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THE DISCHARGE OF *IN SITU* NEMATOCYSTS OF THE ACONTIA OF *AIPTASIA MUTABILIS* IS A Ca^{2+}-INDUCED RESPONSE

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Summary

The ionic events leading to discharge of *in situ* nematocysts were investigated in acontia excised from *Aiptasia mutabilis* Gravenhorst. The effect on discharge of various ionic solutions and ion channel blockers was tested. In the absence of Ca^{2+} in the medium no discharge was elicited, whatever the composition of the medium. In the presence of $10\,mmol\,l^{-1}$ Ca^{2+} total discharge was induced by NaSCN, NaI, choline iodide and KI, whereas KCl induced both discharge and extrusion of undischarged nematocysts. The latter effect was prevented by La^{3+} but not by 4-aminopyridine (4-AP) and tetraethylammonium (TEA^{+}). Alcian Blue induced total discharge. NaCl and choline chloride were ineffective. The discharge induced by lyotropic anions depended on Ca^{2+} concentration and was prevented by the Ca^{2+} channel blockers La^{3+}, Co^{2+} and Cd^{2+}, but not by verapamil. It is proposed that the discharge of *in situ* nematocysts is caused by Ca^{2+} conductance through the cell membrane of either the nematocyte or the supporting cell. Furthermore, cyclic AMP and cyclic GMP do not seem to be involved as second messengers in the discharge process. The combined effects of the metabolic poisons dinitrophenol (DNP) and monoiodoacetic acid did not affect the discharge process.

Introduction

The physiological mechanisms involved in the discharging response of nematocytes, the stinging cells of cnidarians, are only partly known. Pantin (1942) observed that both mechanical and chemical stimuli are involved in inducing the discharge of the nematocyst. Recently, it was shown that the receptive function is performed by two distinct classes of chemoreceptors (Giebel *et al.* 1988; Thorington and Hessinger, 1988) that, in turn, 'tune' the mechanoreceptors (Watson and Hessinger, 1989) sensitive to the movements of the prey. The entire receptive system consists of a combination of the cnidocytes and the supporting cells. On the basis of these investigations it is to be expected that the response of nematocytes would be mediated by changes in membrane potential, as in other receptors.

Key words: nematocytes, discharge, Ca^{2+}, *Aiptasia mutabilis*.

However, it was observed that, although voltage-dependent ionic currents are detectable in these cells, so that in some species they produce Na^+-driven action potentials, the discharging response is not correlated with experimentally induced changes in membrane potential (Anderson and McKay, 1987; McKay and Anderson, 1988). Therefore, the events that link the activation of receptors with the discharge of nematocysts are still unknown. The ionic composition of the medium seems to be relevant in this process. Blanquet (1970) observed that in *Aiptasia pallida in situ* nematocysts of acontia, filamentous mesenterial structures containing nematocytes (Yanagita and Wada, 1959), are discharged more actively by isotonic and hypotonic solutions of KCl than by solutions of NaCl. More recently, McKay and Anderson (1988) observed that cnidocytes in the tentacle of *Anthopleura elegantissima* were discharged by substitution of artificial sea water (ASW) with isosmotic KCl, provided that Ca^{2+} was present in the ASW. KCl, in contrast, was ineffective when the tissue had previously been bathed in Ca^{2+}-free ASW. Furthermore, they observed that the discharge of tentacles suspended in the latter medium can be obtained by treatment with $0.5\,mol\,l^{-1}$ KCl plus $0.17\,mol\,l^{-1}$ $CaCl_2$. The discharge induced by KCl was ascribed to the permeation of K^+, since it was inhibited by 4-aminopyridine (4-AP). Although the possible role of Ca^{2+} in K^+-induced discharge was not discussed, these results suggest a function for Ca^{2+} that is accessory or synergistic to the discharging effect of K^+. The requirement for Ca^{2+} for the response of cnidoblasts had previously been suggested by Lenhoff and Bovaird (1959), although experimental data were not given. Also, Yanagita (1973) observed that in *Charybdea rastonii*, unlike other species, the discharge of tentacle nematocysts was elicited by Ca^{2+}, and he suggested that Ca^{2+} acts *via* cell mechanisms. A possible role for Ca^{2+} in the process of discharge of *in situ* cnidae is intriguing, since this cation has been generally recognized as a powerful inhibitor of the discharge of isolated nematocysts (Blanquet, 1970; Lubbock and Amos, 1981; Salleo, 1984; Salleo *et al.* 1983, 1988*a*,*b*, 1990). If Ca^{2+} has a role in the discharge of nematocysts *in situ*, it is expected to implicate cell structures involved either in reception or in transduction and not the resting nematocyst. Calcium could play various roles in nematocytes, as it does in other cells. As a counterion of negative fixed charges on the outer membrane surface, it could interfere with ionic permeability. Furthermore, following permeation into the intracellular compartment, calcium could: (i) activate contractile proteins already described in nematocytes (Wood and Novak, 1982; Hessinger and Ford, 1988), (ii) act as a second messenger on an unidentified trigger mechanism of discharge, or (iii) favour the adhesion and fusion of the membrane surrounding the resting nematocyst with the plasma membrane of the nematocyte, thereby inducing the exocytosis of the capsule (Lubbock *et al.* 1981).

