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Original article

Involvement of the NO/cGMP/PKG/K_{ATP} pathway and endogenous opioids in the antinociceptive effect of a sulphated-polysaccharide fraction extracted from the red algae, *Gracilaria caudata*

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ABSTRACT

We investigated the antinociceptive activity of a sulphated-polysaccharide (PLS) fraction extracted from the sea algae *Gracilaria caudata* against carrageenan-induced hypernociception in mice and the mechanism underlying the antinociceptive activity. Mechanical inflammatory hypernociception was measured with an electronic version of the Von Frey® test. Mice were treated with PLS (2.5, 5.0, and 10 mg/kg, 0.5 mL, *p.o.*) 1 h before carrageenan treatment. L-Noarg (100 ng/paw; 50 μL) was administered 1 h before carrageenan administration. L-Arg (L-Arginine; 200 mg/kg; 0.5 mL, *p.o.*) was administered 10 min before L-Noarg injection. ODQ (8 μg/paw), KT 5823 (1.5 μg/paw), glibenclamide (160 μg/paw), and naloxone (1.0 μg/paw) were administered 30 min before carrageenan administration. PLS reduced carrageenan-induced hypernociception (300 μg/paw, 50 μL, intraplantar injection). PLS-induced analgesia was reversed by L-Noarg, and the effect of L-Noarg was prevented by L-Arg. The soluble guanylyl cyclase inhibitor ODQ, the protein kinase G inhibitor KT 5823, the K_{ATP} blocker glibenclamide, and the opioid receptor antagonist naloxone, significantly reversed ($P < 0.05$) the antinociceptive effect of PLS. The present study showed an intrinsic peripheral antinociceptive action of PLS administration in mice. This antinociceptive effect seemed to be mediated by activation of the NO/cGMP/PKG pathway followed by the opening of K_{ATP} channels, and depended on endogenous opioids.

1. Introduction

Marine red algae have a high biological diversity and live in a variety of environments [1]. These marine organisms are sources of numerous new compounds with multiple pharmacological properties [2,3]. At present, the number of substances being isolated from such sources is growing. Most of their chemical structures have been elucidated, and they are being investigated for their potential to meet various biological objectives, as well as to expand the scientific knowledge base in the area of naturally bioactive compounds [1,4].

One of the most important substances isolated from algae is sulphated polysaccharide. Sulphated polysaccharides (PLS) are complex macromolecular constituents of the extracellular matrix, and they evidently play an important role in the mechanical, osmotic, and ionic regulation of these organisms [3,5,6]. Investigation of these biomolecules has been steadily increasing in recent years owing to their broad potential for development as anti-viral, anticoagulant, antioxidant, gastroprotective, anti-inflammatory, and antinociceptive agents [7–13]. However, the involvement of endogenous opioids and the NO/cGMP/PKG/K_{ATP} pathway in the antinociceptive effect of PLS extracted from sea red algae has not been demonstrated.

Inflammatory hyperalgesia results from the sensitization of primary afferent neurons, which is better described as hypernociception (decrease in nociceptive threshold) in animal models [14]. This effect is induced by inflammatory mediators such as prostaglandins (PGs), which directly sensitize peripheral nociceptive neurons [15–17]. In fact, following carrageenan administration in the rat paw, an initial formation of bradykinin occurs, which induces a subsequent proinflammatory cytokine

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cascade that triggers the release of PGs and sympathetic amines [18].

During inflammatory pain induced by carrageenan, the NO/cGMP/PKG/K_{ATP} pathway and endogenous opioids can produce an antinociceptive effect. Nitric oxide (NO) activates the guanylate cyclase enzyme, which is directly responsible for an increase in the intracellular levels of cyclic guanosine monophosphate (cGMP) [19]. Subsequently, cGMP induces the opposite effect of cyclic adenosine monophosphate (cAMP) [20] and promotes antihypernociception. Sachs et al. demonstrated that activation of protein kinase G (PKG) by cGMP is necessary for the opening of ATP-sensitive potassium channels (K_{ATP}) in analgesia [21]. Endogenous opioids produce local analgesia during acute peripheral inflammation by activation of peripheral opioid receptors and the nitric oxide/cyclic guanosine monophosphate (cGMP) pathway [22–24].

Thus, considering that the sea red algae are important sources of new chemical substances with potential antinociceptive effects, this study sought to evaluate the effect of a PLS against carrageenan-induced hypernociception in mice, and to investigate the possible involvement of the NO/cGMP/PKG/K_{ATP} signalling pathway and endogenous opioids in its antinociceptive effect.

