Pharmacological Reports 70 (2018) 385–389

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original article

Antinociceptive and pronociceptive effect of levetiracetam in tonic pain model

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ARTICLE INFO

Article history:
Received 1 February 2017
Received in revised form 28 August 2017
Accepted 25 September 2017
Available online 27 September 2017

Keywords:
Levetiracetam
Antinociceptive
Pronociceptive
Tonic pain
Rat

ABSTRACT

Background: Levetiracetam (LEV) is a novel anticonvulsant with proven antinociceptive properties. However, the antinociceptive and pronociceptive effect of this drug has not yet been fully elucidated in a tonic pain model.
Methods: Thirty-six male rats (Wistar) were randomized into six groups and underwent the formalin test as follows: rats in the control group were administered 50 μL of 1% formalin in the paw; sham-group rats were administered 50 μL of saline in the paw to mimick the application of formalin; the four experimental groups were administered LEV intragastrically (*ig*) (50, 100, 200 and 300 mg/kg), and 40 min later 50 μL of 1% formalin was injected in the paw.
Results: LEV exhibited antinociceptive effect in the 300 mg/kg LEV group ($p < 0.05$) and a pronociceptive effect in the 100 mg/kg LEV group ($p < 0.05$) and in the 50 mg/kg LEV group ($p < 0.001$).
Conclusions: The antinociceptive and pronociceptive effect of LEV in a tonic pain model is dose-dependent.

Introduction

The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" [1]. However, because of the vast complexity of this phenomenon, this definition does not cover its whole biological and chemical components. Even though pain provides us with an alarm system that allows us to recognize potential threats or dangers, when it becomes pathological (chronic pain, cancer-related pain, phantom limb pain), it becomes necessary to control the pain. Because of the complexity of pain, pain management has proven to be challenging.

Levetiracetam (LEV) is a novel antiepileptic drug (AED) that has favorable pharmacological characteristics as follows: low potential for interaction; short elimination half-life; no active metabolites, and no major negative effects on cognition [2]. LEV is mainly employed in the treatment of epilepsy, but several studies have proved that it also possesses neuroprotective [3] and antinociceptive effect, the latter with some controversy. Researchers have tested the antinociceptive effect of LEV in an animal model for inflammatory pain with the proinflammatory compound carrageenan and have also used the model of painful diabetic neuropathy, employing the "hot-plate test" and the radiant-heat "tail-flick test" [4–8]. However, to date, the antinociceptive and pronociceptive effect have not been demonstrated in a tonic pain model.

The formalin test is a model of tonic pain that is valid and widely employed for the investigation of drugs with antinociceptive and antihyperalgesic activity [9], in addition to it being a model that has a great resemblance to clinical conditions [10]. The formalin test is different from the majority of models of pain in that it is possible to measure the way an animal responds to moderate, persistent pain caused by the injured tissue. The formalin test provides a more valid model for clinical pain than tests with phasic mechanical or thermal stimuli; further a formalin injection induces a state of tonic pain that comprises a better approximation to clinical conditions [11].

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https://doi.org/10.1016/j.pharep.2017.09.007
1734-1140/

To date, the mechanism of action of LEV has not been fully elucidated. There is evidence that it binds to synaptic vesicle protein SV2A, where it is hypothesized to interfere with neurotransmitter release [12]. LEV has a broad spectrum of molecular targets; it inhibits neuronal hypersynchronization and inhibits N-type Ca^{2+} channels [13,14]. There is a lack of information on the dosage of LEV in a tonic pain model. Therefore, the main purpose of this research was to assess the antinociceptive and pronociceptive effect of LEV in a tonic pain model by using the formalin test.

Materials and methods

Thirty-six male Wistar rats (*Rattus norvegicus*) (250 g) were used; the animals were maintained under controlled laboratory conditions under a 12-h light/12-h dark cycle at 22 °C and were given food and water ad libitum. The animals were treated according to the Guide for the Care and Use of Experimental Animals and under Official Mexican Standard NOM-062-ZOO-1999.10 [15]. Similarly, all experiments complied with the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals [16].

To evaluate the antihyperalgesic effect of LEV in a tonic model of pain, the rats were divided into the following six groups: Control group (n = 6) was administered 50 μL of a 1% formalin solution subcutaneously (*sc*) in the dorsal hind-paw; Sham group (n = 6) was administered 50 μL of a saline solution through an *sc* injection to mimic the application of formalin; the experimental groups were treated with a single dose of LEV (Keppra™, ucb L059, (S)-α-ethyl-2-oxo-pyrrolidine acetamide, UCB Pharma, Torino, Italy) each one with one dose (50, 100, 200, and 300 mg/kg). LEV was administered intragastrically (*ig*) through a special cannula (Gavage needle 22 g with a ball tip needle to prevent damage of the esophagus and prevent it from passing through the glottal opening into the trachea) for the rat species 40 min prior to the *sc* injection of 50 μL of a 1% formalin solution.

