https://doi.org/10.31925/farmacia.2022.4.20 **ORIGINAL ARTICLE**

SYSTEMIC AMPICILLIN DIMINISHES FORMALIN-INDUCED NOCICEPTION IN RATS

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Abstract

The purpose of the present study was to evaluate the antinociceptive effect of the ampicillin using the behavioural nociceptive response induced by formalin in rats. We analysed single and repeated pre-treatment with increasing doses of ampicillin. Acute intraperitoneal administration of ampicillin (100 - 800 mg/kg bw, ip, reduced the second, but not the first phase of formalin-induced flinching behaviour, which was interpreted as antinociception. Moreover, repeated pre-treatment with ampicillin (100 - 800 mg/kg bw, ip) for 1, 3 and 7 days reduced the second phase of the formalin test. Our data indicate that ampicillin induces anti-nociception in the rat formalin test after acute and repeated treatment. Since previous studies reported that ampicillin upregulates glutamate transporter 1 (GLT-1), our data suggest that ampicillin induces antinociception through GLT-1 upregulation.

Rezumat

Scopul prezentului studiu a fost de a evalua efectul antinociceptiv al ampicilinei folosind ca model experimental testul formalinei la șobolan. Am analizat tratamentul în doză unică și repetată cu doze progresive de ampicilină. Administrarea acută intraperitoneală de ampicilină (100 - 800 mg/kgc, ip), a redus a doua, dar nu prima fază a comportamentului reactiv indus de formol, care a fost interpretat ca antinocicepție. Mai mult, pre-tratamentul repetat cu ampicilină (100 - 800 mg/kgc, ip) timp de 1, 3 și 7 zile a redus faza a doua a testului cu formalină. Rezultatele indică faptul că ampicilina induce antinocicepția în testul cu formalină la șobolan după tratament acut și repetat. Întrucât studiile anterioare raportează că ampicilina reglează în sens pozitiv transportorul de glutamat 1 (GLT-1), datele noastre sugerează că ampicilina induce antinocicepția prin reglarea GLT-1.

Keywords: ampicillin, β -lactam antibiotics, glutamate, inflammatory pain

Introduction

Glutamate is a neurotransmitter that contributes to transmission of noxious signals into the dorsal horn spinal cord and other structures. There is evidence that diverse chemical mediators, including glutamate, are released in the central terminal of the nociceptors [1, 2]. In pathological conditions, glutamate is found in vesicles at the central terminal and released into the synaptic gap where it can act on both presynaptic and postsynaptic receptors [2]. Once released, glutamate binds AMPA and NMDA receptors promoting hyper-excitability, synaptic plasticity and nociception [3, 4]. Released glutamate is removed from the synaptic cleft, particularly by glutamate transporters (GLT) [5]. In this sense, mounting evidence indicates that chronic pain leads to a downregulation of glutamate transporters (GLT), such as GLT-1, and increased glutamate concentration [6]. Therefore, the administration of drugs that increase the expression of GLT-1 is an alternative to produce antinociception [7].

The β-lactam drugs are broad spectrum antibiotics that act on both Gram-positive and Gram-negative pathogens. Several studies have reported that β-lactam drugs are able to induce antinociceptive activity [8-11] besides their antimicrobial activity. Ampicillin, a semisynthetic penicillin [12] available since 1960 [18], has shown antinociceptive effect in the hot-plate test [10].
Interestingly, ampicillin has been reported to upregulate of astroglial [13, 14] and brain [15] GLT-1 expression. In the present study, we sought to extend these investigations on the antinociceptive effects of β-lactam drugs. Therefore, we tested the possibility that ampicillin after intraperitoneal administration may produce antinociceptive effect evaluated in a model of persistent pain state.

Materials and Methods

Animals

All experiments were conducted on adult female Wistar rats (body weigh 180 - 200 g). Rats were group-housed in polypropylene cages and maintained under

standard lighting (12-h light/dark, lights were turned on at 6 a.m. Experiments were carried out during light phase. Both food and water were available *ad libitum*. All procedures were carried out according to guidelines for research on experimental pain in animals [16], Mexican law for the use of laboratory animals (NOM-062-ZOO-1999) [17] and approved by the Institutional Committee for the Care and Use of Animals (02-2019).