To identify the physiological mechanisms involved in the process of discharge it is important, therefore, to ascertain whether Ca^{2+} plays any role in the discharge of *in situ* nematocysts and, if so, whether its role is primary or subordinate, intra- or extracellular. The results of this investigation, performed by changing the ionic composition of the suspension medium, as well as by applying various drugs active

on ion channels, strongly suggest that the discharge of *in situ* nematocysts of acontia of *Aiptasia mutabilis* is a Ca^{2+}-induced cell response that depends directly on the permeation of Ca^{2+} into the intracellular compartment of either nematocytes or supporting cells.

Materials and methods

The investigation was performed on acontial filaments because they bear large numbers of nematocysts along their entire length (Yanagita and Wada, 1959). The acontium was removed by excision from specimens of the sea anemone *Aiptasia mutabilis* collected in lake Faro, a seawater pond whose salinity ranges between 34 and 37 ‰, maintained in an aquarium at 23 °C and fed weekly with prawn meat.

The importance of obtaining both a rapid and complete substitution of the suspension medium and the reproducibility of mechanical stimulation during the flow of solutions was taken into account. For this purpose a cut acontial segment (length approx. 4 mm) was placed under a dissecting microscope in a glass channel filled with sea water and was fixed at each end by penetrating the tissue with the sharp tips of two glass rods worked by micromanipulators. In placing the acontium segment parallel to the length of the channel, both stretching and slackening of the tissue were carefully avoided. In this condition both slow and coordinated contractile activity of the acontium and ciliary movement could be clearly observed. If some nematocysts discharged during this procedure, the acontium was discarded. To replace the solutions, one end of the channel was connected with the output of a constant-flow pump (Sage Instruments model 351) driving the solutions along the channel. The solution was not recirculated. The calculated average speed of the fluid along the channel was 16 mm s^{-1} so that, in a 4 cm long channel, the medium could be replaced completely in a few seconds.

All experiments started with the substitution of sea water with ASW (McKay and Anderson, 1988) of the following composition (in mmol l^{-1}): NaCl, 466; KCl, 9.7; $CaCl_2$, 10; $MgCl_2$, 24; $MgSO_4$, 28. The tissue was allowed to equilibrate in this medium for 10 min. The various test solutions (shown in Table 1) were then applied at a constant flow that was not stopped until the end of the 5 min observation period. Treatment with Ca^{2+}-free test solutions was preceded by the removal of ASW, using flowing Ca^{2+}-free ASW containing 0.5 mmol l^{-1} EGTA to avoid any possible contamination with Ca^{2+}. The removal of Ca^{2+} from the medium was confirmed by the complete immobilization of the tissue.