2. Materials and methods

2.1. Algae material and extraction of the polysaccharide fraction

Specimens of the red algae *Gracilaria caudata* J. Agardh (Rhodophyta, Gracilariales) were collected in August 2008 from the Atlantic coast northeast of Brazil (Fleixeira Beach, Trairi-Ceará). The sample was identified with the help of a professor (Dr Mohammed Yusuf, Department of Biology, Federal University of Ceará) and the specimen was preserved at the herbarium of the Biology Department, Federal University of Ceará, Fortaleza, Ceará, Brazil.

After collection, the algae were cleaned of epiphytes, washed with distilled water, and stored at −20 °C. For extraction of polysaccharides, 5 g of dried *G. caudata* tissue was ground into a fine powder and stirred for 2 h in distilled water (1.5% w/v) at 100 °C. After filtration and concentration of the solution, polysaccharides were precipitated with ethanol (1:3 v/v) and the precipitate was washed with acetone and dried under hot air. The polysaccharide fraction was then re-dissolved in distilled water (1.5% w/v) and the process of precipitation, washing, and drying was repeated. The final polysaccharide fraction thus derived is referred to here as "PLS".

The technique described in Dubois et al. was used for determination of carbohydrates. The distribution of molecular weights of the polysaccharides was determined by gel permeation chromatography (GPC) in Shimadzu detectors with refractive index and UV–visible at 254 nm at room temperature with Ultrahydrogel column (7.8 × 300 mm), flow 0.5 mL/min.

2.2. Animals

Swiss male mice weighing 20 to 25 grams were obtained from the animal house of the Federal University of Ceará (UFC). All treatments and surgical procedures were performed in accordance with the Guide for care in use of laboratory animals of the National Institutes of Health (Bethesda, MD, USA) and the project was approved by the Ethics Committee in Research of Universidade Federal do Piauí (No Protoloco. 23111.011989/11-33).

2.3. Mechanical hypernociception

The term hypernociception (increased nociception) was used to describe the behavioural response induced by mechanical pressure in rats. Hyperalgesia was induced by a subcutaneous injection of carrageenan (300 μg/paw) into the plantar surface of the mice hindpaw and measured by the paw pressure test described by Cunha et al. [25].

A digital analgesiometer (Insight®, Brazil) with a cone-shaped, rounded tip paw-presser was used to apply a linearly increasing force to the right hindpaws of the mice. The nociceptive threshold was measured in the right paw and determined by the average of three consecutive trials recorded before (zero time) and 3 and 4 h after carrageenan (50 μL; 300 μg/paw) injection (peak effect). Hyperalgesia was calculated from the difference between these two averages (Δ of nociceptive threshold) and expressed in grams. To reduce stress, the mice were habituated to the apparatus 1 day prior to the experiments.

2.4. Evaluation of the antinociceptive activity of the polysaccharide

In assessing the antinociceptive activity of the polysaccharide from *Gracilaria caudata*, hypernociception in the paw of Swiss mice was induced by carrageenan. The animals ($n = 5$) were divided into the following groups: saline, carrageenan, or carrageenan plus indomethacin, with the test substance administered (PLS of *G. caudata*) at different doses (2.5, 5, and 10 mg/kg, respectively). The treatments with PLS (500 μL, *i.p.)* were intraperitoneally administered 1 h before the induction stimulus (50 μL/paw) by subcutaneous injection of carrageenan (300 μg/paw) in the subplantar region of the right hindpaw. In this test, the animals were transferred to individual cages and maintained for 30 min; they were then conditioned to receive mechanical stimuli at time zero (baseline), which was before the hyperalgesic stimuli, and 3 or 4 h after administration of the stimulus (carrageenan).

2.5. Role of NO in the antinociceptive effect of PLS

The animals were pretreated with PLS (2.5 mg/kg· *i.p.*) or L-Noarg (non selective NOS inhibitor, 100 ng/paw; 50 μL) 1 h before carrageenan (50 μL; 300 μg/paw) injection. Other experimental groups were treated in the same way, but L-arginine (NOS substrate, 200 mg/kg; 0.5 mL; *i.p.*) was given 10 min before L-Noarg injections. In the untreated PLS group, L-Noarg alone was also injected before carrageenan administration. The nociceptive threshold was then measured in the right paw and determined as described above.

