

## GADOLINIUM IS A POWERFUL BLOCKER OF THE ACTIVATION OF NEMATOCYTES OF *PELAGIA NOCTILUCA*

ALBERTO SALLEO, GIUSEPPINA LA SPADA AND ROSA BARBERA

*Institute of General Physiology, University of Messina, Via Sperone 31, 98016 Messina,  
Italy*

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### Summary

The activation properties of *in situ* nematocytes of *Pelagia noctiluca* (Scyphozoa) were investigated by physical contact with a gelatin probe that, besides stimulating the nematocyte battery, retains the discharged nematocysts, thereby allowing a quantitative evaluation of the response. In oral arms previously treated with  $2 \text{ mmol l}^{-1} \text{ La}^{3+}$  the discharge was inhibited. This result confirms the  $\text{Ca}^{2+}$ -dependence of nematocyte activation. A similar inhibitory effect was induced by treatment with  $20 \text{ } \mu\text{mol l}^{-1} \text{ Gd}^{3+}$ , a powerful blocker of mechanosensitive ion channels. It is therefore proposed that  $\text{Ca}^{2+}$ -permeable mechanosensitive channels are involved in the activation of nematocytes.  $50 \text{ } \mu\text{mol l}^{-1} \text{ Gd}^{3+}$  added to the gelatin probe was effective in otherwise untreated oral arms. This result suggests that  $\text{Gd}^{3+}$  could be useful in preventing stings from harmful Cnidaria.

### Introduction

Nematocytes are the stinging cells of Cnidaria, used for defence and for prey capture. They contain a capsule, termed the nematocyst, that, given the correct stimulus, everts a long tubule (filament) through which the venomous content of the capsule is injected into the foreign integument. Such a response is called nematocyst discharge. Nematocyte excitation requires both mechanical and chemical stimuli (Pantin, 1942). Thorington and Hessinger (1988) and Giebel *et al.* (1988) identified two classes of chemoreceptors in the tentacles of *Aiptasia pallida*, one sensitive to low molecular weight amino compounds and the other to *N*-acetylated sugars. The chemoreceptive sites seem to be located on the supporting cells surrounding the nematocyte rather than on the nematocyte itself (Watson and Hessinger, 1989). Watson and Hessinger (1989, 1991) have proposed that both contact-sensitive and vibration-sensitive mechanoreceptors are present in sea anemones and that the chemoreceptors sensitize the former and tune the latter towards lower frequencies.

The transduction mechanisms activated by mechanical and chemical stimuli remain uncertain because the small size of most nematocytes is not favourable for experiments. The electrophysiological studies performed by Anderson and McKay (1987) on

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*Chrysaora quinquecirrha*, *Cladonema* sp. and *Physalia physalis* revealed that discharge is not triggered by membrane depolarization. Furthermore, isolated nematocytes do not respond well to stimuli *in vitro*. Santoro and Salleo (1991a,b) and Salleo *et al.* (1993) observed that *in situ* nematocytes of acontia of *Aiptasia mutabilis* and *Calliactis parasitica* do not discharge in  $\text{Ca}^{2+}$ -free media, but are able to discharge at  $\text{Ca}^{2+}$  concentrations of  $0.1\text{--}30\text{ mmol l}^{-1}$ , and that the  $\text{Ca}^{2+}$  channel blockers  $\text{La}^{3+}$ ,  $\text{Cd}^{2+}$  and  $\text{Co}^{2+}$  prevent discharge. It was therefore concluded that an inflow of  $\text{Ca}^{2+}$  could couple the stimulus to the discharge response. Recently, Watson and Hessinger (1992) have suggested that cyclic AMP could be the second messenger in the supporting cells involved in this coupling.

