Morphological and functional study of the marginal sphincter of the sea anemones *Phymactis clematis* and *Aulactinia marplatensis* from intertidal of Mar del Plata, Argentina

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ABSTRACT. It was made the characterization of marginal sphincter to the species *Phymactis clematis* (Drayton *in* Dana, 1849) and *Aulactinia marplatensis* (Zamponi, 1977), from intertidal ecosystem through their morphogical and functional study. The species *P. clematis* has a circumscript sphincter of palmate type. This muscle is constituted by a mesogloeal axis and several mesogloeal subaxes. Axis as well as subaxes give a support to the endoderm which border is smooth. *Aulactinia marplatensis* has a circunscript sphincter pinnate type. The axis has a truncated cone shape while in *P. clematis* the shape is cylindrical on its origin and it is bifurcated at the end. Both species experiments were carried out using the isolated muscles. They were stimulated at increasing KCl concentrations ranging from 20 to 200 mM. The results were analysed in the form of dose-response curves expressed in tension in grams force vs concentration. Contractil force increases in a sigmoid form to increasing KCl concentrations. The correlation between morphology and function and the differences shown in both species would be related to their intertidal distribution.

KEYWORDS. Actiniaria, marginal sphincter, morphology, contractility.

RESUMEN. Estudio morfológico y funcional del esfínter marginal de las anémonas de mar *Phymactis clematis* y *Aulactinia marplatensis* del intermareal de Mar del Plata, Argentina. Se realizó la caracterización de las anémonas de mar *Phymactis clematis* (Drayton *in* Dana, 1849) y *Aulactinia marplatensis* (Zamponi, 1977) del ecosistema intermareal mediante estudio morfológico y funcional. La especie *P. clematis* tiene un esfínter circunscripto de tipo palmado. Este músculo está constituido por un eje mesogloeal y varios subejes mesogloeales. Tanto el eje como los subejes dan soporte al endodermo cuyo borde es liso. La especie *A. marplatensis* tiene un esfínter circunscripto de tipo pinnado. El eje tiene forma de cono truncado mientras que en *P. clematis* es cilíndrico en su origen bifurcándose en su parte final. Los experimentos fueron llevados a cabo usando el músculo aislado de ambas especies. Estos fueron estimulados a concentraciones crecientes de KCl en un rango de 20 a 200 mM. Los resultados fueron analizados mediante curvas dosis-respuesta expresados en tensión en gramos fuerza vs concentración. La fuerza contráctil se incrementó en forma sigmoidea. La correlación entre la morfología y función y las diferencias mostradas en ambas especies podrían estar relacionadas con su distribución en el intertidal.

PALABRAS CLAVE. Actiniaria, esfínter marginal, morfología, contractilidad.

The column of sea anemone is constituted of muscle fibers and inmediately or a short distance below the margin, the circular muscle sheet is concentrated into a special band: the marginal sphincter (Stephenson, 1928). This structure presents characteristics of smooth muscle (Patronelli *et al*., 1998). The sphincter closes the aperture at the top of the column when the disc and tentacles have been retracted**.** According to the disposition of mesogloeal axis and subaxes the sphincter can be classified as circumscript, circumscript-diffuse or diffuse (Manuel, 1981). These morphological types may present structural and physiological differences depending on the species located in intertidal or depth environment (Patronelli *et al*., 2004). In the intertidal ecosystem of Mar del Plata (Argentina), sea anemones are distributed in protected, partially protected or exposed areas. The different conditions in these areas such as pressure of the water column or dashing waves promote different responses in the sphincter (Patronelli *et al*., 1987).

In Mar del Plata coast two species like *Phymactis clematis* (Drayton *in* Dana, 1849) and *Aulactinia marplatensis* (Zamponi, 1977) occur widely with a great resistance to dominant conditions (Acuña *et al*., 1995, 1998).

The aim of this work is to make a comparative study of the marginal sphincter of both species by morphological and functional analysis.

