Marine and Freshwater Behaviour and Physiology
December 2005; 38(4): 269–274

Taylor & Francis
Taylor & Francis Group

Calcium antagonists inhibit the discharge of cnidae in response to electrical stimulation in the giant tropical sea anemone *Heteractis crispa* Ehrenberger (Anthozoa)

TYGE DAHL HERMANSEN[1], MICHAEL ARVEDLUND[2], & G. CURT FIEDLER[3]

[1]*The Quality of Life Research Center, Copenhagen, Denmark,* [2]*Tropical Biosphere Research Center, University of the Ryukyus, Sesoko Station, Okinawa, Japan, and*
[3]*University of Maryland, University College, Asia Division, Kanagawa, Japan*

(Received 1 August 2005; in final form 11 October 2005)

Abstract
The mechanisms involved in cnidocyst discharge among cnidarians are not well understood. In this study we examined the discharge mechanism in an anthozoan. We examined the effect of the Ca^{2+} antagonists (Mg^{2+}, Co^{2+} and La^{3+}), a Ca^{2+} agonist (Sr^{2+}), and two cations (Ca^{2+} and K^{+}) upon cnidocyst discharge, using electrical stimulation applied directly to sea anemone tentacles. In our experiment we used the sea anemone *Heteractis crispa* (Class: Anthozoa, Family: Stichodactylidae), a known host for anemonefishes, as a model organism. Our results show that the Ca^{2+} antagonists effectively inhibit, while all the other cations enhance the cnidocyst discharge of *H. crispa*. These results are comparable to those from a similar study on the hydrozoan, *Hydra vulgaris*, which also used the electrostimulation method. Our results demonstrate for the first time that the discharge mechanisms in Anthozoa and Hydrozoa are comparable, and support existing hypotheses concerning the importance of Ca^{2+} in cnidocyst discharge.

Keywords: *Cnidaria, Anthozoa, cnidocyst, discharge, calcium, electrical stimulation*

Introduction

The cnidocyte is a specialized cell that secretes a cnidocyst (Lenhoff and Hessinger 1988). A lid on the top of the cnidocyst opens during discharge, and the cnidocyst is ejected in a movement in which it is inverted (Tardent 1988) and expels its contents. Cnidocysts and cnidocyst discharge among various cnidarians have been objects of study for many years (see e.g., Glaser 1909, Mariscal 1974, 1984, Lenhoff and Hessinger 1988, Shick 1991, Ozbek et al. 2002). Investigations of cnidocysts via mechanical and chemical stimulation

Correspondence: Tyge Dahl Hermansen, The Quality of Life Research Center, Teglgårdstræde 4-8, DK-1452 Copenhagen K, Denmark. Tel.: +45 33141113. Fax: +45 33141123. E-mail: Tyge.dahl.hermansen@a.cirque.tv; TD@livskvalitet.org

ISSN 1023–6244 print/ISSN 1029–0362 online/05/040269–274
DOI: 10.1080/10236240500479768

have been carried out in several experiments. For example, Santoro and Salleo (1991) examined the effect of La^{3+} and Co^{2+} on acontia cnidocysts of the sea anemone *Aiptasia mutabilis* using flowing test solutions; Blanquet (1970) used changing concentrations of agents and varying pH values to determine effects of Mg^{2+}, and other agents on acontia cnidocysts of the sea anemone *Aiptasia pallida*. Unfortunately, the methodology in these experiments is unclear and difficult to standardize with other study organisms. In contrast, the electrostimulation method used by Gitter et al. (1993, 1994) on *H. vulgaris* is very easily adapted to other cnidarian species and is used in our study.

Electrical stimulation has been used in a number of published studies on cnidae (e.g., Smith et al. 1974, Holstein and Tardent 1984, Aerne et al. 1991, Gitter et al. 1993, 1994). Gitter et al. (1993) developed a method to investigate *H. vulgaris* tentacle nematocyst discharge by applying electrical stimuli directly to the tentacles with two electrodes. They isolated a tentacle in a chamber containing a water bath including the dissolved agents they wished to use as treatment stimuli. During electrical stimulation of the isolated tentacles they closely observed the results through a microscope. Using this method, Gitter et al. (1994) found that relatively moderate Mg^{2+}, Co^{2+} and La^{3+} concentrations inhibit nematocyst discharge in *H. vulgaris*.