As far as mechanoreception is concerned, a particular class of ion channel, the stretch-activated (SA) channels (Morris, 1990), is expected to be involved. Besides mechanoreception, these channels are involved in cell volume regulation (Filipovic and Sackin, 1991; McCarty and O'Neil, 1992). Among the various subtypes of SA channels, the cationic subtype has been most extensively investigated. A powerful and specific blocking effect of gadolinium on cationic SA channels has been described by Yang and Sachs (1989). This lanthanide is effective at micromolar concentration and has the further advantage of not permeating the cell. The SA channels have been investigated in various taxa, from procaryotes to mammals, with the notable exception of coelenterates. We reasoned that, since mechanoreceptors must be stimulated to trigger a  $\text{Ca}^{2+}$ -dependent discharge of nematocytes, a blocking effect of  $\text{Gd}^{3+}$  could suggest a role for SA channels in the discharge process. Since most investigations on the activation properties of *in situ* nematocytes have been performed on Anthozoa, the present experiments were performed on *Pelagia noctiluca* (Scyphozoa), a species in which the properties of *in situ* nematocyte discharge have not previously been investigated.

### Materials and methods

The experiments were performed on 40 specimens of *Pelagia noctiluca* Forskål collected in the Straits of Messina, Italy. The animals were placed immediately in 20 l tanks filled with sea water maintained at  $20\text{--}22^\circ\text{C}$ . Sea water was replaced after 24 h. All tests were performed within 48 h of collection. Only specimens that showed active swimming movements were used.

Experiments were carried out on excised oral arms; these were placed in Sylgard-coated Petri dishes, rinsed repeatedly with artificial sea water (ASW) and fixed to the Sylgard base at both ends with two *Opuntia* spines. The ASW had the following composition (in  $\text{mmol l}^{-1}$ ): NaCl, 520; KCl, 9.7;  $\text{MgCl}_2$ , 42.6;  $\text{CaCl}_2$ , 10, and was buffered at pH 7.65 with  $5\text{ mmol l}^{-1}$  imidazole. The composition of ASW was derived from that described by McKay and Anderson (1988), except for the lack of sulphate, which was omitted to avoid any possible interaction with the lanthanides, and a higher NaCl concentration to reach the osmotic pressure of sea water in the Straits of Messina. To test whether the nematocyst discharge in this species is a  $\text{Ca}^{2+}$ -dependent process, as it is in Anthozoa (Santoro and Salleo, 1991a,b), the blocking effect of  $2\text{ mmol l}^{-1}$   $\text{LaCl}_3$

was investigated.  $\text{GdCl}_3$  was added to ASW at a concentration of  $20 \mu\text{mol l}^{-1}$  to test the effectiveness of this lanthanide in blocking discharge. The excised oral arms were incubated for 30 min either in ASW or in ASW containing the blocker. Physical contact with a gelatin probe, as proposed by Giebel *et al.* (1988), was used for stimulating the nematocytes. The gelatin (Sigma, type B) was dissolved in ASW at a concentration of 30 % (w/v). One end of a nylon wire was dipped in gelatin to form a bead approximately  $200 \mu\text{m}$  in diameter. The probe was operated with a micromanipulator under a microscope. Single nematocyte batteries, easily identified by their colour, were touched once for about 2 s with the gelatin bead, into which the tubules of the discharged nematocysts penetrated. The number of discharged nematocysts of the holotrichous isorhiza type was estimated by the following method (Giebel *et al.* 1988). Immediately after each test, the probe was placed in a microtitration well containing  $80 \mu\text{l}$  of 1 % Trizyme (Amway Products), a mixture of a proteolytic enzyme and detergent (a generous gift from Dr D. A. Hessinger). After 4 h at room temperature, the gelatin was completely hydrolyzed and the discharged holotrichous isorhiza nematocysts released into the titration well were counted under an inverted microscope. In each test, the response of 10–15 nematocyte batteries was investigated by using one probe for each battery. Eight tests on different animals on different days were performed for each experimental condition. Control tests ( $N=21$ ) were generally performed on oral arms excised from the same animal that was used for the experiments. The number of nematocysts discharged from single batteries in each oral arm was averaged. The frequency distribution of the mean discharge response observed in each test is shown in Fig. 1.

