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Antinociceptive activity of sulfated carbohydrates from the red algae *Bryothamnion seaforthii* (Turner) Kütz. and *B. triquetrum* (S.G. Gmel.) M. Howe

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Abstract

We report the antinociceptive activity, determined by the writhing, formalin and hot-plate tests in mice, of crude (F0/60), lectin and carbohydrate fractions isolated by ammonium sulfate precipitation (0 to 60%) from *Bryothamnion seaforthii* and *B. triquetrum*, species of red algae. Not only fraction F0/60 but also lectins from both species significantly inhibited acetic acid-induced abdominal contractions after intraperitoneal or oral administrations. In the formalin test, lectins (1 and 5 mg/kg, *ip*, and 5 to 20 mg/kg, *po*) inhibited the 1st and 2nd phases (5 and 20 min, respectively), but the effect occurred predominantly on the 2nd phase. The effects of the lectins were totally or partially reversed by naloxone (2 mg/kg, *sc*) in the 1st and 2nd phases, respectively. Experiments performed with lectins in the absence and presence of avidin (1 mg/kg, *ip*) and D-mannose (1 mg/kg, *ip*) showed that avidin did not interfere with the effect of *B. seaforthii* lectin but partially reversed the effect of *B. triquetrum* lectin. D-Mannose completely reversed the effects of both species. F0/60 fractions from both algae significantly increased the latency time in response to thermal stimuli, and naloxone reversed antinociception, indicating the involvement of the opioid system in both the peripheral and central effects of the fractions. In the writhing test, the carbohydrate fractions were the most active, inhibiting the contractions by 71 and 79% (*B. triquetrum*) and by 46 and 69% (*B. seaforthii*) at doses of 1 and 5 mg/kg, *ip*, respectively. Sulfated carbohydrate fractions of *B. seaforthii* and *B. triquetrum*, containing only about 5% protein as contaminants, are probably responsible for the antinociceptive effects of these red algae.

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- *Bryothamnion triquetrum*
- Carbohydrates
- Antinociceptive effect

Introduction

Lectins are ubiquitous glycoproteins with specific and reversible carbohydrate-binding activity widely found in nature, including plant and algal tissues (1,2). Although the functions of these proteins in plants are just beginning to be understood, lectins are becoming very important in the development of glycobiology (3). At present, the term lectin is used in a broad sense to denote all types of carbohydrate-binding proteins that do not catalyze reactions with their ligands or that are antibodies (4).

The proteins present in algae which are capable of binding to and agglutinating cells were first identified by Boyd et al. (5) and designated as marine algal hemagglutinins. However, algal lectins were recently termed phycolectins (6,7) and represent a group of proteins which preferentially bind glycoproteins and share some other characteristics such as relatively low molecular weights, no metal requirement for hemagglutination, and occurrence in monomeric forms.

Certain phycolectins have potent biological activities *in vitro*, including specific and nonspecific agglutination of erythrocytes (8), stimulation of mitogenic activity towards T lymphocytes from mouse spleen or human peripheral blood lymphocytes (9-11), inhibition of ADP or collagen-induced human platelet aggregation (6), and induction of neutrophil migration *in vivo* and *in vitro* (12). In addition, a lectin-gold conjugate has also been used as a new histochemical reagent (13).

An extract from the marine red alga *Bryothamnion seaforthii* (Turner) Kütz. agglutinates trypsin-treated erythrocytes from rabbits, chickens, and cows (14), and an extract from *Bryothamnion triquetrum* (S.G. Gmel.) M. Howe agglutinates enzyme-treated erythrocytes from rabbits, chickens, goats, pigs, and humans (15,16). These investigators found that ammonium sulfate-precipitated fractions (F0/60) from both species contain lectins as one of their major constituents. There are two studies on the purification of lectins from the F0/60 of both species carried out with similar experimental protocols. Their general properties as shown by Calvete et al. (17) suggested that these protein molecules are not different from those described previously (16).

Although some substances produced by algae have antiviral, antifungal, antibacterial, hemolytic and toxic activities, little is known about the involvement of algal lectins in physiological functions. Preliminary studies have revealed that phycolectins from algae of the Brazilian northeastern coast present antinociceptive activities in several experimental models of nociception (18,19).

