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The Antinociceptive Effect of *Lonchocarpus Araripensis* Lectin is Mediated by Endocannabinoid Receptors

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ABSTRACT

The involvement of endocannabinoid receptors in the antinociceptive activity of *Lonchocarpus araripensis* lectin (LAL) was investigated in the model of carragenan-induced hypernociception. Swiss mice received LAL (10 mg/kg) by intravenous (i.v.) route 30 min before subcutaneous (s.c.) injection of carrageenan in the paws. Animals were treated with the antagonists of CB1 (AM251) or CB2 (AM630) cannabinoid receptors 30 min before the lectin. LAL inhibited the hypernociception induced by carrageenan (1 h: 11.87 ± 0.61 g; 3 h: 9.7 ± 0.70 g). AM251 (1 h: 4.75 ± 0.41 g; 3 h: 4.5 ± 0.53 g) and AM630 (1 h: 5.67 ± 0.56 g; 3 h: 5 ± 0.68 g) reversed the inhibitory effect of LAL. The antinociceptive effect of *Lonchocarpus araripensis* lectin is mediated by endocannabinoid receptors.

Keywords: Dalbergieae lectin; hipernociception; cannabinoid receptors

INTRODUCTION

Lectins are considered a heterogeneous group of proteins that exhibit specific and reversible carbohydrate-binding activities with biochemical properties and that act as cell recognition elements in a wide range of biological systems [1].

The lectin isolated from *Lonchocarpus araripensis* (LAL), belonging to Dalbergieae tribe, recognizes and specifically binds to N-acetyl-D-glucosamin [2]. Several effects have already been evidenced for LAL, such as vasorelaxant [3], anti-inflammatory [2, 4], and antinociceptive [5, 6], also a protective effect on sepsis model [7]. In respect to the antinociceptive activity, LAL has a pleiotropic effect in several nociceptive pathways, being associated to the modulation of inflammatory and/or hypernociceptive mediators [5, 6]. However, there are no studies regarding LAL antinociception in the cannabinoid system.

The involvement of the endocannabinoid system is also related to nociceptive pathways, being involved in the analgesic activity of drugs used to treat pain, such as paracetamol [8], indomethacin and ibuprofen [9].

Besides, agonists of cannabinoid receptors present inhibitory effect in animal models of pain [10, 11]. CB1 receptors are expressed primarily in central and peripheral neurons, while CB2 receptors, mainly in immune cells [12, 13, 14, 15].

This study evaluated the participation of CB1 and CB2 cannabinoid receptors in the antinociceptive activity of the lectin isolated from *Lonchocarpus araripensis* seeds in the mice model of hypernociception.

MATERIALS AND METHODS

Lectin

LAL was isolated and purified from the extract obtained from *Lonchocarpus araripensis* BENTH seeds. belonging to the Dalbergieae tribe, the Papilionoideae subfamily and the Leguminosae family. by affinity and ion exchange chromatography [2].

Drugs and reagents

Lambda carrageenan was diluted in sterile saline (0.9% NaCl), AM251 and AM630 were diluted in dimethyl sulfoxide (10% DMSO). All drugs were purchased from Sigma (St. Louis, Missouri, USA).

Animals

Swiss male mice (25-30 g) were maintained in adequate environmental conditions (12 h/12 h dark/white cycles, 25°C), receiving water and food ad libitum. Experimental protocols were conducted according to international ethic principles (National Institute of Health - NIH nº 85-23, revised in 2011) and approved by the Ethic Committee for the use of Experimental Animals of the State University of Ceará (CEUA/UECE nº 2127461/2015)

Hypernociception model: mechanical allodynia

Animals were treated with LAL (10 mg/kg) by intravenous (i.v.) route 30 min before intraplantar subcutaneous (s.c.) injection of carrageenan (300 µg/paw/50 µl). AM-251 (80 µg/paw) and AM-630 (25 µg/paw) were used as CB1 and CB2 cannabinoid receptor antagonists, respectively, 30 min before LAL.

Mice were individually placed in boxes of elevated wire mesh platforms to allow access to the ventral surface of hind paws, in which were applied 6 consecutive mechanical pressures, using a polypropylene tip (0.5 mm diameter) coupled to digital algesimeter. The paw withdrawal response (g) was determined before (basal value), 60 and 180 min after injection of carrageenan. The reduced intensity force required to evoke paw withdrawal is indicative of hypernociception [16].

Statistical analysis

Statistical differences were determined by analysis of variance (one-way ANOVA) followed by Bonferroni test. Parametric data was expressed as Mean ± SEM (n= 8/group). P <0.05 was considered significant.