Using the same cnidae inhibitors and stimulators as Gitter et al. (1994), we conducted a comparative study using the sea anemone *H. crispa*, adapting their method to the much larger tentacles of our study species. We successfully applied this method to anthozoans and our data with the sea anemone is similar to that described for *Hydra*. We show that relatively moderate concentrations of the Ca^{2+} antagonists Mg^{2+}, Co^{2+} and La^{2+} enhance cnidae discharge, whereas the Ca^{2+} agonist Sr^{2+}, and K^{+} have the opposite effect. To our knowledge, this is the first such comparison between nematocyst discharge in Hydrozoa and Anthozoa.

Materials and methods

Healthy specimens of the sea anemone *H. crispa*, imported from Indonesia, were purchased from pet shops in Copenhagen, Denmark, and kept in aquaria at 24–27°C, illuminated with a Philips daylight tube light 2×36 watt, on a 12L : 12D light regime. All aquaria were aerated and equipped with artificial seawater and an under-gravel filter.

The experimental protocol was a modification of the method described by Gitter et al. (1993). A transparent PVC vessel (80 mm × 50 mm × 8 mm) and two stimulus electrodes were connected to a pulse generator (August Krogh Institute), and an oscilloscope (HAMEG 100 MHz Analog/Digital HM 1007). The silver electrodes were 50 mm long, 2 mm in diameter and mounted in a wooden shaft (Figure 1A). The vessel was placed under a Zeiss 20× stereomicroscope, then filled with seawater and appropriate solutes for the various ion treatments. A tentacle from *H. crispa* (Figure 1B) was isolated using a thread loop, excised from the body with a pair of scissors, and placed in the vessel. The following salts (at 8 and 32 mM) were used for experimental treatments: $CaCl_2$, KCl, $SrCl_2$, $MgCl_2$, $CoCl_2$ and $LaCl_3$.

Cnidocyst discharge was provoked by touching the tentacles with electrodes and applying an electrical stimulus using the pulse generator. We found it necessary to touch individual tentacles with both electrodes to get cnidocyst discharge. A millimeter scale was fastened under the bottom of the transparent vessel, and the electrodes were placed on opposite sides of the tentacle at a point where it was 5 mm in diameter. Electrical impulses were supplied as one electrical impulse with duration of 1 ms. Electrical amplitude was measured in volts and the electrical fields for each discharge are given in $V\,cm^{-1}$.