However, other constituents from marine algae might also be responsible for some of their pharmacological activity. This is the case for sulfated polysaccharides which have been shown to present anticoagulant and antithrombotic properties (20,21). Another study (22) has shown that polysaccharides from the red alga *Porphyridium* sp present hypocholesterolemic activity.

The aim of the present study was to further characterize the antinociceptive activity present in the crude fraction (F0/60) and in the lectin and carbohydrate fractions isolated from *B. seaforthii* and *B. triquetrum* in several experimental models of nociception in mice, in an attempt to elucidate the active constituents and their mechanism of action.

Material and Methods

Plant material

Alga specimens *Bryothamnion seaforthii* (Turner) Kütz. and *Bryothamnion triquetrum* (S.G. Gmel.) M. Howe were collected along the northeastern Brazilian coast (Flexeiras Beach, Trairi, CE, Brazil), brought to the laboratory in water-ice bags and kept at -20ºC until use. Algae were classified by Dr. A.C.M. Fortes-Xavier, Department of Biol-

ogy, Federal University of Ceará, and exiccatae were deposited at the Prisco Bezerra Herbarium of the Federal University of Ceará (voucher No. 30.850).

Drugs

Bovine serum albumin, avidin, naloxone, cetylpyridinium chloride and papain were purchased from Sigma (St. Louis, MO, USA). Morphine sulfate was from Cristalia do Brasil S/A (Itapira, SP, Brazil), mannose was from Merck (Darmstadt, Germany) and cysteine was from Riedel-de-Haen (now Sigma-Aldrich, Munich, Germany). Formalin and acetic acid were purchased from Reagen and Vetec (Duque de Caxias, RJ, Brazil). All other drugs used were of analytical grade.

Animals

Male or female Swiss mice (25 g) from the Animal House of the Federal University of Ceará, maintained on a 12-h light/dark cycle with free access to water and food were used in all experiments. Experiments were carried out according to the "Guide for the Care and Use of Laboratory Animals" of the National Academy of Sciences, 1996, USA. The research project was approved by the University Hospital Ethics Committee of the Federal University of Ceará, Brazil.

Preparation of F0/60 ammonium sulfate fractions and isolation of lectins

For the preparation of F0/60 fractions and isolation of lectins from *B. seaforthii* and *B. triquetrum*, a previously described method (16) was used. The algae were thawed, rinsed with distilled water, ground to a fine powder under liquid nitrogen, stirred for 4 h with three volumes of 20 mM sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl, filtered through nylon tissue and centrifuged at 7,000 *g* for 30 min at 4ºC.

The supernatant was acidified, left to stand for 16 h at 6-8ºC, centrifuged, and adjusted to pH 7.0, followed by the addition of ammonium sulfate until 60% saturation. After 16 h, the precipitated proteins were recovered by centrifugation (F0/60), resuspended in distilled water, dialyzed and applied to a DEAE-cellulose column. The column was equilibrated and eluted in one step with 20 mM sodium phosphate buffer, pH 7.6, followed by elution with 1 M NaCl in the same buffer. Active fractions (PI-DEAE) from both species of algae did not adsorb to the column, and were pooled and rechromatographed on the same column. Lectin fractions were combined, dialyzed against water, and lyophilized (PI-DEAE). The procedure for the isolation of *B. seaforthii* and *B. triquetrum* lectins is a simple and reproducible one, and provides an average yield of 10-15 mg protein/kg of fresh algae. The preparations (PI-DEAE) were submitted to SDS-PAGE, presenting single bands. These lectins were shown to contain on average 7% (*B. seaforthii*) and 50% (*B. triquetrum*) carbohydrates (23). The solubility in water of the carbohydrate fraction of *B. seaforthii* was about 50% and that of *B. triquetrum* was more than 90%. The crude protein fraction (F0/60) and lectins (PI-DEAE) were dissolved in distilled water before use.

Preparation of the carbohydrate fractions

Collected fresh algae were washed with distilled water and dried in an oven at 35ºC. Twenty grams of dried algae cut into small pieces were suspended in 1% sodium hypochlorite and washed with distilled water. The dried tissue was then suspended in 500 ml of 100 mM sodium acetate buffer, pH 6.0, containing 5 mM cysteine, 5 mM EDTA and 0.4% (w/v) papain, and incubated at 60ºC for 24 h. The incubation mixture was filtered, and the supernatant saved. The residue was washed with distilled water and filtered, and the two filtrates were combined.