To evaluate the effectiveness of ionic species in causing nematocysts to discharge, we assumed that a discharging agent would induce the same response in all mature nematocytes in the acontium. There are hundreds of such nematocytes in segments like those investigated. Only the discharge of microbasic b-mastogophores was taken into account, because the smaller basitrichs are more difficult to observe at low magnification. The discharging response to ions was considered, therefore, as an 'all-or-nothing' effect. A solution was considered to be effective when it induced within 2 min the discharge of almost all capsules along the entire

length of the acontial segment between the two anchoring tips. Such a response is referred to below as 'total' discharge. The discharge of some capsules and the lack of any response are referred to as 'partial' and 'absent', respectively.

All test solutions, NaCl, NaSCN, NaI, KCl, KI, choline chloride and choline iodide were applied at a concentration of 553 mmol l^{-1}, iso-osmotic with ASW. We tested the possible effect of the lyotropic anions SCN^- and I^- on the basis of the known ability of the former to induce changes in some ion channel characteristics, such as causing a shift in the voltage dependence of delayed rectifier K^+ channels (Kao and Stanfield, 1968) and of Na^+ channels (Dani *et al.* 1983) as well as an increase in Ca^{2+} permeability of sarcoplasmic reticulum (Ohnishi and Ebashi, 1963). In Ca^{2+}-free ASW, Ca^{2+} was replaced by an iso-osmotic equivalent amount of Na^+ and 0.5 mmol l^{-1} EGTA was added. In Na^+-free ASW, Na^+ was replaced by choline. Ca^{2+}, added as $CaCl_2$, was at the same concentration in all solutions as that in ASW, namely 10 mmol l^{-1}. To test the effect of Ca^{2+} concentration, the following concentrations were also used, both in ASW and in NaSCN: 10, 1, 0.1 and 0.01 mmol l^{-1}. Furthermore, the effect of the calcium ionophore A23187 (Prusch, 1980) was investigated by adding it at a concentration of 50 μg ml^{-1} to NaCl solution in the presence of 0.1 mmol l^{-1} Ca^{2+}. The ionophore was dissolved in ethanol. 0.5% ethanol was also added to the control solution, namely NaCl+0.1 mmol l^{-1} Ca^{2+}. In these tests the observation was continued for 60 min.

The following ion channel blockers were added both to ASW and to test solutions: 2 mmol l^{-1} $LaCl_3$ (Requena *et al.* 1985), 5 mmol l^{-1} $CoCl_2$, 2 mmol l^{-1} $CdSO_4$, 0.2 mmol l^{-1} verapamil (Fedulova *et al.* 1985), 10 mmol l^{-1} 4-aminopyridine (4-AP) and 20 mmol l^{-1} tetraethylammonium chloride (TEA^+) (Hudspeth and Lewis, 1988).

To test for a possible role of surface-bound Ca^{2+}, 0.01% Alcian Blue (Prusch and Hannafin, 1979) was added to the following test solutions: KCl, KCl+Ca^{2+}, NaCl and NaCl+Ca^{2+} (see Table 1).

The possible role of cyclic AMP and cyclic GMP as second messengers in the process of discharge was investigated by treating the acontial tissue with either dibutyryladenosine 3′,5′-cyclic monophosphate (dbcAMP) or dibutyrylguanosine 3′,5′-cyclic monophosphate (dbcGMP) at a concentration of 3 mmol l^{-1}. As the dibutyryl analogue nucleotides are able to permeate plasma membranes (Saumon *et al.* 1989), they are effective when applied extracellularly. The importance of energy expenditure in the process of discharge was investigated by treating the tissue with both 0.2 mmol l^{-1} dinitrophenol (DNP) and 2 mmol l^{-1} monoiodoacetic acid for 120 min before testing the responsiveness of nematocytes. During this time both spontaneous contractile activity of the acontia and ciliary motility were completely abolished. Besides these metabolic poisons, other drugs used in this investigation also induced changes in contractile activity. Such changes are described below, because they indicate the pharmacological effectiveness of the drugs.