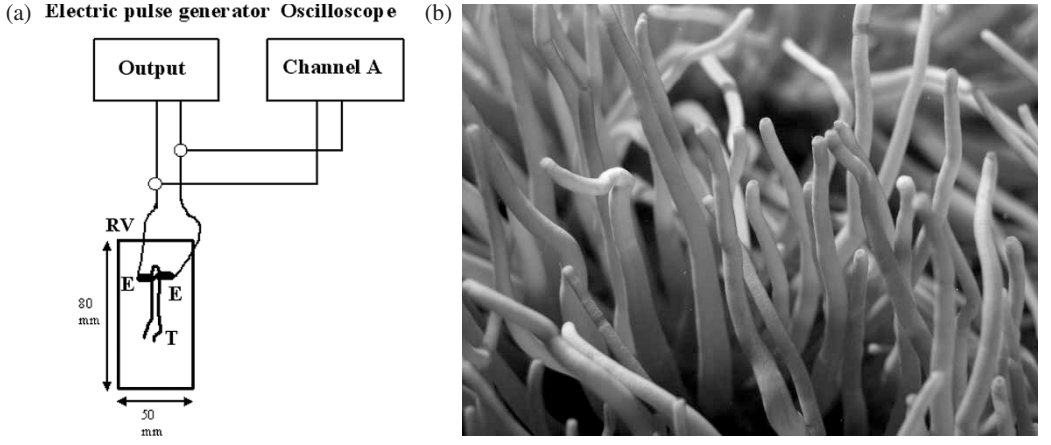

Figure 1. (a) Experimental setup for electric stimulation of cnidae discharge. Under a light microscope (not shown), a single isolated *H. crispa* tentacle (T) was placed with an electrode (E) arranged on each side, in a research vessel (RV; 80 mm length × 50 mm width × 8 mm height) containing seawater. The electrodes were composed of silver, measured 50 mm long, 2 mm in diameter, and were mounted in a wooden shaft. The figure is not drawn to scale. (b) Close-up of a *H. crispa* sea anemone showing the delicate tentacles from a shallow coral reef in southern Japan, at 5 m depth (M. Arvedlund).

Table I. ANOVA table for voltage.

	DF	Sum of square	Mean square	F-value	*p*-value	Lambda	Power
Voltage category	15	302.446	20.163	164.709	<0.0001	2470.630	1.000

For the control treatment, $n = 12$, and for all other treatments, $n = 6$. All molar concentrations are indicated as the amount added to the actual molar concentrations of the respective ions already present in seawater. Control treatments used seawater without the addition of extra ions.

During each experimental replicate, we recorded the minimum voltage needed to discharge cnidocysts at the electrodes. Hence, inhibition of cnidocyst discharge is indicated by voltage levels higher than, and discharge enhancement by voltage levels lower than control values. Variance of means among treatments was compared with a one-way ANOVA test (Table I) and then significant differences between treatments were determined using a Fisher's PLSD post hoc test (for significant *p*-values see Figure 2).

Results and discussion

Our data show that test solutions with Ca^{2+} antagonists (La^{3+}, Mg^{2+}, Co^{2+}) require higher voltages (Table I lists ANOVA results for voltage) for cnidocyst discharge than for control, agonist (Sr^{2+}), Ca^{2+}, or K^{+} treatments (Figure 2). However, this effect was not uniform among the antagonists and was concentration dependent. The 8 mM Mg^{2+} and Co^{2+} treatments threshold voltages were almost the same as the control (6.60 V), while the 32 mM treatments showed significantly higher thresholds (7.64 and 8.56 V cm^{-1}; $p = 0.0124$ and $p < 0.0001$, respectively). The 8 and 32 mM La^{3+} treatments displayed the highest voltage thresholds (12.76 and 13.00 V cm^{-1}, respectively) and were significantly greater than for all