Sulfated polysaccharides were precipitated with 17 ml 10% cetylpyridinium chloride. After standing at room temperature for 48 h, the mixture was centrifuged at 2,500 *g* for 20 min at 4ºC. The sulfated polysaccharides in the pellet were washed with 600 ml of 0.05% cetylpyridinium chloride and centrifuged (2,500 *g*, 20 min). The remaining pellet was then dissolved with 150 ml 2 M NaCl:ethanol (100:15, v/v) and precipitated with 300 ml absolute ethanol. After 24 h at 4ºC, the precipitate was collected by centrifugation (2,500 *g*, 20 min at 4ºC), washed with 300 ml 80% (v/v) ethanol, followed by the same volume of absolute ethanol and finally by the same volume of acetone. The final precipitate was dried at room temperature. The yield was 21 and 12% for *B. seaforthii* and *B. triquetrum*, respectively. Carbohydrate fractions were dissolved in distilled water before use.

Evaluation of antinociceptive activity

The writhing, formalin, and hot-plate tests were applied to mice. In all cases, the crude protein fraction F0/60, lectins (PI-DEAE) or carbohydrate fractions were dissolved in distilled water, which was also used in an equivalent volume in controls.

Writhing test. Male and female Swiss mice were treated with fractions F0/60 (0.5 to 10 mg/kg, *ip*, and 1 to 40 mg/kg, *po*), lectins (0.5 to 5 mg/kg, *ip*, and 0.5 to 10 mg/kg, *po*), carbohydrate fractions (0.1 to 5 mg/kg, *ip*), 30 min (for intraperitoneal administration, *ip*) or 60 min (for oral administration, *po*) before receiving a 0.6% acetic acid injection (10 ml/kg, *ip*), and the number of contractions was recorded after 5 min for 20 min (24). Controls received the same volume of distilled water. In another set of experiments, the writhing test was performed with lectins in the absence and presence of avidin (1 mg/kg, *ip*) or D-mannose (1 mg/kg, *ip*). In this case, avidin or mannose alone or previously mixed with the lectin was heated at 37ºC for 1 h before administration. The test was then performed 30 min later as described above.

Formalin test. Twenty microliters of 1% formalin was injected into the right hind paw of male Swiss mice (25 g), and the licking time was recorded during the first 5 min (1st phase) and after 20 min (2nd phase) for 5 min each time. Animals were pretreated with F0/60 (0.5 to 10 mg/kg, *ip*, or 10 to 20 mg/kg, *po*) or lectins (1 to 5 mg/kg, *ip*, or 5 to 20 mg/kg, *po*), 30 or 60 min for intraperitoneal or oral administration, respectively. Naloxone (2 mg/kg, *sc*), an opioid antagonist, was injected 15 min before F0/60, lectins, or morphine (5 mg/kg, *ip*) used as standard (25,26).

Hot-plate test. Male Swiss mice (25 g) were preselected according to their reactions to thermal stimuli (jumping or licking of hind limbs when placed on a hot plate at 55ºC). The cut-off point was set at 30 s, and animals presenting a higher latency time were discarded (27). Latency times were recorded immediately before and 30, 60 and 90 min after F0/60 (10 to 20 mg/kg, *ip*) or lectin (5 to 10 mg/kg, *ip*, or 10 mg/kg, *po*) administration. To detect a possible involvement of the opioid system, animals were pretreated with naloxone (2 mg/kg, *sc*) 15 min before injection of the fractions, lectins, or morphine (5 mg/kg, *ip*) used as standard.

Statistical analysis

Data were analyzed by ANOVA and by the Dunnett test as the *post hoc* test, and considered significant at $P<0.05$.

Results

The results presented in Table 1 show that F0/60 (0.5 and 10 mg/kg, *ip*, and 10 and 40 mg/kg, *po*) and the lectin (0.5 and 1 mg/kg, *ip* and *po*) from *B. seaforthii* were equally potent in causing inhibition of the acetic acid-induced abdominal contractions in mice.

In both cases, the effect was greater after intraperitoneal (68 to 98% decreases) as compared to oral administration (44 to 74% decreases), and maximum inhibition was observed with the dose of 0.5 mg/kg of either F0/60 or lectin administered *ip*. A similar profile was observed after the administration of F0/60 and lectin from *B. triquetrum*. In this case, inhibition ranging from 65 to 88% was seen after *ip* administration of F0/60 or lectin (1 and 5 mg/kg). After oral administration, F0/60 (1 and 5 mg/kg) inhibited abdominal contractions by 64 and 78%, respectively, while *B. triquetrum* lectin (5 and 10 mg/kg) was somewhat less efficient (49 and 56% inhibition, respectively).