2.6. Role of soluble Guanylate Cyclase (GC) in the antinociceptive effect of PLS

The animals were pretreated with PLS (2.5 mg/kg, *i.p.*) 1 h before carrageenan administration. Thirty min after PLS administration, the soluble guanylyl cyclase inhibitor ODQ (8 μg/paw; 50 μL, intraplantar injection) or dimethyl sulphoxide (diluent) were injected into the plantar surface. Thirty min after ODQ administration, carrageenan (50 μl; 300 μg/paw) was administered in the right paw of the mice. In the untreated PLS group, ODQ alone was also injected before carrageenan or PGE_2. The nociceptive threshold was then measured in the right paw and determined as described above.

2.7. Participation of protein kinase G (PKG) in the antinociceptive effect of PLS

The animals were pretreated with PLS (2.5 mg/kg, *i.p.*) 1 h before carrageenan administration. Thirty min after PLS administration, KT 5823 (a selective inhibitor of protein kinase G, 1.5 μg/paw; 50 μL, intraplantar injection) or dimethyl sulphoxide (diluent) were injected. Thirty min after KT 5823 administration, carrageenan (50 μL; 300 μg/paw) was administered in the right paw of the mice. In the untreated PLS group, KT 5823 alone was also

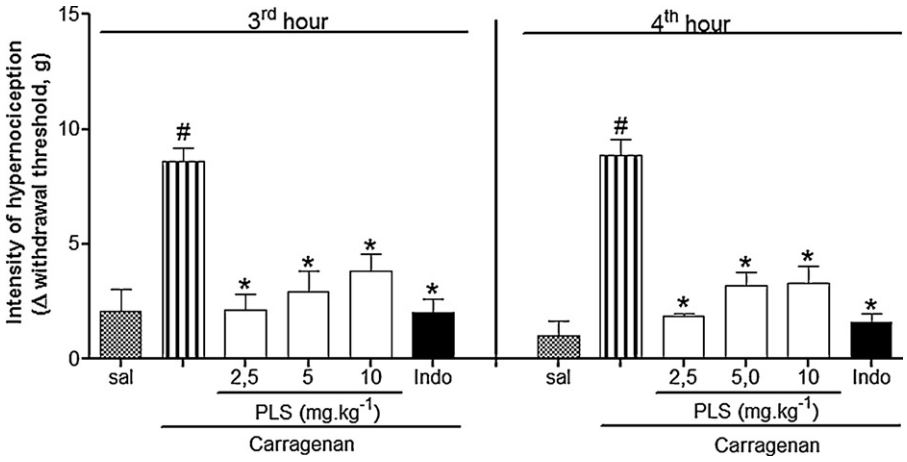

Fig. 1. Role of polysaccharide (PLS) treatment on carrageenan-induced hypernociception. Male Swiss mice were treated with PLS (2.5, 5, and 10 mg/kg, *i.p.*) or indomethacin (10 mg/kg, *i.p*). After 1 h, carrageenan (300 μg/paw; 50 μL) was injected and paw hypernociception was measured 3 and 4 h after carrageenan injection. The results are expressed as mean ± SEM for a minimum of 6 animals per group. (*) $P < 0.05$ compared to saline + carrageenan group; (#) $P < 0.05$ compared to saline group. (Anova followed by Bonferroni's *t*-test.)

injected before carrageenan. The nociceptive threshold was then measured in the right paw and determined as described above.

2.8. Role of ATP-dependent potassium channels (K_{ATP}) in the antinociceptive effect of PLS

The animals were pretreated with PLS (2.5 mg/kg, i.p.) 1 h before carrageenan administration. Thirty min after PLS administration, Glibenclamide (an ATP-sensitive potassium channel blocker, 160 μg/paw; 50 μL, intraplantar injection) or 2% Tween 20 (diluent) were injected. Thirty min after Glibenclamide administration, carrageenan (50 μL; 300 μg/paw) was administered in the right paw of the mice. In the untreated PLS group, Glibenclamide alone was also injected before carrageenan. The nociceptive threshold was then measured in the right paw and determined as described above.

2.9. Role of endogenous opioids in the antinociceptive effect of PLS

The animals were pretreated with PLS (2.5 mg/kg, i.p.) 1 h before carrageenan administration. Thirty min after PLS administration, naloxone (a non specific opioid receptor antagonist; 1 μg/paw; 50 μL intraplantar injection) or saline were injected. Thirty min after naloxone administration, carrageenan (50 μL; 300 μg/paw) was administered in the right paw of the mice. In the untreated PLS group, naloxone alone was also injected before carrageenan administration. The nociceptive threshold was then measured in the right paw and determined as described above.