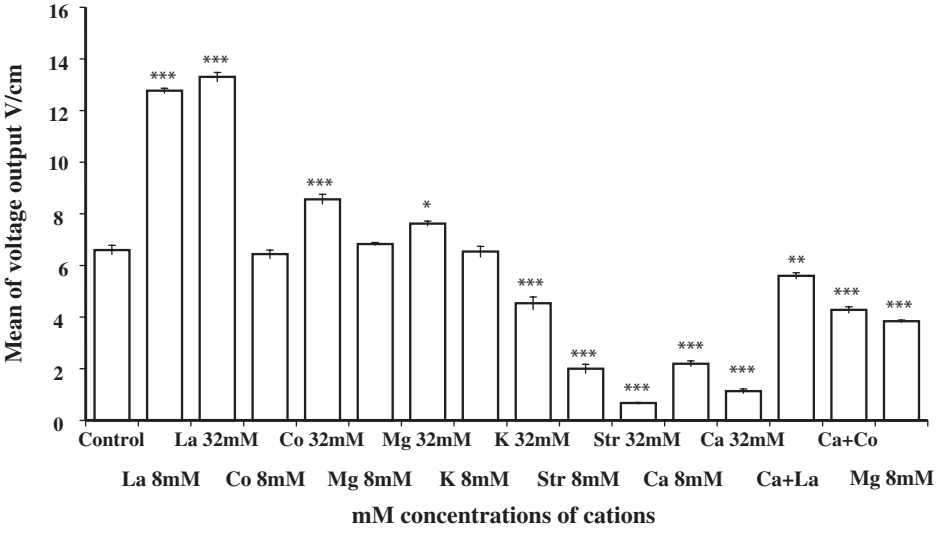

Figure 2. Threshold cnidocyst discharge field strength for treatments with various dissolved cations in seawater. Single cation treatments (at 8 mM and 32 mM) include: La^{3+}, Co^{2+}, and Mg^{2+} (Ca^{2+} antagonists); K^{+}, Sr^{2+} (a Ca^{2+} agonist) and Ca^{2+}. Paired cation treatments include: Ca + La, Ca + Co, and Ca + Mg, each at 8 mM; these show the competitive interaction (if any) between calcium and its antagonists. The 8 mM and 32 mM La^{3+} treatments displayed the highest voltage thresholds (12.76 and 13.00 V cm^{-1}, respectively) and were significantly greater than for all other treatments ($p < 0.0001$). However, the 8 mM Mg^{2+} and Co^{2+} treatments threshold voltages were almost the same as the control (6.60 V), while the 32 mM treatments showed significantly higher thresholds (7.64 and 8.56 V cm^{-1}; $p = 0.0124$ & $p < 0.0001$, respectively). The control treatment used unmodified seawater. For all cation treatments, $n = 6$; for the control treatment, $n = 12$ replicates. Data indicate the mean threshold voltage needed for discharge. Error bars indicate standard errors of the mean. Stars above bars are used to indicate treatments which are significantly different from the control (Fisher's PLSD): $*p = 0.0124$, $**p = 0.0079$, $***p < 0.0001$.

other treatments ($p < 0.0001$). La^{3+} was clearly the strongest inhibitor, perhaps too strong to be explained by surface charge neutralization of the cell membrane. K^{+} enhanced cnidocyst discharge only slightly less than Ca^{2+}. These results show the same inhibition/stimulation pattern as for cation treatments as were observed in the hydra, *H. vulgaris* (Gitter et al. 1994). Figure 2 illustrates the effect of the cations compared to the control. It displays the same pattern as observed in the hydra (Gitter et al. 1994) where La^{3+} shows the highest inhibition, followed by Co^{2+}, then Mg^{2+}. Similarly, Ca^{2+} enhances cnidocyst discharge more than K^{+} (showing a K^{+} induced depolarization in *H. vulgaris*), while the Ca^{2+} effect is mimicked by Sr^{2+}. Our results agree with the findings of Santoro and Salleo (1991), who examined the effect of La^{3+} and Co^{2+} on acontial cnidocysts of the sea anemone *Aiptasia mutabilis*, and found that both hampered discharge. Blanquet (1970) tested the effect of Mg^{2+} on acontial cnidocysts of the sea anemone *Aiptasia pallida*, but found that Mg^{2+} inhibited discharge, which is counter to our results and those of Gitter et al. (1994) as well as Santoro and Salleo (1991).

Strontium is known to substitute for calcium and pass through Ca^{2+} channels (Gitter et al. 1994). We show that the Sr^{2+} and Ca^{2+} treatments have significantly lower ($p < 0.0001$) thresholds than all other treatments (Sr^{2+}: 0.66 and 2.00 V cm^{-1}; Ca^{2+}: 1.14 and 2.20 V cm^{-1} for 8 mM and 32 mM respectively), but do not differ from each other at

their respective concentrations. This is also the same pattern as found by Gitter et al. (1994) for the discharge of *Hydra* nematocysts.

Treatments combining Ca^{2+} and the three antagonists (Mg^{2+}, Co^{2+} and La^{3+}) displayed (Figure 2) significantly lower voltage thresholds than the control ($5.50\,V\,cm^{-1}$, $p = 0.008$; $3.90\,V\,cm^{-1}$, $p < 0.0001$; $2.13\,V$, $p < 0.0001$, respectively). However, all three antagonists + Ca^{2+} treatments had higher threshold voltages than both the 8 mM and 32 mM Ca^{2+} treatments ($p < 0.0001$ for all). This indicates that even in the presence of excess Ca^{2+} ions, the effect of these antagonists upon cnidocyst discharge is competitive. Gitter et al. (1994) also found a competitive effect between Ca^{2+} and its inhibitors.