To verify whether damage to the tissue made some treatments ineffective, the

test solution in some experiments was removed and the tissue was washed again with ASW before applying a putative discharging agent (see Table 3).

All solutions were buffered at pH 7.8 with 10 mmol l^{-1} imidazole. Reagents used were of analytical grade and were purchased from Sigma, except for salts of La^{3+}, Co^{2+} and Cd^{2+}, which were purchased from Aldrich. Finally, tests were performed on isolated nematocysts. The capsules were isolated from acontial tissue in 1 mol l^{-1} glycerol (Yanagita, 1973), filtered and washed in ASW by repeated centrifugation, and suspended in this medium (Salleo *et al.* 1983). The isolated capsules were then treated with 550 mmol l^{-1} NaSCN plus 10 mmol l^{-1} $CaCl_2$.

Results

The effectiveness of the various ionic species in inducing nematocyst discharge is shown in Table 1. Treatment with either KCl or NaCl was completely ineffective in the absence of Ca^{2+}. This result is in agreement with those of McKay and Anderson (1988). When KCl was substituted for ASW in the presence of 10 mmol l^{-1} Ca^{2+}, partial discharge and extrusion of undischarged capsules occurred along the entire length of the acontial segment. The contractile activity of the acontium also increased noticeably. The addition of Alcian Blue to KCl solution was ineffective. In contrast, when the dye was added with KCl in the presence of Ca^{2+}, a sudden discharge was observed that was completed within 15 s. In this case no extrusion was observed. When the K^+ channel blockers 4-AP and TEA^+ were added to ASW, powerful contractions but no discharge were observed. Mass discharge was elicited by subsequent treatment with KCl in the presence of Ca^{2+}. Both effects, namely contraction and discharge, were suppressed by La^{3+}.

NaCl was ineffective even in the presence of 10 mmol l^{-1} Ca^{2+} and of Alcian Blue. In some tests a sporadic and gradual discharge occurred after 2 min. However, discharge was elicited by KI+Ca^{2+} in acontia previously suspended in Na^+-free ASW (see Table 1).

The lyotropic anions SCN^- and I^-, applied in combination with 10 mmol l^{-1} Ca^{2+}, elicited within 15 s the simultaneous discharge of all capsules over the entire length of the acontial segment (see Table 1). This response was preceded by brisk and powerful contractions of the tissue. Since no significant difference in effectiveness was observed between the two lyotropic anions, they were used interchangeably as discharging agents in subsequent tests. The response to lyotropic anions was induced with either K^+ or Na^+ as the monovalent cation and even in conjunction with the non-permeant cation choline. Furthermore, the effectiveness of lyotropic anions in the presence of Ca^{2+} was also confirmed in acontia treated with both 4-aminopyridine and TEA^+ to prevent conductance through K^+ channels. In contrast, when Ca^{2+} was absent from the medium, the lyotropic anions had no effect on either contractile activity or nematocyst discharge. The effect of Ca^{2+} was observed to depend on concentration. Ca^{2+}

