Neuroscience Letters 556 (2013) 93–98

Contents lists available at ScienceDirect

Neuroscience Letters

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

Oral gabapentin treatment accentuates nerve and peripheral inflammatory responses following experimental nerve constriction in Wistar rats

Carlos C. Câmara[a,b], Heitor F. Ramos[a], Alan P. da Silva[a], Celina V. Araújo[c], Antoniela S. Gomes[e], Mariana L. Vale[e], André Luiz R. Barbosa[e], Ronaldo A. Ribeiro[e], Gerly A.C. Brito[d,e], Carlos Maurício C. Costa[a], Reinaldo B. Oriá[c,d,*]

[a] *Laboratory of Experimental Neurophysiology, Department of Physiology and Pharmacology, School of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil*
[b] *Laboratory of Neurophysiology, School of Veterinary, Federal and Rural University of the SemiArid, Mossoro, RN, Brazil*
[c] *Laboratory of Tissue Healing, Ontogeny and Nutrition, Institute of Biomedicine of the Brazilian SemiArid, Federal University of Ceara, Fortaleza, CE, Brazil*
[d] *Department of Morphology, School of Medicine, Federal University of Ceara, Fortaleza, CE, Brazil*
[e] *Laboratory of Inflammation and Cancer, Department of Physiology and Pharmacology, School of Medicine, Federal University of Ceara, Fortaleza, CE, Brazil*

HIGHLIGHTS

- A nerve pro-inflammatory effect of gabapentin treatment was identified.
- Gabapentin increased carrageenan-induced paw edema and peritoneal cell migration.
- Concern of gabapentin widespread use in systemic inflammatory diseases was raised.

ARTICLE INFO

Article history:
Received 8 August 2013
Received in revised form
30 September 2013
Accepted 3 October 2013

Keywords:
Gabapentin
Sciatic nerve
Neuropathic pain
Cytokine
Inflammation

ABSTRACT

Gabapentin (GBP) is an anti-convulsive drug often used as analgesic to control neuropathic pain. This study aimed at evaluating whether oral GBP treatment could improve nerve inflammation response after sciatic nerve constriction in association with selected pain and motor spontaneous behavior assessments in Wistar rats. We evaluated nerve myeloperoxidase (MPO) and inflammatory cytokines on the 5th day post-injury, time in which nerve inflammation is ongoing. In addition, the role of GBP on carrageenan-induced paw edema and peritoneal cell migration was analyzed. GBP was given by gavage at doses of 30, 60 and 120 mg/kg, 60 min prior to chronic constriction of the sciatic nerve (CCSN) and during 5 days post-injury, 12/12 h. CCSN animals treated with saline were used as controls and for behavioral and inflammation assessments untreated sham-operated rats were also used. On the 5th day, GBP (60 and 120 mg/kg) alleviated heat-induced hyperalgesia and significantly increased delta walking scores in CCSN animals, the latter suggesting excitatory effects rather than sedation. GBP (60 mg/kg) significantly increased nerve MPO, TNF-α, and IL-1β levels, comparing with the saline group. GBP (120 mg/kg) reduced the anti-inflammatory cytokine IL-10 nerve levels compared with the CCSN saline group. Furthermore, GBP (60 and 120 mg/kg) increased carrageenan-induced paw edema and peritoneal macrophage migration compared with the CCSN saline group. Altogether our findings suggest that GBP accentuates nerve and peripheral inflammatory response, however confirmed its analgesic effect likely due to an independent CNS-mediated mechanism, and raise some concerns about potential GBP inflammatory side effects in widespread clinical use.

1. Introduction

Neuropathic pain is a direct consequence of injury or disease causing dysfunction of the peripheral and central nervous system at different levels, mostly affecting the middle-aged and elderly with an escalating global burden.

Treatment for neuropathic pain is still not satisfactory for most of the patients especially those with a more severe condition,

* Corresponding author at: Institute of Biomedicine of the Brazilian Semiarid, School of Medicine, Federal University of Ceara, Rua Coronel Nunes de Melo, 1315, CEP: 60430-270 Fortaleza, CE, Brazil. Tel.: +55 08533668239.
E-mail address: rbo5u@virginia.edu (R.B. Oriá).

0304-3940/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2013.10.010

highlighting the need for novel and more efficacious therapy for pain relief [23]. Mechanisms underlying pain relief with the use of anticonvulsants and antidepressants are a promising target for therapy improvements [11]. Previously, we have studied the beneficial effects of vigabatrin (gamma-vinyl-GABA) and other analgesic anticonvulsants (carbamazepine, phenytoin and valproic acid) in a model of neuropathic pain induced by sciatic nerve constriction in rats [1].

Gabapentin (GBP) (1-(aminomethyl)cyclohexaneacetic acid), one structural derivative of the gamma-amino butyric acid (GABA), in addition to being an anti-convulsive drug, is now further considered as an effective therapeutical drug for some forms of neuropathic and post-surgical pain [26]. Gabapentin effects of reducing allodynia may be associated with specific binding to calcium-voltage dependent $\alpha 2\delta$ subunits, reducing calcium cell influx with changes in neurotransmitter release [13] or activation of protein kinase-G-K+ channel pathways [24]. Several studies have shown gabapentin-related analgesic effects in experimentally induced neuropathic pain [7,15]. However, studies associating GBP with the inflammatory response following sciatic nerve constriction are still scarce. This is an important issue since nerve inflammation may influence drug efficacy during neuropathic pain treatment [30].