We observed another effect of the Ca^{2+} antagonists on the tentacles themselves. Treatments using 32 mM of the cations Co^{2+} and La^{3+} resulted in shrinkage of the tentacles after a period of 5–10 min. For both cations, the shrinking effect appeared reversible. We found that when tentacles were returned to standard seawater, they regained some of their normal thickness, though they did not recover completely.

This is the first study to examine the effect of several potential inhibitors on cnidae discharge evoked by electrical stimulation applied directly to sea anemone tentacles. We show that the cnidae discharge inhibitors La^{3+}, Mg^{2+} and Co^{2+}, are effective in the sea anemone, *H. crispa*. These results and those of all the cation treatments (Sr^{2+}, K^{+}, Ca^{2+}) in our experiment (Figure 2) match those of Gitter et al. (1994) in their study of nematocyst discharge in the hydra, *H. vulgaris*. This suggests that the discharge mechanisms among Anthozoa and Hydrozoa are similar and that the electrostimulation method may be useful in studying cnidocyst discharge in other cnidarians.

Acknowledgements

Thanks to the staff of the August Krogh Institute, Copenhagen University for general help, for technical support from Henrik Albertsen, for comments to improve the manuscript from Lis Engdahl Nielsen, Thea Marie Brolund, and the peer-reviewers. The experiments comply with the current laws of Denmark.

References

Aerne BL, Stidwell RP, Tardent P. 1991. Nematocyst discharge in *Hydra* does not require the presence of nerve cells. J. Exp. Zool. 258:137–141.

Blanquet R. 1970. Ionic effects on discharge of the isolated and in situ nematocysts of the sea anemone, *Aiptasia pallida*: A possible role of calcium. Comp. Biochem. Physiol. 35:451–461.

Gitter AH, Oliver D, Thurm U. 1993. Streptomycin inhibits nematocyte discharge in *Hydra vulgaris* by blockage of mechanosensitivity. Naturvissenschaften 80:273–276.

Gitter AH, Oliver D, Thurm U. 1994. Calcium- and voltage-dependence of nematocyst discharge in *Hydra vulgaris*. J. Comp. Physiol. A 175:115–122.

Glaser OC. 1909. The physiology of nematocysts. Baltimore, MD: Williams & Wilkins Co.

Holstein T, Tardent P. 1984. An ultra high-speed analysis of exocytosis: Nematocyst discharge. Science 223: 830–833.

Lenhoff HM, Hessinger D. 1988. The biology of nematocysts. San Diego: Academic Press.

Mariscal RN. 1974. Nematocysts. In: Mariscal L, Lenhoff HM, editors. Coelenterate biology: Reviews and new perspectives. New York: Academic Press. pp 129–178.

Mariscal RN. 1984. Cnidaria: Cnidae. In: Bereiter Hahn J, Matoltsy AG, Richards KS, editors. The biology of the integument. Berlin: Springer-Verlag. pp 57–68.

Ozbek S, Engel U, Engel J. 2002. A switch in disulfide linkage during minicollagen assembly in hydra nematocysts or how to assemble a 150-bar-resistant structure. J. Struct. Biol. 137:11–14.

Santoro G, Salleo A. 1991. The discharge of in situ nematocysts of the acontia of *Aiptasia mutabilis* is a Ca^{2+}-induced response. J. Exp. Biol. 156:173–175.

Shick JM, editor. 1991. A functional biology of sea anemones. London: Chapman & Hall.

Smith S, Oshida J, Bode H. 1974. Inhibition of nematocyst discharge in *Hydra* fed to repletion. Biol. Bull. 147:186–202.

Tardent P. 1988. History and current state of knowledge concerning the discharge of cnidae. In: Hessinger DA, Lenhoff HM, editors. The biology of nematocysts. San Diego: Academic Press. pp 309–332.