Formalin-induced nociception test

Nociception was assessed using the formalin test [18, 19]. After about 30 minutes of acclimation, the rats received an injection of formalin (1%) into the dorsal surface of the right hind paw. Flinching of the injected paw was considered as the nociceptive behaviour. Flinches were recorded during in 1-minute intervals every five minutes for 60 minutes after delivery of formalin. [19]. The acute (phase 1; 0 - 10 min) and tonic response (phase 2; 15 - 60 min) were identified. Upon completion the test, rats were removed from the chamber and euthanized.

Drugs

Ampicillin (AMSA Laboratory, Mexico) was used as a commercial presentation and dissolved in saline solution (0.9% NaCl). The drug solutions were prepared the same day as the experiment.

Experimental design

Two treatment schemes, acute and repeated, were carried out, for which it was necessary to use 10 groups consisting of six rats each group, a control group was used for each scheme (acute and repeated) with the administration of the vehicle with the objective of standardizing the formalin test of each one. Increasing doses of ampicillin (100, 200, 400 and 800 mg/kg, i.p.) were administered to obtain dose-response curves. In the acute protocol, rats received ampicillin or vehicle 20 min before formalin injection (1%). In the repeated treatment protocol series, each rat received vehicle or ampicillin each 24 h for 1, 3, or 7 days, and the formalin test was performed 1 day later, on days 2, 4 and 8, respectively. Each animal participated in only one determination of nociception. Rats were euthanized after the test.

Data analysis

The area under the curve (AUC) was calculated from the flinches time course. AUC is an expression of the duration and intensity of antinociceptive effect during overall time evaluated. AUC was calculated by the trapezoidal rule. Dose-response curves are shown as AUC for phase 1 and 2. The AUC was transformed to percentage of antinociception by the next equation:

$$\% \text{ antinociception} = \frac{(\text{AUC Control} - \text{AUC Drug})}{(\text{AUC Control})} \text{ x } 100,$$

where, AUC Control: represents the group that were administered only with vehicle and later received 1% formalin.

Data were recorded in and analysed using GraphPad Prism 9.1.0 Software (San Diego, California, USA). All data were analysed using a one-way analysis of variance (ANOVA) with a Bonferroni *post-hoc* test. Values with a $p < 0.05$ were considered to be statistically significant.

Results and Discussion

Antinociceptive activity of acute pre-treatment with ampicillin

The formalin produced the typical flinching behaviour of the injected paw. Immediately after the administration of formalin the phase 1 started and in about 10 minutes was gradually diminished and then started the phase 2, lasted about 50 min (Figure 1A). Acute intraperitoneal administration of ampicillin, but not vehicle, reduced flinching behaviour, which was interpreted as antinociception (Figure 1B). Rats received vehicle or ampicillin (100, 200, 400, or 800 mg/kg) ip 20 min prior to an injection of 1% formalin (50 µL), moreover, ampicillin reduced the flinching behaviour only during phase 2 (Figure 1C). The maximum antinociceptive effect of acute treatment with ampicillin was about 30% with the dose of 800 mg/kg, ip (Figure 1D).

One-day pre-treatment with ampicillin reduces nociception induced by formalin

Intraperitoneal pre-treatment (1 day) with ampicillin (800 mg/kg, ip) induced an antinociceptive effect on phase 2, but not in phase 1 (Figure 2A and Figure 2B). In addition, 1 day-pre-treatment with ampicillin dose-dependently (100 - 800 mg/kg, ip) reduced formalin-induced nociception during phase 2 (Figure 2C and Figure 2D).

Three and seven days-pre-treatment with ampicillin reduces formalin-induced nociception

Pre-treatment (3 or 7 days) with systemic injection of ampicillin (800 mg/kg, ip) produced a dose-dependent (100 - 800 mg/kg, ip) antinociceptive effects only in phase 2 (Figure 3 and Figure 4).