Hurley et al. after giving a single dose of GBP (300 mg/kg) via gavage, 2.5 h following carrageenan-induced intra-plantar edema in Wistar rats, did not find significant acute edema change [16].

In this current study we explored whether prolonged GBP treatment modulated inflammatory responses following sciatic nerve constriction in rats by assessing the involvement of nerve inflammatory cytokines, associating these outcomes with selected pain-related and motor behaviors. In addition, the role of GBP on carrageenan-induced paw edema and peritoneal cell migration was assessed to evaluate a peripheral inflammatory response.

2. Materials and methods

Protocols from this study were approved by the Animal Care and Use Committee of the Federal University of Ceara and were in accordance with the Brazilian College for Animal Experimentation (COBEA) and the International Association for the Study of Pain (IASP) guidelines.

2.1. Animals

160 male Wistar rats weighing between 250 and 300 g from the Department of Physiology and Pharmacology vivarium at the Federal University of Ceará were used in this study. Rats were housed in a temperature-controlled room ($26 \pm 2\,^{\circ}C$) with free access to water and chow diet in a 12 h/12 h light/dark cycles. All surgical procedures were performed in the laboratory of Experimental Neurology in the Physiology and Pharmacology department at the Federal University of Ceara.

2.2. Sciatic nerve chronic constriction

We used the chronic constriction of the sciatic nerve (CCSN) model to induce the experimental neuropathy, described by Bennett and Xie, 1988 [2] and modified by Sommer and Myers [32]. Animals were anesthetized with intraperitoneal injection of tribromoethylene (25 mg/kg), following trichotomy and anti-sepsis of the surgery field. A 15-mm longitudinal incision of the right thigh, at the level of the femoral trocater of the posterior limb, was used to access and expose the sciatic nerve after gluteus and femoral biceps dissection. We used three 4-0 cat-gut loose ligatures on the right paw sciatic nerve, distanced approximately 1 mm between the ligatures and proximal to the sciatic trifurcation inducing a slight nerve ischemia. In the left thigh, the sciatic nerve was exposed, but remained untouched and surgery closed afterwards. Skin and muscular layers were sutured with a 5-0 mononylon thread.

2.3. Drugs and treatment regimens

Gabapentin (GBP) (1-(aminomethyl)cyclohexaneacetic acid, C9H17NO2) (Neurontin®, Pfizer) capsules were dissolved in 0.9% saline solution and then given orally by gavage every 12 h during a 5 day-treatment course. GBP doses of 30, 60, and 120 mg/kg were used based on a previous study showing its safety use and analgesic effect in a model of neuropathic pain [18]. As the maximum GBP effect occurs 60 min after oral administration [17], the first dose was given 60 min prior to the nerve surgery and 60 min before each behavior test. Following the last behavior test on the 5th day post-injury the animals were euthanized and the sciatic nerve was removed to conduct histological and molecular biology studies.

2.4. Spontaneous motor behaviors

To find out whether GBP could ameliorate motor behaviors after sciatic nerve injury and rule-out a possible sedation effect, cohort animals were kept in a wooden cage (100 cm × 50 cm × 50 cm) for a 5-min acclimation time. The observing cage (with Plexiglas) with the floor covered with wood shavings was placed in a slightly illuminated and silent room for behavioral testing. Positioned in front of the cage, the observer could identify each behavioral component and record it using a computer software (Comporta®) designed by Prof. Marcus Vale (Federal University of Ceará, Fortaleza, CE, Brazil). Each animal was observed for resting-sleeping and walking behavior and observed during a 30 min-time. The first observation was 60 m before the surgery (baseline) and then on the 5th day post-surgery and 60 m after the last treatment. A delta mean behavior value was derived from baseline (prior to surgery) and 5th day post-surgery time points. Cumulative resting-sleeping behavior was measured (in seconds) if the animals were found lying immobile on the bedding. Cumulative walking behavior was also measured (in seconds). Experimenters were unaware of the identity of the experimental groups.

2.5. Thermal test

In order to confirm the GBP analgesic effect on the CCSN, we evaluated pain-induced responses to thermal stimuli. Tests using noxious (46 °C) stimulation were performed involving immersion of the rat's hind paw in a bath until the withdrawal or struggle was observed. After 15 s of exposure (cut-off), the thermal stimulus was removed and the withdrawal latency was determined. The tests were performed on day zero (preoperative day) and on the 5th postoperative day 60 min after GBP-treatment. A delta score was calculated for further analyses.

2.6. Nerve inflammation markers

In order to evaluate GBP effect on nerve inflammation, we obtained snap-frozen sciatic nerves following CCSN for nerve cytokine measurements by immunoenzymatic assays and by immunohistochemistry.

2.6.1. ELISA assays for nerve cytokines

CCSN animals treated with GBP (60 and 120 mg) or saline were sacrificed on the 5th day post-nerve injury and a 20-mm-long sciatic nerve segment, proximal to the ligatures, was harvested and stored in a −80 °C freezer until the assay was performed. The tissue collected was homogenized and processed. The detection of TNF-α, IL-1β, and IL-10 concentrations was determined by ELISA, as described previously [20]. Values are expressed as picograms/milliliter (pg/ml).