In the formalin test (Figure 1A and B), *ip* administration of F0/60 (0.5 and 1 mg/kg) from *B. seaforthii* caused a significant inhibition of the 2nd phase of the response only with the high dose. No effect was detected on the 1st phase. However, F0/60 was effective on both phases, after the oral administration of 10 and 20 mg/kg, producing inhibitions of 36 and 55% (1st phase), and 58 and 88% (2nd phase), respectively. The results obtained with *B. seaforthii* lectin were similar to those obtained with F0/60. Thus, after *ip* administration of 1 and 5 mg/kg, significant inhibitions were detected during the 2nd phase (34 and 73%, respectively). The oral administration was also effective although at higher doses (10 and 20 mg/kg). A similar pharmacological profile was observed with F0/60 and the lectin from *B. triquetrum*.

The formalin test was also performed in the absence and presence of naloxone, an opioid antagonist. The effect of morphine and both lectins was totally reversed by naloxone in the 1st phase. Although the effect of morphine was also reversed in the 2nd phase of the formalin test, 24% (*B. seaforthii*) and 30% (*B. triquetrum*) inhibition of the licking time remained after combination of the lectins with naloxone (Figure 2A and B).

The objective of the next experiment was to demonstrate the role of avidin and D-mannose in the antinociceptive effects of the lectins in the acetic acid-induced contractions in mice. While the antinociceptive activity of *B. seaforthii* lectin was unaltered after its administration with avidin, the effect was completely reversed after its administration with D-mannose. On the other hand, administration with avidin partially reversed the antinociceptive effect of *B. triquetrum* lectin alone. However, similarly to the lectin from *B. seaforthii*, the administration of the lectin from *B. triquetrum* with D-mannose also reversed its antinociceptive effect, and

Table 1. Effects of F0/60 and lectin fractions from *Bryothamnion seaforthii* (BS) and *B. triquetrum* (BT) on acetic acid-induced abdominal contractions in mice.

Group	No. of contractions/20 min	% Inhibition	
		Mean	Range
Control (vehicle)	42.8 ± 1.56 (32)	-	-
BS F0/60			
0.5 mg/kg, *ip*	6.0 ± 1.64 (13)*	86	82.8-89.4
10.0 mg/kg, *ip*	0.7 ± 0.34 (10)*	98	97.7-99.1
10.0 mg/kg, *po*	23.9 ± 2.39 (9)*	44	40.8-47.8
40.0 mg/kg, *po*	11.3 ± 1.64 (6)*	74	70.9-76.5
BS lectin			
0.5 mg/kg, *ip*	13.7 ± 3.05 (13)*	68	62.2-74.0
1.0 mg/kg, *ip*	9.8 ± 1.98 (7)*	77	73.4-81.1
0.5 mg/kg, *po*	15.8 ± 2.17 (18)*	63	59.5-67.0
1.0 mg/kg, *po*	20.0 ± 1.60 (11)*	53	51.3-55.3
BT F0/60			
1 mg/kg, *ip*	11.1 ± 2.77 (12)*	74	68.7-79.8
5 mg/kg, *ip*	5.2 ± 1.28 (15)*	88	85.4-90.5
1 mg/kg, *po*	15.3 ± 3.24 (10)*	64	58.3-70.6
5 mg/kg, *po*	9.5 ± 3.06 (8)*	78	71.6-84.5
BT lectin			
1 mg/kg, *ip*	15.0 ± 2.46 (18)*	65	60.6-69.6
5 mg/kg, *ip*	9.3 ± 1.97 (23)*	78	74.5-82.3
5 mg/kg, *po*	21.7 ± 3.76 (10)*	49	42.6-56.5
10 mg/kg, *po*	19.0 ± 3.49 (6)*	56	49.3-62.4

Values are reported as means ± SEM for the number of animals shown in parentheses. The data reported as range indicate the minimum and maximum values of the percentage of inhibition, indicating the dispersion of the mean of each group (No. of contractions/20 min).
*P<0.05 *vs* control (ANOVA and Dunnett as a *post hoc* test).

the number of abdominal contractions was equal to that observed with D-mannose (Table 2).