2.10. Statistical analysis

Results are presented as mean and standard error of the mean for groups of six animals each. Differences between experimental groups were compared by Analysis of Variance (Anova) followed by Bonferroni's *t*-test. The significance level was set at $P < 0.05$.

3. Results

3.1. Effect of PLS treatment on carrageenan-induced hypernociception

On the Fig. 1 we demonstrated that PLS treatment reduced the carrageenan-induced hypernociception (carrageenan, 3rd h: 8.60 g ± 0.56 g/4th h: 8.84 g ± 0.70 g; PLS 2.5 mg.kg^{-1}, 3rd h: 2.14 g ± 0.65/4th h: 1.84 ± 0.12 g; PLS 5.0 mg/kg, 3rd h: 2.90 g ± 0.81/4th h: 3.20 g ± 0.55 g; PLS 10 mg/kg, 3rd h: 3.84 g ± 0.75/4th h: 3.28 ± 0.65 g). Fig. 1 shows that the PLS fraction prevented carrageenan-induced hypernociception in a dose-dependent manner, reaching maximal effect at a dose of 2.5 mg/kg (3rd hour: 2.14 g ± 0.65/4th hour: 1.84 ± 0.12 g). This dose was selected for studying the possible mechanisms of action involved in PLS-mediated intracellular signalling.

3.2. Involvement of NO in the antinociceptive effect of PLS

Fig. 2 demonstrates that pretreatment with a non specific NOS inhibitor (L-Noarg) reversed (3rd hour: 8.50 g ± 1.01 g/4th hour: 9.02 g ± 0.09 g) the antinociceptive effect induced by PLS administration (3rd hour: 1.15 g ± 0.54 g/4th hour: 1.00 g ± 0.59 g). In animals not treated with PLS, L-Noarg did not increase the inflammatory hypernociception induced by carrageenan. In another group, L-arginine was able to reverse (3rd hour: 3.00 g ± 0.51 g/4th hour: 2.70 g ± 0.55 g) the effect of L-Noarg on inflammatory hypernociception induced by carrageenan.

3.3. Involvement of cGMP in the antinociceptive effect of PLS

As shown on Fig. 3, ODQ (a soluble guanylyl cyclase inhibitor) treatment of animals reversed (3rd hour: 11.52 ± 0.74 g/4th hour: 11.22 g ± 0.79 g) the antinociceptive effect induced by PLS administration (3rd hour: 3.74 ± 1.01 g/4th hour: 2.88 ± 1.07 g). In animals not treated with PLS, ODQ did not increase the inflammatory hypernociception induced by carrageenan.

3.4. Involvement of protein kinase G in the antinociceptive effect of PLS

As shown in Fig. 4, KT 5823 (a selective inhibitor of protein kinase G) treatment of animals reversed (3rd hour: 12.20 ± 1.32 g/4th hour: 11.84 ± 0.42 g) the antinociceptive effect induced by PLS administration (3rd hour: 4.95 ± 1.11 g/4th hour: 3.72 ± 1.22 g). In animals not treated with PLS, KT 5823 did not increase the inflammatory hypernociception induced by carrageenan.