MATERIAL AND METHODS

Specimens of the sea anemones *Phymactis clematis* and *Aulactinia marplatensis*, were collected from the middle littoral in the rocky zone of Punta Cantera, Mar del Plata (38°05'S and 57°38'W), Argentina. The samples, all around the same size (30 mm in basal diameter), to avoid another variable, were caught in autumn-winter, period whereon the responses are optimal to mechanical stimuli, discarding those specimens with typical spawning position (Patronelli *et al*., 2002). Both species were collected in the same area under the same environmental conditions and in different positions on the substrate. *Aulactinia marplatensis* is distributed on the vertical walls on the rocks, unlike *P. clematis* whose distribution is extended over plane and horizontal hollows. The organisms were maintained at room temperature in an aquarium with decanted and aerated sea water. They were used between one and seven days after collection.

Morphological study. The sphincter analysis was made by dissection techniques through longitudinal plane of the organism and it was placed in a Petri dish with fixed solution (formaldehyde solution neutralizated 40% or absolute alcoholic solution). The sphincter was observed upon stereoscopic microscope; by objectives of different resolutions the following meristic dates were measured: length and width of sphincter, of mesogloeal axis and mesogloeal subaxes.

Physiological study. Sphincter extraction and preparation were carried out according to the techniques described by PATRONELLI *et al.*, 1987. The variations of tension were measured by a force transducer connected to a polygraph. The muscles were stimulated at different KCl concentrations (20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 mM) and their responses were analysed in the form of dose-response curves expressed in tension in grams force (gr) vs concentration. Data are presented as average values ± standard error of experiments. Statistical analysis was performed using the Student's t test. *P* values < 0.05 were considered statistically different.

RESULTS

Morphological study. The species *P. clematis* has a circumscript sphincter of palmate type (Fig. 1) which is between 1.06 to 1.67 mm long and between 0.76 to 1.21 mm wide. This muscle is constituted by a mesogloeal axis and several mesogloeal subaxes. Axis as well as subaxes give a support to the endoderm which border is smooth. The mesogloeal axis is between 0.30 to 1.06 mm long and between 0.15 to 0.60 mm wide. It is possible to count a number of more than 5 subaxes (6-8) which dimensions are between 0.07 to 0.91 mm long and 0.07 to 0.30 mm wide. The species *A. marplatensis* has a circumscript sphincter pinnate type (Fig. 2) which is between 1.21 to 1.76 mm long and between 1.06 to 1.36 mm wide. This muscle is constituted by a mesogloeal axis and many subaxes. Axis and subaxes give a support to endoderm of sphincter which border is folded. The mesogloeal axis is 0.30 to 0.76 mm long while its width is between 0.60 to 0.91 mm (n= 25). This axis has a truncated cone shape while in *P. clematis* the shape is cylindrical on its origin and bifurcated at the end. The mesogloeal subaxes are numerous which number is more than 9 and their dimensions are between 0.45 to 1.21mm long and 0.15 mm wide (n= 25).

Physiological study. Contractil force increases in a sigmoid form to increasing KCl concentrations (Fig. 3, 4). In *P. clematis* the maximal developed force obtained with 200 mM KCl was 2.68 gr ± 0.20 gr (n=22). In *A. marplatensis* maximal response reached was 1.33 gr ± 0.24 gr (n=20) obtained with 180 mM KCl (Fig. 5). With 200 mM KCl the response was minor which value was 1.04 gr ± 0.10 gr ($p < 0.01$). The contraction was more sustained and the relaxation period was longer.