Figure 3A and B shows the effects of F0/60 and lectins in the hot-plate test. A significant increase (ranging from 29 to 78%) in the latency to respond to thermal stimuli was seen 30 min after F0/60 administration (20 mg/kg, *ip*), as compared to controls at the same time, and the effect lasted up to 90 min.

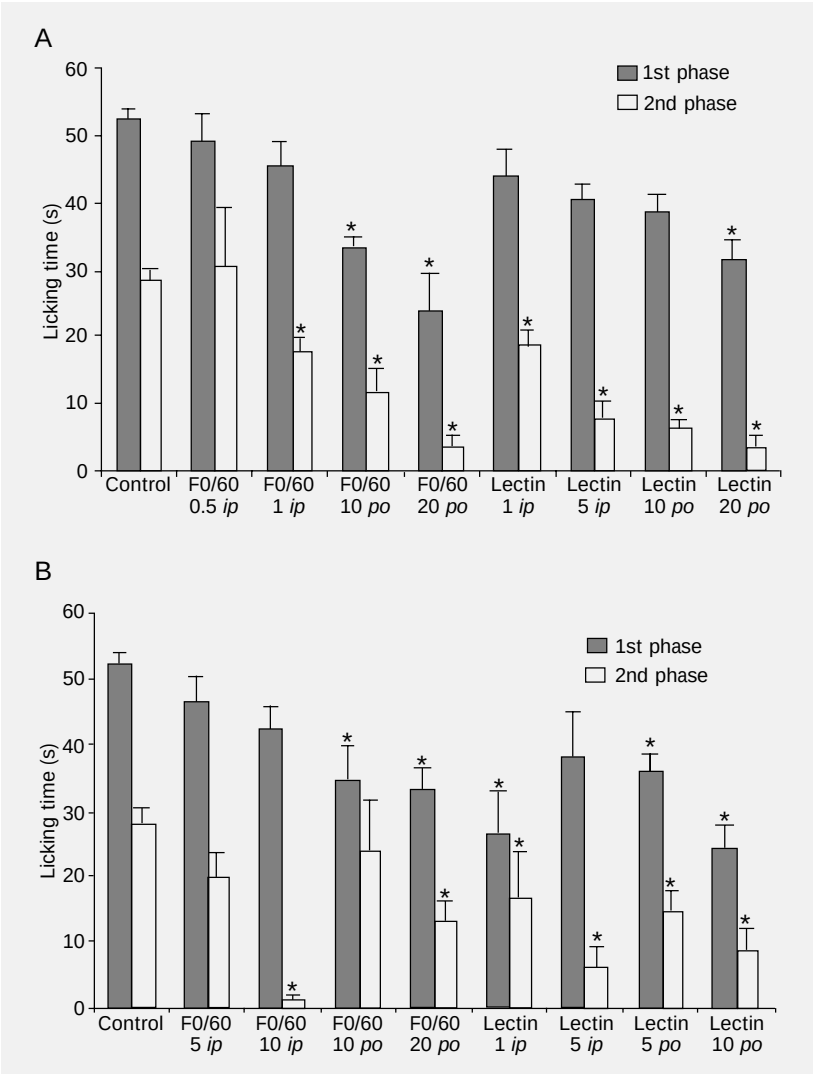

Figure 1. Effects of the crude F0/60 and lectin fractions from *Bryothamnion seaforthii* (A) and *B. triquetrum* (B) on the formalin test in mice. Licking time (± SEM) from 6 to 38 mice. Animals received fractions 30 (*ip*) or 60 (*po*) min before the intraplantar administration of 1% formalin, and measurements were done during the first 5 min (1st phase) and after 20 min (2nd phase) for 5 min each. Numbers under fractions indicate dose in mg/kg and route of administration. *P<0.05 *vs* control (ANOVA and Dunnett as a *post hoc* test).

A significant effect was also observed with *B. seaforthii* lectin after the administration of 10 mg/kg, *ip* (30, 50 and 58% increases at 30, 60 and 90 min, respectively) as compared to controls at the same times. Oral administration of *B. seaforthii* lectin (10 mg/kg, *po*) was also effective. Within the same dose range, *B. triquetrum* lectin presented similar effects. Morphine (5 mg/kg, *ip*), used as standard, increased latency time by 149, 171 and 88%, 30, 60 and 90 min after injection, respectively. Pretreatment with naloxone reversed the antinociception caused by F0/60 and lectin fractions.