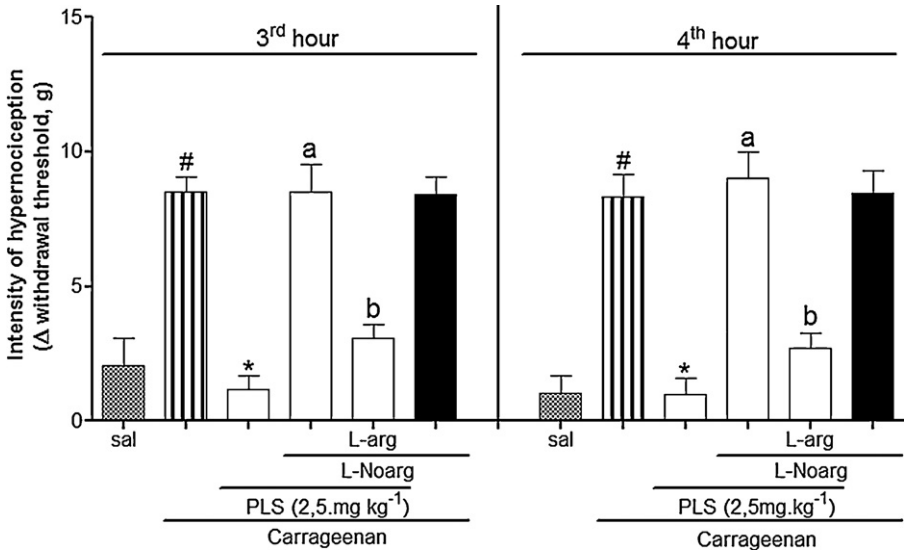

Fig. 2. Involvement of NO in the antinociceptive effect of polysaccharide (PLS). Mice were pretreated with an injection of PLS (2.5 mg/kg, *i.p.*) 1 h before carrageenan administration (300 μg/paw, 50 μL;). L-Noarg (100 ng/paw; 50 μL) was administered 1 h before carrageenan administration in the right paw of the mice. L-Arg (L-Arginine; 200 mg/kg; 0.5 mL) was administered 10 min before L-Noarg injection. The analgesic effect of PLS in the right hindpaw was measured 3 or 4 h after carrageenan-induced hypernociception. (#) Indicates statistically significant differences between the carrageenan group and the saline group. (*) Indicates statistically significant differences between the PLS + carrageenan group and the carrageenan group. (a) Indicates statistically significant differences between the PLS + L-Noarg + carrageenan group and the PLS + carrageenan group. (b) Indicates statistically significant differences between the PLS + L-Noarg + L-arg + carrageenan group and the PLS + L-Noarg + carrageenan group. The results are expressed as mean ± SEM from a minimum of 6 animals per group. $P < 0.05$ indicates statistical significance (Anova/Bonferroni).

3.5. Involvement of K_{ATP} channels in the antinociceptive effect of PLS

As shown on Fig. 5, Glibenclamide reversed (Glib; 3rd hour: 11.94 ± 0.64 g/4th hour: 11.62 ± 1.00 g) the peripheral antinociceptive effect induced by PLS administration (3rd hour: 4.94 ± 1.11 g/4th hour: 3.70 ± 1.21 g). In animals not treated with PLS, Glib did not increase the inflammatory hypernociception induced by carrageenan.

3.6. Involvement of endogenous opioids in the antinociceptive effect of PLS

As shown on Fig. 6, our results clearly demonstrated that pretreatment with a non specific receptor opioid blocker (naloxone) reversed (3rd hour: 9.41 ± 0.80 g/4th hour: 10.15 ± 0.91 g) the peripheral antinociceptive effect induced by PLS administration (3rd hour: 4.92 ± 1.11/4th: 3.74 ± 1.20 g). In animals not treated with PLS, naloxone did not increase the inflammatory hypernociception induced by carrageenan.

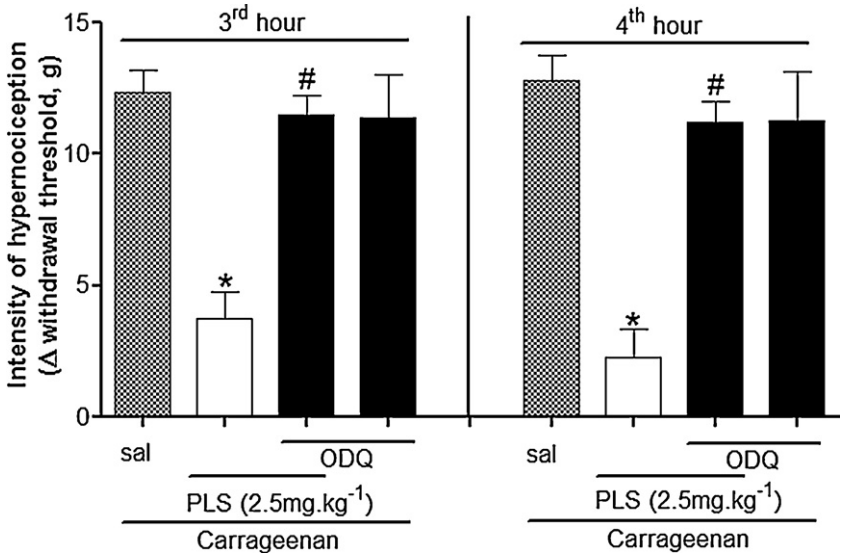

Fig. 3. Involvement of cGMP in the antinociceptive effect of polysaccharide. Mice were pretreated with an injection of PLS (2.5 mg/kg, *i.p.*) 1 h before carrageenan administration (300 μg/paw, 50 μL). ODQ (8 μg/paw; 50 μL) was administered 30 min before carrageenan treatment. The analgesic effect of PLS in the right hindpaw was measured 3 or 4 h after carrageenan-induced hypernociception. (*) Indicates statistically significant differences between the PLS + carrageenan group and the carrageenan group. (#) Indicates statistically significant differences between the PLS + ODQ + carrageenan group and the PLS + carrageenan group. The results are expressed as mean ± SEM from a minimum of six animals per group. $P < 0.05$ indicates statistical significance (Anova/Bonferroni).