RESULTS

AM251 and AM630 reverses LAL antihypernociceptive effect

Carrageenan reduced the nociceptive threshold in response to mechanical stimulation of the animal paws at 1 h (carrageenan: 3.05 ± 0.32 *vs*. saline: 10. 62 ± 0 98 g) and 3 h (carrageenan: 2.75 ± 0.31 *vs*. saline: 9.37 ± 0.41 g) after its administration. LAL inhibited the hypernociception induced by carrageenan (1 h: 11.87 ± 0.61 g; 3 h: 9.7 ± 0.70 g) and the previous administration of AM251 (1 h: 4.75 ± 0.41 g; 3 h: 4.5 ± 0.53 g) or AM630 (1 h: 5.67 ± 0.56 g; 3 h: 5 ± 0.68 g) reversed the inhibitory effect of LAL (Fig. 1).

DISCUSSION

The present study demonstrates that the lectin isolated from *Lonchocarpus araripensis* (LAL) inhibits the hypernociception induced by carrageenan in mice, an effect that was reversed by the pretreatment with antagonists of endocannabinoids receptors.

It has been previously shown that LAL reduces the hypernociception induced with initial and final mediators that participate in the carrageenan effect, such as TNF-α and PGE2, respectively [5]. Besides, LAL has a direct action on the nociceptor, preventing the development of the hypernociceptive state via decrease of total Na+ current produced in rat dorsal root ganglion [5]. The present study demonstrates that the antinociceptive effect of LAL may have the participation of cannabinoid receptors, since previous treatment with the antagonists of AM251 (CB1) and AM630 (CB2) reversed the lectin antinociceptice effect. Some studies highlight the involvement of the endocannabinoid system in the analgesic activity of drugs widely used in the clinical practice to treat pain, like paracetamol [8], indomethacin and ibuprofen [9]. Also, it has already been demonstrated the role of endocannabinoids in the modulation of hypernociception by electrophysiological and behavioral aspects [12, 17, 18, 19, 20]. CB1 agonists are known to inhibit membrane excitation and Ca^{2+} conductance and also to increase potassium conductance, inducing an antinociceptive effect [21], in addition to stimulate nitric oxide synthesis [22]. In this line, the LAL antinociceptive effect involves the activation of the L-arginine/NO/GMPc/K+ATP pathway [6] and the decrease in the total Na+ current [5]. Moreover, CB2 agonists play an important role in the control of the inflammatory process, especially in the initial events that modulate the communication between endothelial cells and leukocytes, leading to inhibition of cell migration [23]. It is well demonstrated that lectins have been used as tools in the study of inflammatory processes as they have the capacity of reversible and specific binding to glycan structures present in the membranes of inflammatory cells [24, 25, 26]. In the other study, LAL presented anti-inflammatory activity via lectin domain, inhibiting neutrophil migration to rat peritoneal cavity and modulating inflammatory mediators [2]. In fact, the antinociceptive effect of LAL was inhibited by the animal's pre-treatment with AM630, proposing that the lectin could be acting as a CB2 receptor agonist or leading to the increase of endocannabinoids synthesis. Therefore, the present study demonstrated, for the first time, the participation of the endocannabinoid system in the antinociceptive effect of LAL, however, there is a need to conduct further studies to better understand how this system are associated to the effect of this lectin.

CONCLUSIONS

In conclusion, the antinociceptive effect of the lectin isolated from

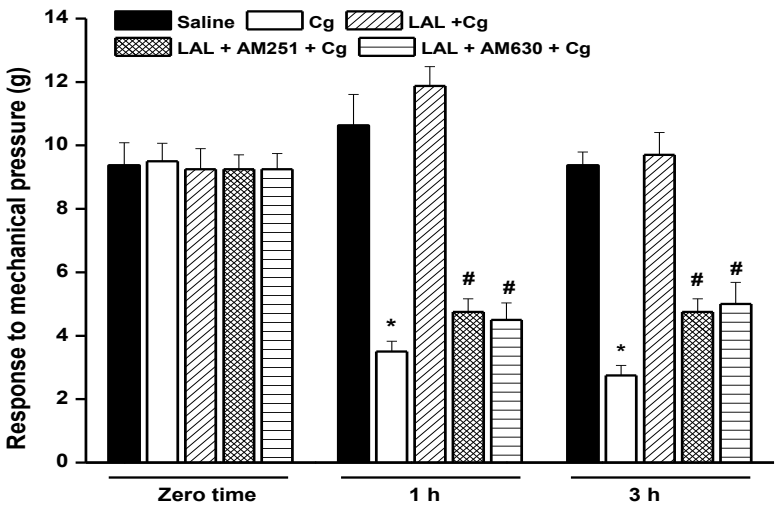

FIGURE 1: AM251 AND AM630 INHIBIT LAL ANTIHYPERNOCICEPTICE EFFECT.

LAL (10 mg/kg) was administered i.v. 30 min before carrageenan (Cg: 300 µg/paw; s.c.). Hypernociception was evaluated 1 h and 3 h after Cg administration by digital analgesimetry. AM251 (80 µg/paw; s.c.) or AM630 (25 µg/paw; s.c.) was administered 30 min before LAL. Mean ± S.E.M. (n=6-8). One-way ANOVA/Bonferroni. *p<0.05 vs. Saline; #p<0.05 vs. LAL.

Lonchocarpus araripensis seems to be mediated by endocannabinoid receptors.

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