Table 3 shows the effects of the carbohydrate fractions from *B. seaforthii* and *B. triquetrum* on the acetic acid-induced contractions. These fractions contain about 7% (*B. seaforthii*) and 50% (*B. triquetrum*) sulfated polysaccharides, as determined by the method of Dubois et al. (23), in addition to approximately 5% protein as contaminant. The yield based on algae dry weight was 21 and 12% for *B. seaforthii* and *B. triquetrum*, respectively. Interestingly, while the carbohydrates present in *B. seaforthii* were about 50% water soluble, those from *B. triquetrum* were 90% water soluble. In both cases, only water-soluble material was used, and the maximum effect (ranging from 63 to 72% inhibition) was observed with the dose of 5 mg/kg, *ip*.

Discussion

Although extracts from marine algae are known to possess several pharmacological properties, including anti-inflammatory activity (28), the present study showed for the first time, to our knowledge, the presence of antinociceptive activity in lectin and carbohydrate fractions from marine algae. Lectins are among the main constituents of algae and present a range of biological properties *in vitro*, including specific and nonspecific agglutination of erythrocytes and stimulation of lymphocyte transformation. Other reports

Table 2. Effect of avidin and D-mannose on the antinociceptive effects of lectins from *Bryothamnion seaforthii* (BS) and *B. triquetrum* (BT) measured as acetic acid-induced abdominal contractions in mice.

Group	Route of administration	No. of contractions
Control	Vehicle	32.8 ± 1.08 (25)
Avidin	*ip*	24.1 ± 2.60 (25)
BS lectin	*ip*	6.3 ± 0.97a (33)
	po	20.0 ± 1.60 (11)
BT lectin	*ip*	4.0 ± 1.93a (6)
	po	17.6 ± 1.96a (5)
BS lectin + avidin	*ip*	5.9 ± 1.13a,b (21)
	po	20.7 ± 2.14 (14)
BT lectin + avidin	*ip*	16.0 ± 1.37a,b (5)
	po	17.4 ± 4.82 (5)
D-mannose	*ip*	20.9 ± 1.82 (25)
BS lectin + D-mannose	*ip*	29.0 ± 3.94 (10)
	po	27.7 ± 1.63 (9)
BT lectin + D-mannose	*ip*	19.3 ± 1.55 (6)
	po	29.0 ± 4.58 (6)

Avidin and mannose alone or mixed with the lectin (1 mg/kg) were previously incubated at 37ºC 1 h before administration, and the test was performed as described in Methods. Values are reported as means ± SEM for the number of animals in parentheses.
a,b = P<0.05 *vs* control and lectin, respectively (ANOVA and Dunnett as a *post hoc* test).

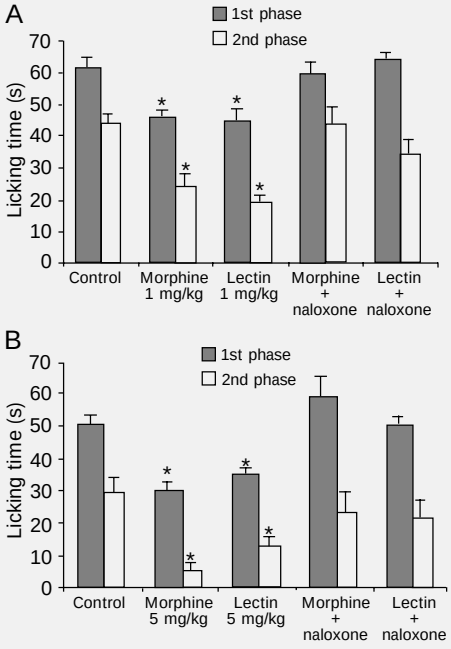

Figure 2. Role of the opioid system in the antinociceptive effects of lectins from *Bryothamnion seaforthii* (A) and *B. triquetrum* (B) in the formalin test in mice. Licking time (mean ± SEM) recorded for 5 to 16 mice. Animals received lectin fractions (1 and 5 mg/kg, *ip*) in the absence and presence of naloxone (2 mg/kg, *sc*, injected 15 min before the lectin), 30 (*ip*) or 60 (*po*) min before the intraplantar administration of 1% formalin solution. Morphine (5 mg/kg, *ip*) in the absence and presence of naloxone (2 mg/kg, *sc*, injected 15 min before morphine) was used as standard. Measurements were made during the first 5 min (1st phase) and after 20 min (2nd phase) for 5 min each time. *P<0.05 *vs* control (ANOVA and Dunnett as a *post hoc* test).