To test the rapidity of action of  $\text{Gd}^{3+}$  in blocking the activation of nematocytes, the oral arms were incubated in control ASW and stimulated with a probe constructed from a gelatin solution made in ASW containing either  $20 \mu\text{mol l}^{-1}$  ( $N=5$ ) or  $50 \mu\text{mol l}^{-1}$  ( $N=10$ )  $\text{GdCl}_3$ . In this case, therefore, the blocker was present only in the test probe.

## Results

Contact with the gelatin probe did not induce discharge of all the holotrichous isorhiza nematocysts present in each battery (20–60), because of the round shape of the probe and the differing degrees of maturation of the cells. The mean number of nematocysts discharged in each oral arm varied to a certain extent (Fig. 1). Nevertheless, the frequency distribution of the mean discharge response in control tests differs clearly from that of the experimental ones. The mean of means for single control tests was  $16 \pm 1.4$  ( $\pm$  S.E.M.).

The results of various blocking experiments are summarized in Fig. 2 as mean of means for each experimental condition. In the oral arms incubated in ASW containing  $2 \text{ mmol l}^{-1}$   $\text{La}^{3+}$ , discharge occurred only occasionally, so that the mean number of discharged nematocysts was only  $1.2 \pm 0.4$  (Fig. 2B). The difference from the control value is highly significant using a *t*-test ( $P < 0.001$ ).

The direct incubation of oral arms in ASW containing  $20 \mu\text{mol l}^{-1}$   $\text{Gd}^{3+}$  reduced the responsiveness of nematocytes to contact with the gelatin probe (Fig. 2C). The mean

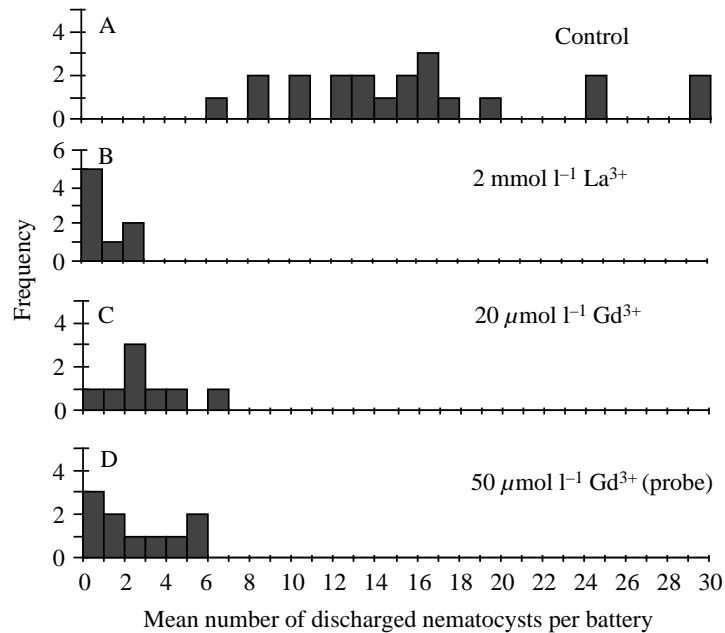


Fig. 1. Frequency distribution of the mean number of discharged holotrichous isorhiza nematocysts per battery in each oral arm. The mean was obtained by averaging the number of nematocysts discharged in each battery. (A) No blocking treatment. (B) Oral arms treated with La<sup>3+</sup>. (C) Oral arms treated with Gd<sup>3+</sup>. (D) Gd<sup>3+</sup> in the gelatin probe; untreated oral arms.