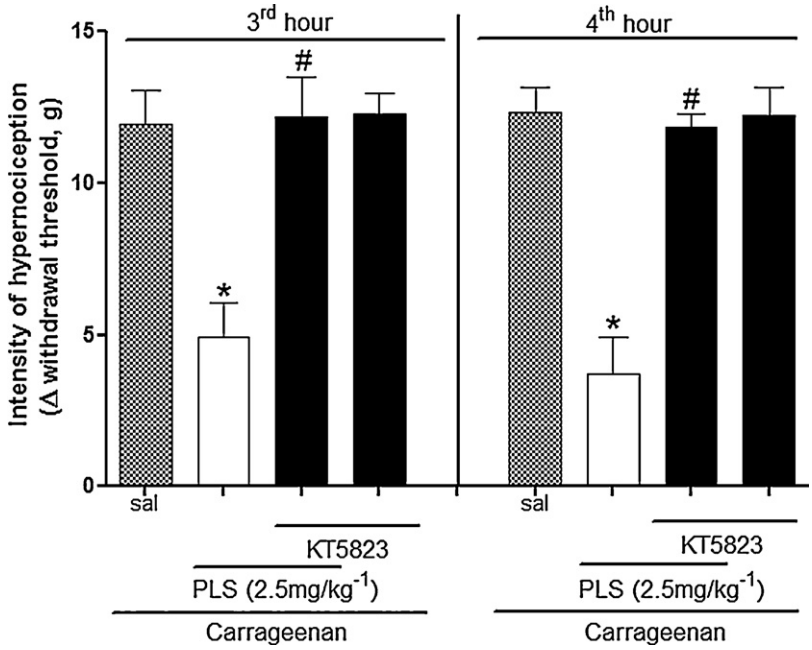

Fig. 4. Involvement of protein kinase G (PKG) in the antinociceptive effect of polysaccharide. Mice were pretreated with an injection of PLS (2.5 mg/kg, *i.p.*) 1 h before carrageenan administration (300 μg/paw, 50 μL). KT 5823 (a selective inhibitor of protein kinase G, 1.5 μg/paw; 50 μL, intraplantar injection) was administered 30 min before carrageenan treatment. The analgesic effect of PLS in the right hindpaw was measured 3 h or 4 h after carrageenan-induced hypernociception. (*) Indicates statistically significant differences between the PLS + carrageenan group and the carrageenan group. (#) Indicates statistically significant differences between the PLS + KT5823 + carrageenan group and the PLS + carrageenan group. The results are expressed as mean ± SEM from a minimum of 6 animals per group. $P < 0.05$ indicates statistical significance (Anova/Bonferroni).

4. Discussion

Natural products of algal origin are used in folk medicine worldwide, and they exhibit a wide range of pharmacological activities. Over the years, natural products have contributed enormously to the development of important therapeutic drugs used currently in modern medicine [25,26]. Polysaccharides extracted from algae can play a relevant role in biomedical and pharmaceutical applications, particularly in the field of drug delivery.

In this study, we sought to investigate, by the use of pharmacological tools and molecular procedures, the possible antinociceptive effect of a PLS fraction extracted from the marine red algae G. *caudata* in mice. Our results demonstrate that PLS produced a peripheral antinociceptive effect in carrageenan-induced mechanical hypernociception, and this effect was at least in part