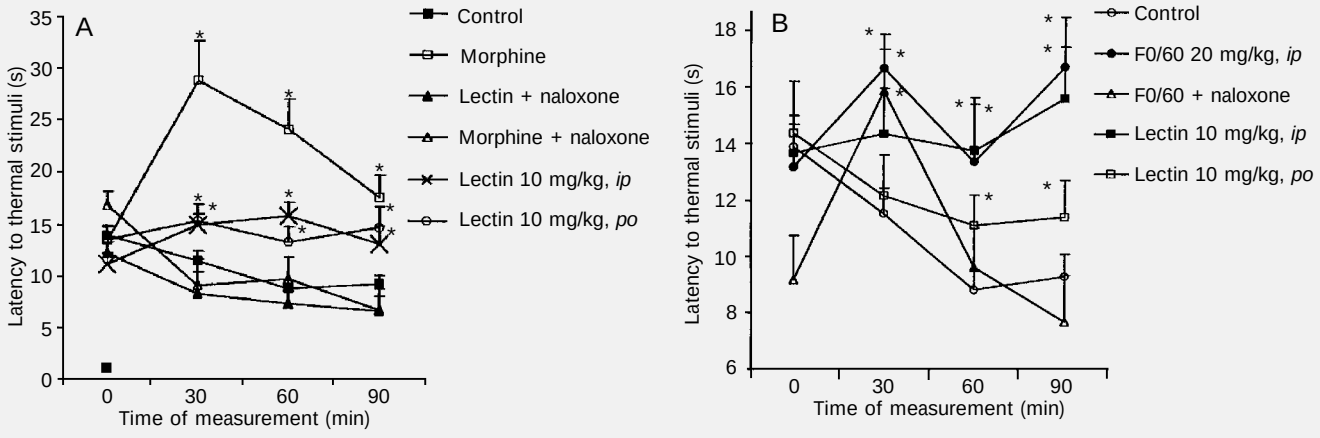

Figure 3. Possible involvement of the opioid system in the antinociceptive effects of F0/60 and lectin fractions from *Bryothamnion seaforthii* (A) and *B. triquetrum* (B) in the hot-plate test in mice. Latency to respond to thermal stimuli (mean ± SEM) recorded for 8 to 10 mice. Latency times were recorded immediately before and 30, 60, and 90 min after the administration of F0/60 or lectin fractions from *B. seaforthii* or *B. triquetrum*, in the absence or presence of naloxone (2 mg/kg, *sc*, injected 15 min before). Morphine (5 mg/kg, *ip*) in the absence or presence of naloxone (2 mg/kg, *sc*) was also used as standard. *P<0.05 *vs* control at the same time point (ANOVA and Dunnett as a *post hoc* test).

(29) have indicated that lectins possess neuromodulatory effects. They are also used as histochemical markers to study the distribution of glycoconjugates in mammalian tissues. Some years ago (13), a new histochemical reagent was developed utilizing colloidal gold coupled to a specific lectin from the green alga *Codium fragile* ssp *tomentosoides*. This lectin-gold conjugate binds to erythrocyte membranes from the human A1 blood group.

Besides hemagglutination activity, lectins from marine algae possess other actions such as the inhibition of human platelet aggregation (6). Lectins are proteins with sugar-binding subunits that recognize and bind to specific carbohydrates present on cell surfaces. The carbohydrate specificity of lectins has made them valuable for a variety of applications. Our study showed a potent antinociceptive activity present in crude fractions of *B. seaforthii* and *B. triquetrum*. Although more potent after *ip* administration, fractions were also active when administered orally.

Table 3. Effect of carbohydrate fractions from *Bryothamnion seaforthii* and *B. triquetrum* on the acetic acid-induced abdominal contractions in mice.