Table 1. *Effect of the medium composition on discharge*

Starting medium	Test solution	Discharge			
		Total	Partial*	Absent	N§
Ca^{2+}-free ASW	KCl	0	0	4	4
Ca^{2+}-free ASW+ Alcian Blue	KCl+Alcian Blue	0	0	3	3
ASW	KCl+Ca^{2+}	0	5†	0	5
ASW+Alcian Blue	KCl+Ca^{2+}+Alcian Blue	3	0	0	3
Ca^{2+}-free ASW	NaCl	0	0	3	3
Ca^{2+}-free ASW	NaCl+Alcian Blue	0	0	3	3
ASW	NaCl+Ca^{2+}	2‡	1‡	6	9
ASW	NaCl+Ca^{2+}+Alcian Blue	0	1‡	2	3
Ca^{2+}-free ASW	NaSCN	0	0	4	4
ASW	NaSCN+Ca^{2+}	13	0	0	13
Ca^{2+}-free ASW	NaI	0	0	5	5
ASW	NaI+Ca^{2+}	5	0	0	5
ASW	Choline chloride+Ca^{2+}	0	0	5	5
Na^{+}-free ASW	Choline iodide+Ca^{2+}	5	0	0	5
ASW	KI+Ca^{2+}	3	0	0	3
Na^{+}-free ASW	KI+Ca^{2+}	5	0	0	5
ASW+La^{3+}	NaSCN+Ca^{2+}+La^{3+}	0	0	4	4
ASW+Cd^{2+}	NaSCN+Ca^{2+}+Cd^{2+}	0	0	3	3
ASW+Co^{2+}	NaSCN+Ca^{2+}+Co^{2+}	0	0	3	3
ASW+verapamil	NaSCN+Ca^{2+}+verapamil	6	0	0	6
ASW+4-AP+TEA^{+}	KCl+Ca^{2+}+4-AP+TEA^{+}	3	0	0	3
ASW+4-AP+TEA^{+}+La^{3+}	KCl+Ca^{2+}+4-AP+TEA^{+}+La^{3+}	0	0	3	3
ASW+4-AP+TEA^{+}	NaSCN+Ca^{2+}+4-AP+TEA^{+}	3	0	0	3

All starting media were applied for 10 min.
Test solutions were applied for 5 min.
* Only a few nematocysts discharged.
† Discharge and extrusion of undischarged nematocysts.
‡ Discharge of few nematocysts started after 2 min and continued gradually.
§ *N*, number of acontia tested.

added to NaSCN solution at a concentration of 1 mmol l^{-1} elicited a delayed discharge that proceeded slowly and was complete within 5 min. At a concentration of 0.1 mmol l^{-1}, massive extrusion was observed instead of discharge. At 0.01 mmol l^{-1}, neither discharge nor extrusion occurred (Table 2). Nevertheless, when the ionophore was applied together with NaCl and 0.1 mmol l^{-1} Ca^{2+}, which were ineffective on their own, a gradual extrusion was observed; extrusion was completed 20–45 min after treatment (Table 2).

Cl^{-} was found not to be involved in the process of discharge since, even in the presence of Ca^{2+}, choline chloride, unlike choline iodide, did not elicit any response.

In all tests, the removal of Ca^{2+} from the medium induced the immediate

Table 2. *Effect of Ca^{2+} concentration in the medium and of A23187*

Starting medium	Test solution	Effect	*N**
ASW (10 mmol l^{-1} Ca^{2+})	NaSCN+10 mmol l^{-1} Ca^{2+}	Discharge	3
ASW (1 mmol l^{-1} Ca^{2+})	NaSCN+1 mmol l^{-1} Ca^{2+}	Delayed discharge†	3
ASW (0.1 mmol l^{-1} Ca^{2+})	NaSCN+0.1 mmol l^{-1} Ca^{2+}	Extrusion	3
ASW (0.01 mmol l^{-1} Ca^{2+})	NaSCN+0.01 mmol l^{-1} Ca^{2+}	None	3
ASW (0.1 mmol l^{-1} Ca^{2+})	NaCl+0.1 mmol l^{-1} Ca^{2+}	None	3
ASW (0.1 mmol l^{-1} Ca^{2+})	NaCl+0.1 mmol l^{-1} Ca^{2+}+A23187	Delayed extrusion‡	3

Starting media were applied for 10 min and test solutions for 5 min.
* *N*, number of acontia tested.
† Discharge completed by 5 min after application of the test solution.
‡ Extrusion completed by 45 min.

suspension of spontaneous contractile activity (Anctil, 1987) in the acontia. Nevertheless, the lack of Ca^{2+} did not seem to damage the tissue. In fact, following treatment with Ca^{2+}-free KCl, NaCl, NaSCN or NaI, which did not elicit any discharge, reincubation in ASW restored the motility and the tissue could be induced to discharge totally by subsequent treatment with lyotropic anions and Ca^{2+} (Table 3).