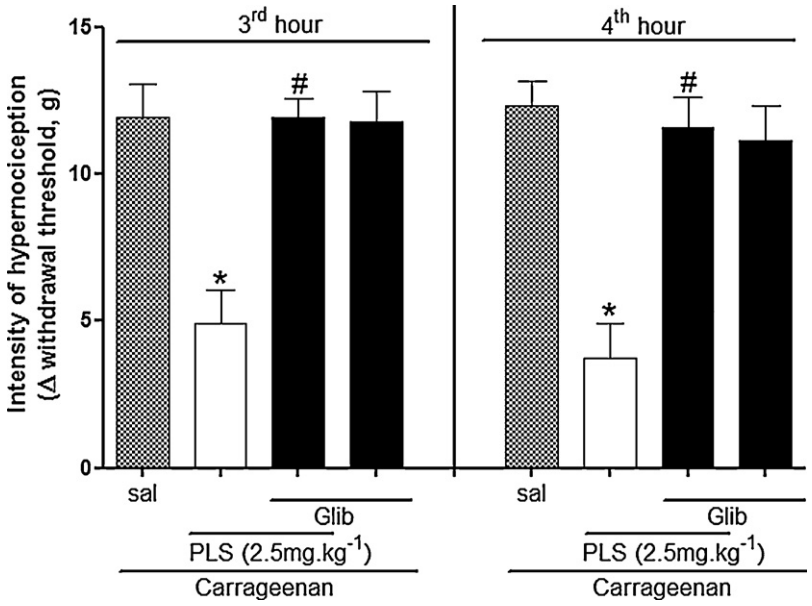

Fig. 5. Involvement of K_{ATP} channels in the antinociceptive effect of polysaccharide. Mice were pretreated with an injection of PLS (2.5 mg/kg, *i.p.*) 1 h before carrageenan administration (300 μg/paw, 50 μL). Glibenclamide (Glib; 160 μg/paw; 50 μL) was administered 30 min before carrageenan treatment. The analgesic effect of PLS in the right hindpaw was measured 3 or 4 h after carrageenan-induced hypernociception. (*) Indicates statistically significant differences between the PLS + carrageenan group and the carrageenan group. (#) Indicates statistically significant differences between the PLS + Glib + carrageenan group and the PLS + carrageenan group. The results are expressed as mean ± SEM for a minimum of six animals per group. $P < 0.05$ indicates statistical significance (Anova/Bonferroni).

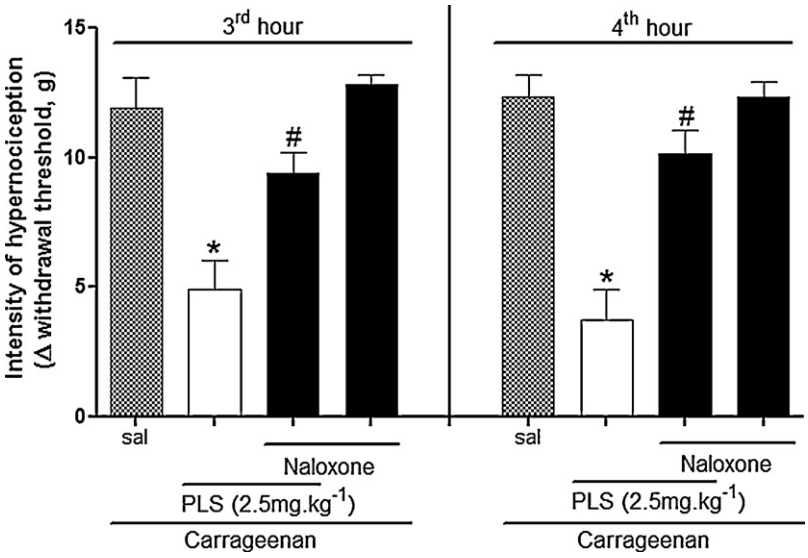

Fig. 6. Involvement of endogenous opioids in the antinociceptive effect of polysaccharide. Mice were pretreated with an injection of PLS (2.5 mg/kg, *i.p.*) 1 h before carrageenan administration (300 μg/paw, 50 μL). Naloxone (1.0 μg/paw; 50 μL) was administered 30 min before carrageenan treatment. The analgesic effect of PLS in the right hindpaw was measured 3 h or 4 h after carrageenan-induced hypernociception. (*) Indicates statistically significant differences between the PLS + carrageenan group and the carrageenan group. (#) Indicates statistically significant differences between the PLS + Naloxone + carrageenan group and the PLS + carrageenan group. The results are expressed as mean ± SEM for a minimum of six animals per group. $P < 0.05$ indicates statistical significance (Anova/Bonferroni).

mediated by activation of the NO/cGMP/PKG/K_{ATP} pathway and opioid system.

Our results demonstrate that PLS produced a peripheral antinociceptive effect in carrageenan-induced mechanical hypernociception. The induction of inflammatory hypernociception is primarily driven by the sensitization of primary nociceptive neurons, an effect that is caused by nociceptor-sensitizing mediators. Carrageenan-induced inflammatory hypernociception depends on the concomitant release and action of cytokines such as IL1β, IL-8, and TNF-α [27]. One possibility was that PLS decreased the inflammatory response by decreasing cytokine release and, subsequently, inflammatory hypernociception. Therefore, we can infer that PLS decreases both inflammation and the hypersensitivity of the nociceptive neuron.