Group	No. of contractions/ 20 min	% Inhibition	
		Mean	Range
Control	27.0 ± 2.03 (32)	-	-
B. seaforthii			
0.1 mg/kg, *ip*	23.1 ± 5.07 (8)	14.4	2.9-27.9
1.0 mg/kg, *ip*	13.9 ± 1.53 (12)*	48.5	46.9-50.3
5.0 mg/kg, *ip*	10.0 ± 3.34 (8)*	63.0	54.2-73.2
B. triquetrum			
0.1 mg/kg, *ip*	23.4 ± 3.90 (7)	13.3	4.6-21.9
1.0 mg/kg, *ip*	9.5 ± 2.60 (8)*	64.8	58.3-72.4
5.0 mg/kg, *ip*	7.7 ± 1.32 (7)*	71.5	69.0-74.4

Values are reported as means ± SEM for the number of experiments in parentheses. The range shows the minimum and maximum values of the percent inhibition, indicating the dispersion of the mean of each group (No. of contractions/20 min). Carbohydrates were extracted as described in Methods. *P<0.05 compared to control (one-way ANOVA and Kruskal-Wallis as a *post hoc* test).

The amount of lectins present in crude fractions of algae was approximately 10%. This means that the effect observed with 10 mg/kg of the crude extract would correspond to that demonstrated with 1 mg/kg of isolated lectins. Surprisingly, the lectins from the two species were not equipotent on a weight basis. Naloxone at least partially reversed the antinociceptive effect of the lectins, indicating the involvement of the opioid system.

Another interesting point was the total blockade of the antinociceptive effect of *B. seaforthii* by D-mannose but not by avidin. This suggests that carbohydrate-binding domains are important for the biological effect of this lectin. In the case of the lectin from *B. triquetrum*, while pretreatment with avidin partially blocked its antinociceptive effect, a total blockade was also observed in the presence of D-mannose.

These results suggest that avidin interferes with the effect of the *B. triquetrum* lectin by interacting with the active sites of the lectin. It can be speculated that these lectins need larger and more complex molecules such as those of avidin in order to produce hemagglutination. It is known that D-mannose does not inhibit the hemagglutinating activity of either lectin. However, the bioavailability of D-mannose residues inside the avidin structure may be a requirement for the lectin-avidin interaction to occur.

Similar results have been reported in the literature for other lectins from marine algae (30). The blockade by avidin of the carbohydrate-binding sites of the *B. triquetrum* lectin suggests the involvement of other carbohydrate residues in the glycoprotein molecule besides D-mannose, which are equally important for the development of its antinociceptive activity. However, in both cases the most important role is played by D-mannose, since this carbohydrate totally blocked the antinociceptive effects seen with lectins from both *B. seaforthii* and *B. tri-*

quetrum.

Both lectins also presented a central effect as revealed by the hot-plate test in mice. Similarly to the results observed with the formalin test, these effects were also reversed by naloxone. As far as we know, this is the first report showing the presence of a central as well as a peripheral analgesic activity in both crude extracts and isolated lectins from two species of red marine algae. The effects were observed at low doses, a fact that makes these lectins potential candidates as analgesic drugs.

On the other hand, sulfated polysaccharides comprise a complex group of macromolecules with a wide range of biological properties such as anticoagulant and antithrombotic ones, which are present in several marine organisms including brown (31) and red algae (20). Recently, Farias et al. (32) characterized the structure of a sulfated p-galactan from the red alga *Botryocladia occidentalis* which presented a potent anticoagulant activity due to enhanced inhibition of thrombin and factor Xa by antithrombin and/or heparin cofactor II.

Recently, we showed (33) that the inactivation of lectins from the F0/60 fraction by its treatment with SDS plus ß-mercaptoethanol did not interfere with the pharmacological effect present in this fraction, indicating that protein molecules are not responsible for the antinociceptive activity. Such data suggest that carbohydrates present in the lectins, and probably bound to them, are responsible for the antinociceptive effect of both *B. triquetrum* and *B. seaforthii* fractions. In addition, considering that the protein content of the carbohydrate fractions is very low (not higher than 10%), it means that the dose of 5 mg/kg would correspond only to 500 µg of protein at most, which is a low dose probably not contributing to the effect seen in the carbohydrate fraction. Preliminary spectroscopy experiments indicate that these carbohydrate components are sulfated polysaccharides.

We conclude that sulfated carbohydrates present in the lectin fractions from *B. seaforthii* and *B. triquetrum* are probably responsible for the antinociceptive effects of the algae.

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