It is important to mention the principal limitation on this study, although we did not measure the expression of GLT-1, it is important for us to describe the frame of the field and this includes the description of the participation of glutamate, glutamate receptors as well as the system that carry out the reuptake of glutamate. We utilized a rat model of persistent pain in order to evaluate antinociceptive activity of ampicillin after systemic administration. The data presented herein demonstrates that acute systemic ampicillin reduces phase 2 of the formalin-induced nociception. To our knowledge, this is the first study about the antinociceptive effect of ampicillin in a model of inflammatory pain. Previously, ampicillin was able to reduce thermal hyperalgesia in forelimb-deafferented rats [10]. Thus, data suggest that ampicillin is effective to reduce inflammatory and neuropathic pain. Interestingly, pre-treatment with ampicillin for 1, 3, or 7 days also produced an antinociceptive effect in the formalin test in a dose-dependent manner. These data are consistent

with previous reports showing the antinociceptive effect of other β-lactam drugs in the formalin test [4, 10, 20], in the model of carrageenan-induced paw inflammatory hyperalgesia [21] and neuropathic pain models [22, 23].

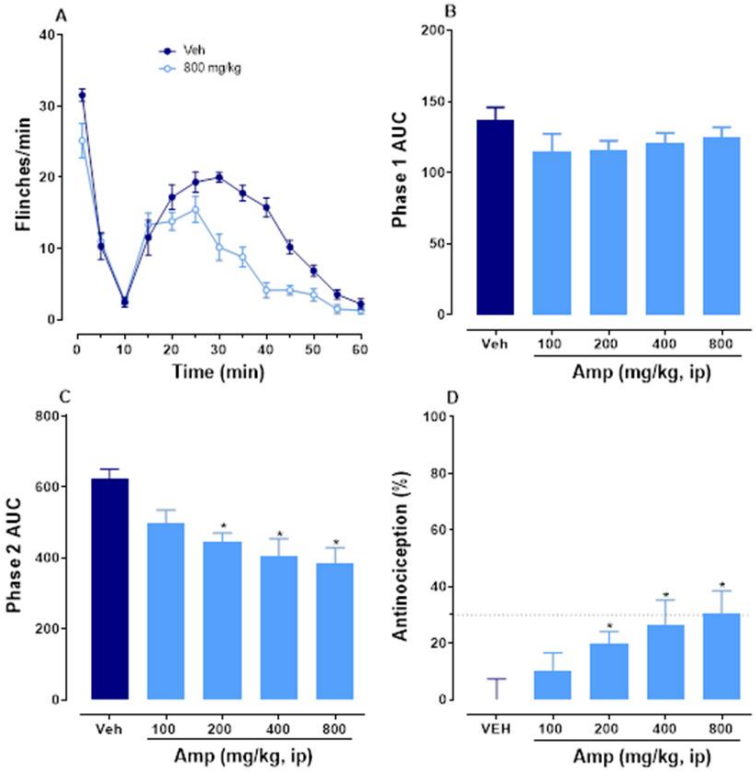

Figure 1.

Antinociceptive activity of acute pre-treatment with ampicillin (Amp)

The mean ± Standard Error of the Mean *per* group (six animals) are considered the data. *Significantly different from the Veh group (1% formalin + vehicle) as determined by one-way analysis of variance, followed by the Bonferroni´s test