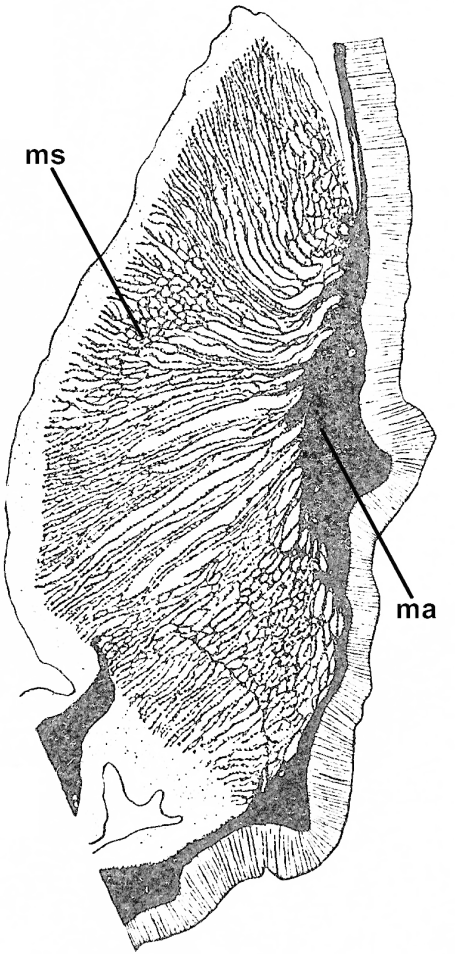

Figure 1. Circumscript sphincter of palmate type of *Phymactis clematis* (Drayton *in* Dana, 1849) (ma, mesogloeal axis; ms, mesogloeal subaxis).

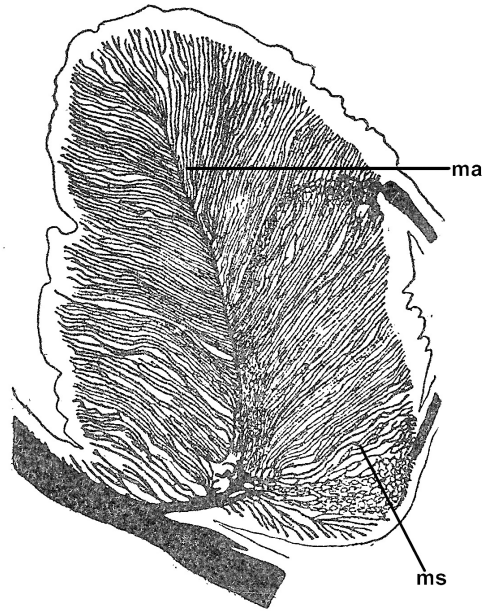

Figure 2. Circumscript sphincter of pinnate type of *Aulactinia marplatensis* (Zamponi, 1977) (ma, mesogloeal axis; ms, mesogloeal subaxis.

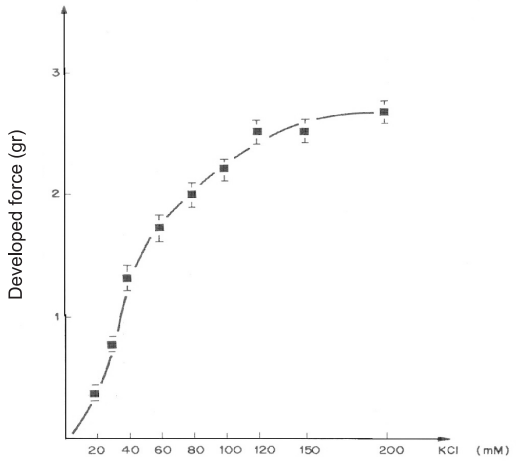

Figure 3. Dose-response curves to increasing concentrations of KCl (mM) in the marginal sphincter of *Phymactis clematis* (Drayton *in* Dana, 1849) (bars represent standard error; gr, grams force; n = 22).