We also provide evidence for the molecular mechanism by which PLS administration promotes analgesia by activation of the NO/cGMP/PKG/K_{ATP} pathway and opioid system. Our findings suggest that the PLS antinociceptive effect is an NO-dependent process, and that blockage of the NO pathway with L-Noarg abrogated the antinociceptive effect of PLS against carrageenan-induced hypernociception. Several studies demonstrated that NO can produce an antinociceptive effect in carrageenan-induced hypernociception [28]. The peripheral analgesic activity of NO in the peripheral nerve termini seemed to be mediated by activation of soluble guanylyl cyclase [29] and subsequent cGMP production. Once synthesized, NO can diffuse within the same cell or neighbouring cells, where it binds to the haeme group of soluble guanylyl cyclase to generate cGMP from GTP [30,31]. According these data we can infer that NO participates of antinociceptive effect of PLS.

By using a pharmacological approach, it was demonstrated that PLS provides a protective effect against carrageenan-induced hypernociception via the NO/cGMP pathway. As shown on Fig. 3, our results demonstrated that ODQ (a soluble guanylyl cyclase inhibitor) treatment of animals reversed the antinociceptive effect induced by PLS administration. Based on the literature data and according to our results, we can infer that the antinociceptive effect of PLS is NO/cGMP pathway dependent.

By using a pharmacological tool, PLS was shown to provide a protective effect against carrageenan-induced hypernociception via the protein kinase G (PKG) pathway. On Fig. 4, our results suggest that KT 5823 (a selective inhibitor of protein kinase G) treatment of animals reversed the antinociceptive effect induced by PLS administration.

During hypernociception, NO activates the guanylate cyclase enzyme that is directly responsible for an increase in intracellular levels of cGMP [21]. Subsequently, cyclic guanosine monophosphate (cGMP) induces the opposite effect of cyclic adenosine monophosphate (cAMP) [22] and promotes anti-hypernociception. Sachs et al. demonstrated that activation of protein kinase G (PKG) by cGMP is necessary for the opening of ATP-sensitive potassium channels (K_{ATP}) in analgesia [23]. According to these data, we can infer that the antinociceptive effect of PLS is NO/cGMP/PKG pathway dependent.

In an attempt to demonstrate the role of K_{ATP} channels in the antinociceptive effect of PLS, as shown on Fig. 5, our results demonstrated that Glibenclamide reversed the peripheral antinociceptive effect induced by PLS administration. The peripheral analgesic activity of the NO/cGMP pathway seems to result from the modulation of K_{ATP} currents. For instance, opioids, NO donors, and cGMP are inhibited by K_{ATP} blockers [17,32,33]. Other studies demonstrated that activation of protein kinase G (PKG) by cGMP is necessary for the opening of ATP-sensitive potassium channel (K_{ATP}) channels in analgesia [23]. According to these data, we can infer that the antinociceptive effect of PLS seemed to be mediated by activation of the NO/cGMP/PKG pathway followed by the opening of K_{ATP} channels.

Another system involved in the analgesic effect of PLS is the opioid system. As shown on Fig. 6, our results clearly demonstrated that pretreatment with a non specific receptor opioid blocker (naloxone) reversed the peripheral antinociceptive effect induced by PLS administration.

It is well known that activation of the NO/cGMP/PKG pathway modulates the peripheral antinociceptive effects induced by certain drugs, including opioids, during inflammatory pain [24,25]. Adding to this, another study demonstrated that the peripheral effect of endogenous opioids seems to act directly on primary nociceptive neurons and stimulate the nNOS/NO/cGMP/PKG/K_{ATP} channel antinociceptive pathway [34,35]. According to these results, we can infer that the antinociceptive effect of PLS seemed to be mediated by activation of the NO/cGMP/PKG pathway followed by the

opening of K_{ATP} channels, and this effect appears to depend on the action of opioids.

In summary, the present study showed an intrinsic peripheral antinociceptive action of PLS administration in mice. This antinociceptive effect seemed to be mediated by activation of the NO/cGMP/PKG pathway followed by the opening of K_{ATP} channels, and depended on endogenous opioids. These observations also raise the possibility that polysaccharides may present new strategies for the treatment of inflammatory pain.

5. Conclusion

In conclusion, our results indicate that sulphated polysaccharide extracted from the seaweed *G. caudate* (PLS) has a potential antinociceptive effect against carrageenan-induced hypernociception and this effect is mediates by activation of the NO/cGMP/PKG/K_{ATP} pathway and dependent on endogenous opioids action.

Disclosure of interest

The authors declare that they have no conflict of interest concerning this article.

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