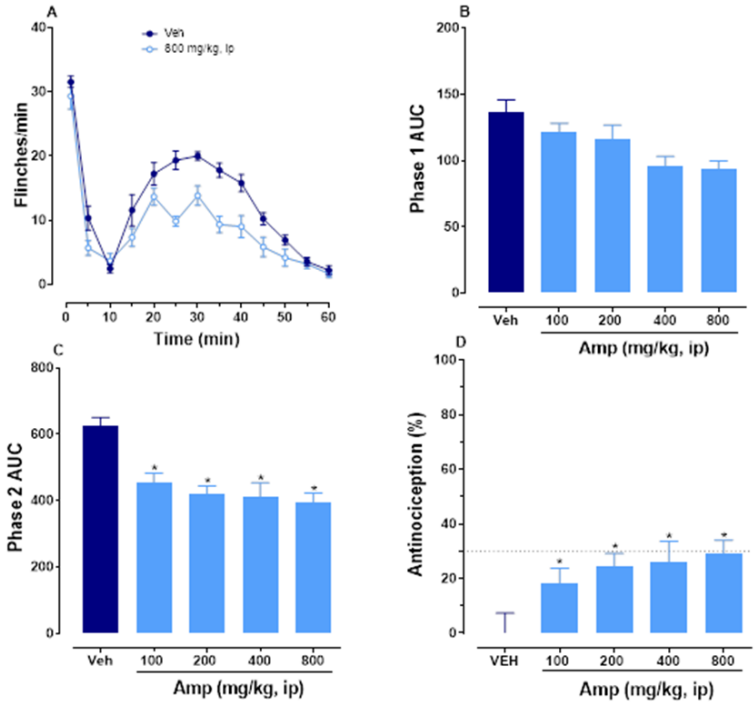

Figure 2.

One-day pre-treatment with ampicillin reduces nociception induced by formalin

The mean ± Standard Error of the Mean *per* group (six animals) are considered the data. *Significantly different from the VEH group (1% formalin + vehicle), as determined by one-way analysis of variance, followed by the Bonferroni´s test

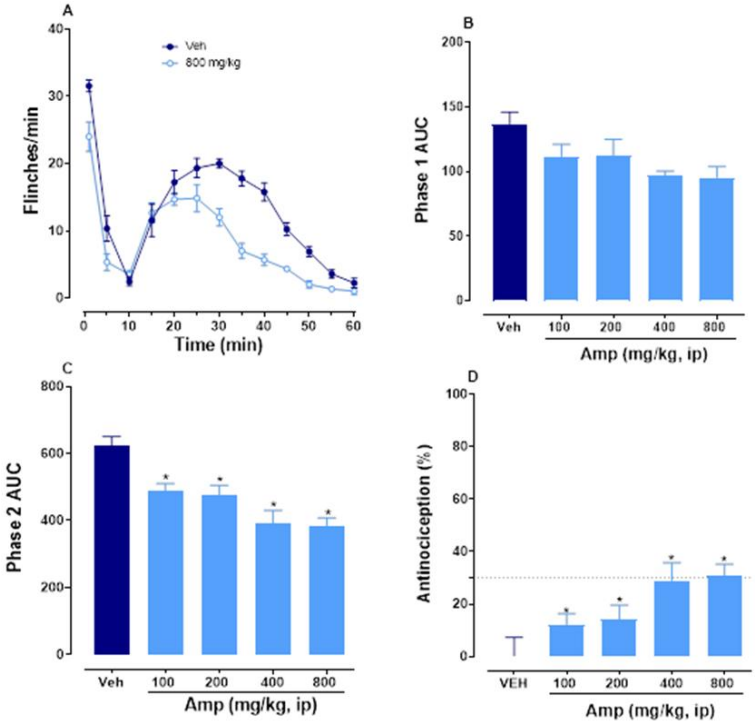

Figure 3.

Three days-pre-treatment with ampicillin reduces formalin-induced nociception

The mean ± Standard Error of the Mean *per* group (six animals) are considered the data. *Significantly different from the VEH group (1% formalin + vehicle), as determined by one-way analysis of variance, followed by the Bonferroni´s test