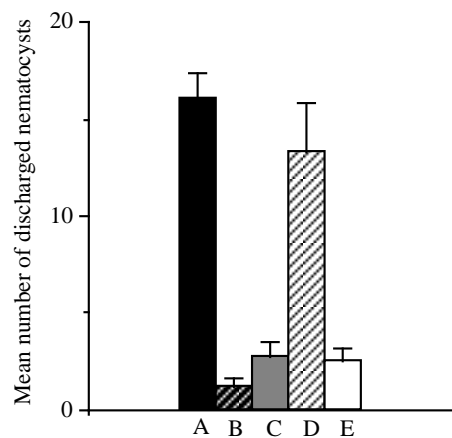


Fig. 2. Mean of means of discharged holotrichous isorhiza nematocysts, obtained by averaging the mean number of nematocysts discharged in all oral arms for each experimental condition. A, untreated oral arms ( $N=21$ ); B, oral arms treated with 2 mmol l<sup>-1</sup> La<sup>3+</sup> ( $N=8$ ); C, oral arms treated with 20 μmol l<sup>-1</sup> Gd<sup>3+</sup> ( $N=8$ ); D, 20 μmol l<sup>-1</sup> Gd<sup>3+</sup> in the gelatin probe ( $N=5$ ); E, 50 μmol l<sup>-1</sup> Gd<sup>3+</sup> in the gelatin probe ( $N=10$ ). In D and E, no blocking treatment was applied to the oral arms. Vertical bars show S.E.M.

number of nematocysts discharged in the batteries on the oral arms treated with  $\text{Gd}^{3+}$  was  $2.8 \pm 0.7$ , a value that also differed significantly from the control ( $P < 0.001$ ).

$20 \mu\text{mol l}^{-1}$   $\text{Gd}^{3+}$  added to the gelatin probe was found to be completely ineffective in preventing the discharge in untreated oral arms (Fig. 2D). However,  $50 \mu\text{mol l}^{-1}$   $\text{Gd}^{3+}$  in the gelatin probe exerted a significant inhibitory effect on the discharge (Fig. 2E). The mean number of discharged nematocysts ( $2.5 \pm 0.7$ ) was also significantly different from the control value ( $P < 0.001$ ).

### Discussion

The method for quantitative assessment of nematocyst discharge proposed by Giebel *et al.* (1988) for tentacles of Anthozoa was also found to be suitable for the nematocyte batteries on the oral arms of *Pelagia noctiluca*. Only the excised oral arms could be used in this experiment because of the spontaneous swimming movements of the animals.

The inhibitory effect of  $\text{La}^{3+}$  on the nematocyst discharge is in agreement with results obtained by Santoro and Salleo (1991a,b) on *in situ* nematocytes of the acontia of *Aiptasia mutabilis* and *Calliactis parasitica*. Such a result suggests that the  $\text{Ca}^{2+}$ -dependence of the discharge process could be a general aspect of this physiological function in Cnidaria. This would be expected if the discharge were an exocytotic process (Lubbock *et al.* 1981). The discharge of few nematocytes in some batteries probably results from an incomplete diffusion of the blockers.

The effectiveness of micromolar  $\text{Gd}^{3+}$  in blocking nematocyst discharge is striking. This lanthanide is considered to be a powerful blocker of SA cationic channels (Yang and Sachs, 1989; McCarty and O'Neil, 1992), although the mechanism of its action is still uncertain. It is therefore likely that its inhibitory effect on nematocyst discharge is exerted *via* SA channels that are expected to operate in contact mechanoreceptors. If  $\text{Gd}^{3+}$  inhibits the discharge by acting on SA channels, it could provide a new tool for investigating mechanoreception in detail in Cnidaria.

The main result of the present investigation is that  $50 \mu\text{mol l}^{-1}$   $\text{Gd}^{3+}$  applied through a gelatin probe, in the absence of any previous treatment of the tissue, prevents discharge of nematocyte batteries in the oral arms of *Pelagia noctiluca*. This result suggests that an amount of  $\text{Gd}^{3+}$  sufficient to block the discharge diffuses from the gelatin to the batteries during the brief time required for stimulus–activation coupling. Besides confirming that  $\text{Gd}^{3+}$  acts at very low concentrations in this system, this result could have an important practical application in preventing stings from those Cnidaria harmful to humans.

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