La^{3+}, Cd^{2+} and Co^{2+} prevented the SCN^--induced discharge (Table 1). Furthermore, they stopped any contractile activity in the acontia. Unlike that of Cd^{2+} and Co^{2+}, the inhibitory effect of La^{3+} on discharge could not be reversed. In fact, when the solution containing this inhibitory cation was removed by rinsing the tissue with ASW, a second treatment with the discharging solution of NaSCN+Ca^{2+} did not induce any discharge. A slow reversibility of the La^{3+} block of Ca^{2+} channels has also been described by Jones and Marks (1989). In contrast, the same procedure was successful when either Cd^{2+} or Co^{2+} had been employed as inhibitors (Table 3).

Treatment with dbcGMP, but not with dbcAMP, induced a noticeable increase in spontaneous contractile activity of the tissue. Nevertheless, neither dibutyryl analogue of these cyclic nucleotides elicited any discharging response in the tissue or changed its responsiveness to SCN^- (Table 4). The blocking action of DNP and monoiodoacetic acid on metabolic processes in the cells caused a complete, progressive immobilization of the tissue without affecting the SCN^--induced discharge (Table 4).

Finally, when NaSCN+Ca^{2+} was applied to already isolated nematocysts no discharge was elicited. This result is in agreement with the findings of Salleo *et al.* (1988*a*).

Discussion

The main result of this investigation is to show that, when Ca^{2+} is absent from the medium, the discharge of acontia nematocysts of *Aiptasia mutabilis* is

Table 3. *Effect of removal of Ca^{2+} blockers on Ca^{2+}-induced discharge*

Starting medium	*N**	First test solution	Discharge	Washing medium	Second test solution	Discharge		
						Total	Partial	Absent
Ca^{2+}-free ASW	4	KCl	0	Ca^{2+}-free ASW–ASW	NaSCN+Ca^{2+}	3	0	0
Ca^{2+}-free ASW	3	NaSCN	0	Ca^{2+}-free ASW–ASW	NaSCN+Ca^{2+}	3	0	0
ASW+La^{3+}	4	NaSCN+Ca^{2+}+La^{3+}	0	ASW	NaSCN+Ca^{2+}	0	0	4
ASW+Cd^{2+}	3	NaSCN+Ca^{2+}+Cd^{2+}	0	ASW	NaSCN+Ca^{2+}	3	0	0
ASW+Co^{2+}	3	NaSCN+Ca^{2+}+Co^{2+}	0	ASW	NaSCN+Ca^{2+}	3	0	0

Starting media were applied for 10 min.
Test solutions were applied for 5 min.
* *N*, number of acontia tested.

Table 4. *Effect of metabolic poisons and of putative second messengers on Ca^{2+}-induced discharge*

Starting medium	Treatment	Test solution	Discharge			*N**
			Total	Partial	Absent	
ASW	ASW+0.2 mmol l^{-1} DNP+IAA	NaSCN+Ca^{2+}	3	0	0	3
ASW	ASW+3 mmol l^{-1} dbcAMP	NaSCN+Ca^{2+}	3	0	0	3
ASW	ASW+3 mmol l^{-1} dbcGMP	NaSCN+Ca^{2+}	3	0	0	3

DNP and IAA were applied for 120 min; mononucleotides were applied for 30 min; test solution was flowed for 5 min.
DNP, dinitrophenol; IAA, iodoacetic acid.
* *N*, number of acontia tested.

completely prevented, whatever the inducing ionic species. Whether the Ca^{2+}-dependence of discharge is a general property of nematocytes, either in tentacles or in acontia, is not yet clear. Nevertheless, the requirement for Ca^{2+} has been suggested for *Hydra* (Lenhoff and Bovaird, 1959), it has been observed on tentacular cnidae of *Charybdea rastonii* (Yanagita, 1973) and *Anthopleura elegantissima* (McKay and Anderson, 1988), and it has been demonstrated in the present investigation for acontial nematocytes of *Aiptasia mutabilis*. It is therefore likely that the same mechanisms are operating in all cnidae. Furthermore, such an interpretation is in agreement with the exocytotic model of discharge proposed by Lubbock *et al.* (1981).