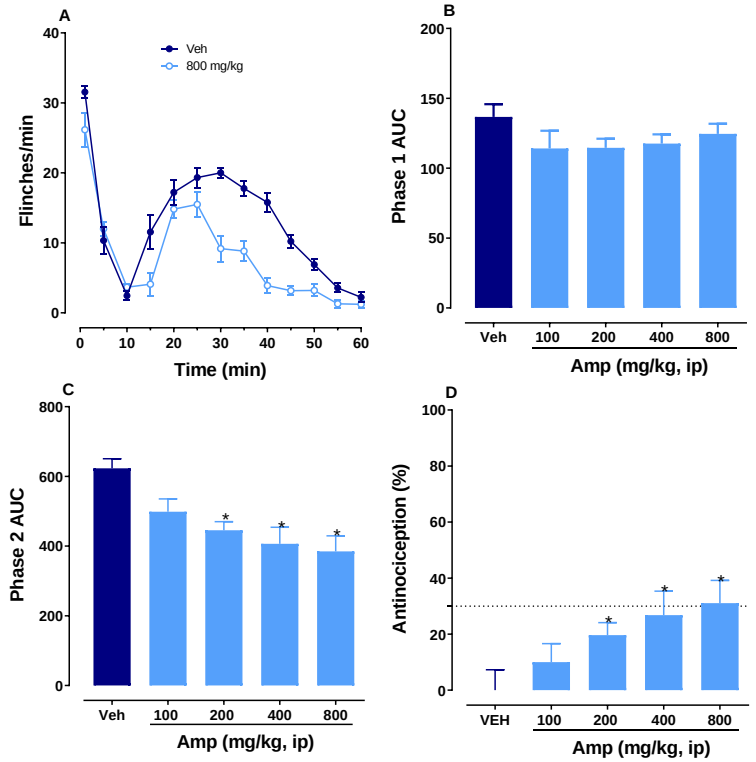

Figure 4.

Seven days-pre-treatment with ampicillin reduces formalin-induced nociception

In all plots, data are the mean ± SEM of six animals *per* group. *Significantly different from the VEH group (1% formalin + vehicle), as determined by one-way analysis of variance, followed by the Bonferroni´s test

The lack of antinociceptive effect in phase 1 with both acute and chronic administration of ampicillin suggests that this drug does not act on the mediators involved in neurogenic pain. There is evidence that the second phase of the formalin test is associated with peripheral as well as central sensitization [24]. Thus, our data suggest that ampicillin is effective to reduce central sensitization in this model.

There is mounting evidence that nerve injury leads to GLT-1 loss or downregulation in the spinal dorsal horn along with hyperexcitability and nociception [8, 25]. Contrariwise, β-lactam drugs, such as ceftriaxone and ampicillin, upregulate GLT-1 and, therefore, decrease extracellular glutamate concentrations and hyper-excitability [23, 26]. Other reports suggest that β-lactam drugs upregulate astroglial GLT-1 and modulate NMDA receptor NR2B subunits and HMGB1-dependent pathways [2, 20, 21, 26].

Thus, it has been postulated that ampicillin and other β-lactam antibiotics induce their antinociceptive effect by restoring nerve injury-induced downregulation of glutamate transporter-1. It is important to note that ampicillin penetrates cerebral spinal fluid following systemic administration, and then the antinociceptive effect observed in the present study could be due to an action at the spinal cord. In support of this, there is evidence indicating that ampicillin induces upregulation of the astroglial GLT-1 in the prefrontal cortex and nucleus accumbens [1, 11, 12, 23]. The latter is consistent with other β-lactam drugs, such as clavulanic acid and ceftriaxone [5, 13].

Previous data indicate that 1% formalin injection induces mixed pain (inflammatory and neuropathic pain) [24], suggesting that this antibiotic is effective against inflammatory and neuropathic pain. The fact that ampicillin and other β-lactam antibiotics are effective in inflammatory and neuropathic pain models supports this suggestion (see above).

The current study provides evidence that single or repeated doses of ampicillin show a dose-dependent antinociceptive effect in the rat formalin test probably by restoring formalin-induced downregulation of GLT-1.

Conclusions

Results indicate that ampicillin induces antinociception in the rat formalin test after acute and repeated treatment. The data presented demonstrate the anti-nociceptive effect of ampicillin on a behavioural way, molecular studies should continue to demostrate the antinoceiception through GLT-1 upregulation.

The data presented herein demonstrates that acute systemic ampicillin reduces phase 2 of the formalin-induced nociception.

The lack of antinociceptive effect in phase 1 with both acute and chronic administration of ampicillin suggests that this drug does not act on the mediators involved in neurogenic pain.

The antinociceptive effect could be due to an action at the spinal cord such as upregulation of the astroglial GLT-1 in the prefrontal cortex and nucleus accumbens.

Conflict of interest

The authors declare no conflict of interest.

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