The model for evaluating nociception employed in this study was the formalin test [17]. Rats were adapted to this test following the same procedure as the test itself 5 days before the injection of formalin. Rats were first adapted for 30 min in a cylindrical plastic chamber to allow them to become accustomed to their surroundings, and a mirror with a 45-degree angle was placed behind the chamber to allow an unimpeded view of the animals' paws. Formalin 1% (50 μL) was injected subcutaneously (*sc*) into the dorsal surface of the dorsal hind-paw of the rat with a 30-gauge needle. The animals were then placed into the chambers, and nociceptive behavior was observed immediately after the formalin injection. Nociceptive behavior was quantified as the number of flinches of the injected paws during 1-min periods every 5 min, up to 60 min after injection. Formalin induces nociception in a biphasic manner, as previously reported [18]. These phases were defined as follows: phase 1, acute phase from 0 to 10 min, and phase 2, tonic phase from 15 to 60 min.

Normality tests were performed for all variables examined. The values obtained were expressed as mean values ± Standard Errors of the Mean (SEM). For the time-course data, student's *t*-test was used, and for the results of the formalin test, one-way Analysis of Variance (ANOVA) was conducted and the *post-hoc* Tukey test was utilized; p values of <0.05 were considered as statistically significant differences between the samples. Statistical analysis was performed using SPSS (ver. IBM SPSS Statistics 19.0.1, USA) statistical software.

Results

Formalin subcutaneously injected into the hind paw induces a biphasic nociceptive response. Following the formalin injection, the Control and Experimental groups demonstrated typical biphasic nociceptive responses. The Sham group showed flinches only in min 0 and 5, which shows that the nociceptive responses were caused by the formalin instead of the puncture of the needle. Figs. 1 and 2 depict the typical curves of the formalin test; the X-axis illustrates the minutes registered and the Y-axis shows the number of flinches recorded. Fig. 1 shows the higher doses employed in this study (200 and 300 mg/kg); when comparing each minute by student's *t*-test, significant differences were found between the Control group and LEV 300 mg/kg group at minutes 20 ($p < 0.01$), 25 ($p < 0.05$), and 35 ($p < 0.05$); significant differences were also found between LEV 200 mg/kg and LEV 300 mg/kg at minutes 15 ($p < 0.05$), 20 ($p < 0.05$) and 25 ($p < 0.05$). The curves presented by the lower doses employed in this study (50 and 100 mg/kg) are depicted in Fig. 2; significant differences were found between the Control group and LEV 50 mg/kg group in minutes 30 ($p < 0.05$), 35 ($p < 0.01$), 40 ($p < 0.01$), 50 ($p < 0.05$) and 60 ($p < 0.01$); significant differences were also found between the Control group and LEV 100 mg/kg only in minute 30 ($p < 0.05$).

Fig. 3 shows the cumulative flinches recorded for each group separated in Phase 1 (0–10 min) and Phase 2 (15–60 min) in the

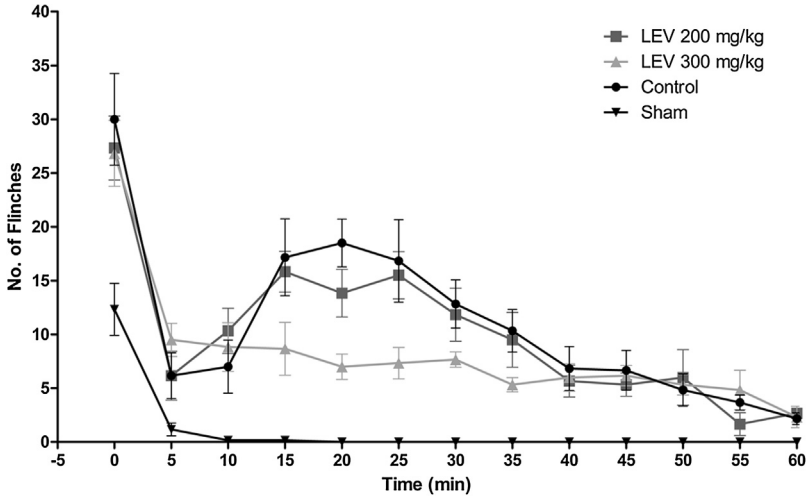

Fig. 1. Effect of high doses (300 and 200 mg/kg) of Levetiracetam (*ig*) on paw flinches induced by injection of 1% formalin. The curves were constructed by plotting the mean of the nociceptive response (flinches) every 5 min for 60 min (n = 6 per group). Data analyzed by Student's *t*-tests and are expressed as Mean ± Standard Errors of the Mean (SEM).