Our results contrast with those of Blanquet (1970), who observed a high responsiveness to KCl in the absence of Ca^{2+}. Since the latter solution was applied to cut acontial filaments suspended in sea water on a depression glass slide, it is possible that a small amount of Ca^{2+}, contained in sea water or in mucus, remained around the filament. This interpretation is confirmed by the fact that, in the experiments performed by McKay and Anderson (1988), KCl without Ca^{2+} substituted for ASW was effective, provided that the ASW contained Ca^{2+}. In the present experiments, tests with Ca^{2+}-free solutions were performed in the absence of residual Ca^{2+}. A comparison of results obtained with Ca^{2+}-free solutions of KCl, NaCl, NaSCN, NaI, KI, choline chloride and choline iodide demonstrates that none of them induces any discharging response in nematocytes *in situ*. With the addition of Ca^{2+} at the same concentration as in ASW, $10\,mmol\,l^{-1}$, the discharge response was induced by KCl, NaSCN, NaI, KI and choline iodide, but not by NaCl and choline chloride. The response to KCl consisted of both discharge and extrusion. The latter phenomenon (the expulsion from the tissue of undischarged capsules) has been considered as part of the discharge process (Yanagita, 1958), although this view has not been generally accepted (Mariscal, 1974). Since the physiological importance of this phenomenon is not clear, the response of the tissue to KCl cannot be considered as a total one. In contrast, a total discharge was obtained when Alcian Blue was added to this test solution in the presence of Ca^{2+}. This dye is expected to displace surface-bound Ca^{2+} and, consequently, to increase permeability to solutes, including Ca^{2+} (Prusch and Hannafin, 1979). However, since KCl is ineffective in the absence of Ca^{2+}, even when Alcian Blue is added, we reasoned that either the discharging response is elicited by K^+ and Ca^{2+} plays a subordinate role or *vice versa*. The second hypothesis seems more likely. In fact, high K^+ concentration causes depolarization, thereby possibly inducing an increase in Ca^{2+} conductance, which, in turn, could cause both contraction of musculoepithelial cells and the discharge of nematocytes. In our experiments, unlike those of McKay and Anderson (1988), treatment with the K^+ channel blockers 4-AP and TEA^+ did not prevent the discharge. Such a discrepancy could depend on the properties of the cell, on the presence of spirocytes in the experiments of McKay and Anderson (1988) or on the method of solution replacement. An alternative explanation is that with a high K^+ concentration in the external medium, the K^+ channels blockers, although preventing K^+ entry, do

not prevent the shift of membrane potential towards less negative values, which, in turn, could cause the opening of Ca^{2+} channels.

