabapentin and nortriptyline (i.p.) in the hot plate assay at days 0 (**a**), 7 (**b**), and 14 (**c**). ● = Theoretical ED_{50} value with 95% CL; ○ = experimental ED_{50} value with 95% CL.

Table 2. Isobolographic parameters for the antinociceptive activity of the combination of nortriptyline and gabapentin administered i.p. to PSNL mice in the hot plate test

	Theoretical ED_{50} (95% CL), mg/kg	Experimental ED_{50} (95% CL), mg/kg	Interaction index
Control mice	8.30 (9.70–7.23)	3.80 (6.15–2.10)	0.457
PSNL mice			
7 days	7.29 (8.31–6.40)	2.27 (2.63–1.95)	0.311
14 days	6.52 (7.25–5.87)	2.78 (2.91–1.65)	0.348

Discussion

Neuropathic pain is produced by injury to the peripheral or the central nervous system. The symptoms of neuropathic pain include persistent pain, hyperalgesia, and allodynia. In the present study, we used a model of PSNL in the mouse to evaluate antinociception by measuring the reaction time of the spinal reflex under exposure to the hot plate assay. Such a reflex is mediated through spinal mechanisms, which are modulated by supraspinal regions as an integrated response [14]. In this study, the intraperitoneal administration of gabapentin or nortriptyline or their combination in a 1:1 proportion of their ED_{50} values induced a dose-dependent antinociceptive activity in the hot plate assay in mice, and nortriptyline was 2.2 times more potent than gabapentin. This result is concordant with the antinociceptive activity of gabapentin demonstrated in other algesiometric tests such as tail flick, formalin (phase 2), carrageenan, neuropathic pain, and cancer-induced bone pain assays [15–17]. In addition, an antinociceptive effect induced by nortriptyline has been reported in chemical (formalin and acetic acid tests) or thermal assays (hot plate and tail flick tests). The results show a stronger antinociceptive effect in chemical tests than in thermal tests [18].

In addition, the antinociceptive efficacy of each drug in the context of emerging neuropathic analgesia demonstrated that the ED_{50} for each of the drugs changed over time as the injury developed. This finding is important, since it implies a significant degree of plasticity in the pharmacological antinociceptive activity of gabapentin and nortriptyline.

The antinociception induced by gabapentin could be explained by the fact that the analgesic activity of this drug is based on several mechanisms of action, including the activation of specific receptors such as GABA, NMDA, and AMPA as well as muscarinic and adenosine receptors. Also, the cellular action of gabapentin might involve binding to the L-amino acid transporter or voltage-dependent calcium channel by activation of the $\alpha_2\delta$ subunit of N-type Ca^{2+} channels [17, 19, 20].

The analgesic effect produced by nortriptyline in this study can be explained by several pharmacological mechanisms by which some antidepressants, including nortriptyline, may produce antinociception. Thus, it has been suggested that nortriptyline blocking of sodium ion channels may contribute to its antinociceptive effect [21]. However, it has also been considered that nortriptyline modulates pain partly by blocking the reuptake of monoaminergic neurotransmitters in noradrenergic and serotonergic systems and partly via the endogenous opioid system [22–24]. Moreover, the antihistaminergic action of tricyclic antidepressants may have a general analgesic effect [25], and they may act as NMDA receptor antagonists [26].

Drugs for neuropathic pain have an incomplete efficacy and dose-limiting side effects when given as a monotherapy. In this study, we assessed the efficacy of the coadministration of gabapentin with nortriptyline compared with each drug given alone. Gabapentin and nortriptyline combined seems to be more efficacious than either drug given alone for neuropathic pain induced by PSNL, since their coadministration induced dose-related antinociception in the hot plate assay and demonstrated a synergy at the 50% level of effect at 7 and 14 days by isobolographic analysis. This synergy is concordant with previous reports using the same combination [27, 28].

The synergism obtained by coadministration of gabapentin with nortriptyline is consistent with the idea that the concurrent administration requires that the drugs combined have different mechanisms, so that decreasing the dose to avoid toxicity still entails increasing or maintaining the same efficacy [29] and all levels of cell function are involved [30]. According to this, it is likely that the synergistic effect is based on different antinociceptive pathways that are activated by combining these drugs.

In conclusion, the data show a synergy in the antinociceptive activity of the 1:1 combination of gabapentin with nortriptyline in a PSNL mouse model of neuropathic pain. This finding suggests that the combination of gabapentin and nortriptyline provides a therapeutic alternative that can be used for neuropathic pain management.

Acknowledgments

This study was partially supported by project DI-76-12/CB from the Universidad Andrés Bello, Santiago, Chile.

Disclosure Statement

The authors declare that they have no competing interests.

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