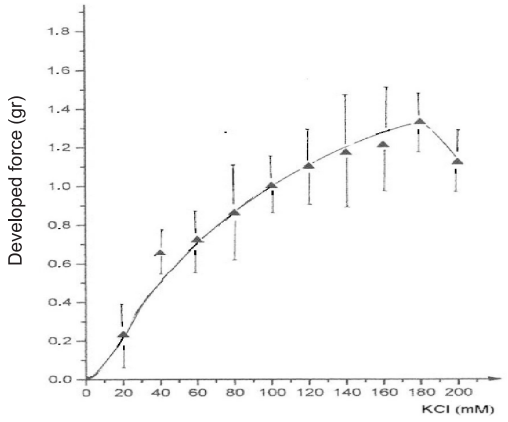

Figure 4. Dose-response curves to increasing concentrations of KCl (mM) in the marginal sphincter of *Aulactinia marplatensis* (Zamponi, 1977) (bars represent standard error; gr, grams force; n = 20).

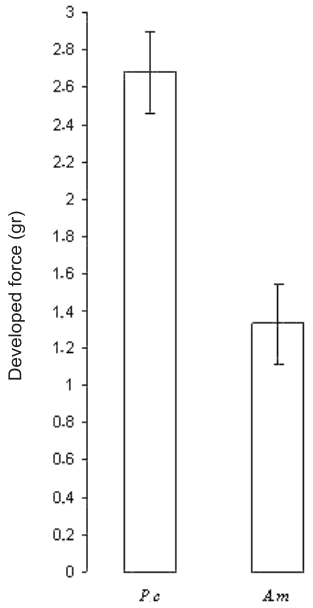

Figure 5. Maximal developed force obtained by stimulation with 200 mM KCl in *Phymactis clematis* (Drayton *in* Dana, 1849) (*Pc*) and 180 mM KCl in *Aulactinia marplatensis* (Zamponi, 1977) (*Am*) (bars represent standard error; gr, grams force).

DISCUSSION

The species *Phymactis clematis* has a smooth edge sphincter of the circumscript type, which presents constant physiological activity even if it is exposed to environmental dissecation (Patronelli *et al*., 1999). *Aulactinia marplatensis* is found in the same study area and its sphincter presents folded edges, making a stronger structure. This morphological adaptation might be related to the localization of the species since that it is distributed on the vertical walls on the rocks, unlike *P. clematis* whose distribution is extended over plane and horizontal hollows where pressure of water is second-rate.

The analysis of sphincter contractility in both species, showed that *P. clematis* developed a higher contractile force than *A. marplatensis* for all KCl concentrations. The contractile force to 200 mM KCl was minor. This event might be related to the higher depolarization which leads to decreasing calcium ions influx, because these ions positively charge the intracellular medium repulsing more calcium ions from entering (Patronelli *et al*., 2000).

Comparing sphincters of both species, morphological and functional differences were found, which might be related with their distribution in intertidal. Sphincters of *P. clematis* populations distributed in exposed areas present great differences compared to specimens from protected environments. The ones which comes from exposed environments have developed sphincters adapted to resist the continuous dashing of waves. Physiological studies correlated well with morphological observations (Patronelli *et al*., 1987).

Instead, in *A. marplatensis* there was no correlation between morphology and function in the sphincters, as it was expected to obtain a higher contractile force, because these are stronger and have a pinnate disposition. These results suggest that *A. marplatensis*, due to its localization is subjected to direct wave impact, and its sphincter instead of developing a higher contractile force than in *P. clematis* uses, as an adaptative strategy, a sustained contraction and a longer relaxation.

As is it described for molluscan smooth muscles, the sphincter maintains a stretch-resistance state for long periods. This phenomenon suggests us that contraction of this smooth muscle produces a "catch" tension. This property is present for the so called "paramyosine muscles" (Kryvi, 1975) among which may be included these sphincters because under biochemical analysis in our laboratory, we have identified a protein with a molecular weight of 106 KDa (data non published) which might correspond to paramyosine. This protein is typical in the thick filament of invertebrates. The sphincter of *A. marplatensis* might present more quantity of this protein than in *P. clematis*, which would allow it to combine the ability to provide relatively free movement activity and a firm position for sustained periods.

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