A prompt discharge of the entire population of nematocysts was elicited equally by the lyotropic anions SCN^- and I^-. Such a response was ascribed exclusively to these anions because it was elicited whether the associated cation was Na^+, K^+ or choline. The other anion tested, Cl^-, was ineffective, since it did not induce any response in conjunction with Na^+ or with choline. As regards the pharmacological mechanism of lyotropic anions, it is noteworthy that their discharging effectiveness depends strictly on the presence of Ca^{2+} in the medium, with a clear concentration dependence. Again, a likely interpretation is that the discharge is elicited by a Ca^{2+} inflow induced by the lyotropic anions. Such an interpretation is also supported by the effect of treatment with Ca^{2+} channel blockers. Although an explanation of their inhibitory effect could be that they act on Na^+ channels, as suggested by Anderson and McKay (1987), it should be stressed that in our experiments Na^+ does not seem to be involved in the discharge process. The mechanism of the observed inhibitory effects of La^{3+}, Cd^{2+} and Co^{2+} on nematocyst discharge is therefore conceivably similar to that exerted by these cations on other Ca^{2+}-dependent physiological processes, in particular the block of Ca^{2+} channels (Tsien *et al.* 1987). Since SCN^- in the presence of Ca^{2+} was completely ineffective when combined with each of the above inhibitors, it is likely that lyotropic anions act by opening Ca^{2+} channels, thereby allowing Ca^{2+} to flow in passively along the electrochemical gradient. An inhibitory effect of di- and polyvalent cations directly exerted on the resting capsules (Salleo, 1984; Salleo *et al.* 1988*b*) can be excluded on the basis of the low concentrations used in the present investigation. The powerful contractions observed following treatment with the lyotropic anions in the presence of Ca^{2+} suggest that an inflow of Ca^{2+} also occurs into the myoepithelial cells as a consequence of the pharmacological action of lyotropic anions. On entering the cells, Ca^{2+} could play any one of the possible roles mentioned above. At present, we can only state that Ca^{2+} does not act directly on the resting nematocyst, as demonstrated by the ineffectiveness of lyotropic anions, applied with Ca^{2+}, in discharging isolated nematocysts. The lack of any inhibitory effect of verapamil, either on discharge or on contractile activity, does not exclude such a mechanism. In fact, Ca^{2+} could enter these cells through receptor-operated channels (Hosey and Lazdunski, 1988), which in some instances are insensitive to verapamil (Bolton, 1979), rather than through voltage-operated ones. The fact that the discharge of *in situ* nematocysts of tentacles could not be elicited following depolarization (McKay and Anderson, 1988), while mechanical stimulation of the tissue accompanied by treatment with either N-acetylated sugars or mucin is effective (Watson and Hessinger, 1989), supports this interpretation.

The extrusion observed in place of discharge following treatment with NaSCN solution containing only $0.1\,mmol\,l^{-1}$ Ca^{2+} suggests that this phenomenon could be a phase of the discharge process, as proposed by Yanagita (1958): this becomes evident when the discharge cannot be completed, possibly owing to a low cytoplasmic Ca^{2+} concentration. Such an interpretation is supported by the

extrusion elicited by NaCl solution plus 0.1 mmol l^{-1} Ca^{2+}, otherwise ineffective, in the presence of A23187. The failure of the ionophore to elicit discharge could depend on the requirement for a cytoplasmic Ca^{2+} concentration higher than that caused by the ionophore-mediated Ca^{2+} entry.

Extracellularly applied dbcGMP, unlike dbcAMP, was found to be effective in increasing the spontaneous motility of the acontial tissue. This result confirms that the drug passed through the plasma membrane of musculoepithelial cells. Anctil (1989) observed the induction by dbcAMP (dbcGMP was not tested) of a rhythmic contractile activity in the coelenterate *Renilla köllikeri*. Such an effect was ascribed to a second messenger function of cyclic AMP associated with serotonin. Since in the present investigation both nucleotides were ineffective in inducing discharge, it can be inferred that they may not be involved as second messengers in the discharging response of nematocysts. Finally, the energy for discharge is completely stored in the resting capsule. The lack of any effect on discharge of metabolic poisons such as DNP and monoiodoacetic acid, besides confirming this generally accepted assumption, suggests that switching off all the active ion transport systems that normally support the membrane potential does not affect the discharging response of nematocytes.

In conclusion, we propose that the discharging response of nematocytes is a Ca^{2+}-induced mechanism, possibly based on an increase in Ca^{2+} conductance through channels that are normally activated by naturally occurring mechanical and/or chemical stimuli, whose effect is mimicked, in our experiments, by the pharmacological action of lyotropic anions. Nevertheless, we do not yet know whether Ca^{2+} elicits the discharge process by acting directly on the nematocytes or on the supporting cells.

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