Cell-to-cell transmission in the activation of in situ nematocytes in acontia of *Calliactis parasitica*

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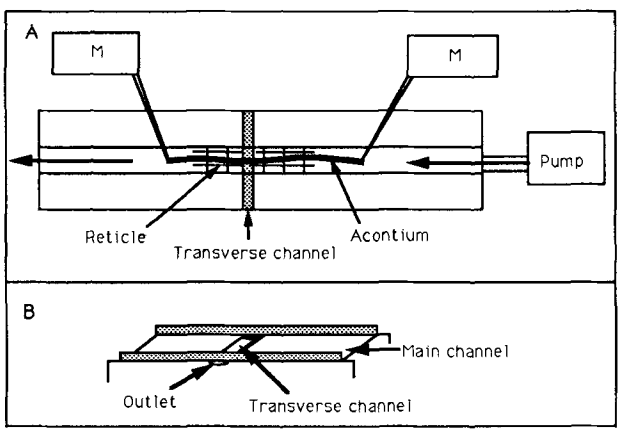
Summary. It is reported that Ca^{2+}-induced discharge of in situ nematocytes of acontia of *Calliactis parasitica* can occur by cell-to-cell transmission along the acontial filament at a speed that averages $9.8 \cdot 10^{-3}$ cm^{-1}. The discharge is preceded by protrusion of nematocytes that proceeds along the acontium at a slightly higher speed.
Key words. Nematocyte; discharge; transmission; Ca^{2+}; *Calliactis parasitica.*

The discharge of nematocysts, the stinging organoids of Coelenterates, Cnidarians, is controlled by partly known mechanisms that include, at least in Anthozoa, the intervention of different types of chemoreceptors located in the supporting cells surrounding the nematocytes [1], the mechanoreception supplied by specialized structures of the nematocyte itself [2], and a process of transduction that induces the discharge of the nematocyst. Although nematocytes were considered previously as independent effectors, recently it has been recognized that some information from supporting cells [1], and possibly from synapses [3], is involved in activating the discharge process. A recent study [4] on the discharge of nematocytes of acontia from *Aiptasia mutabilis* revealed that a mass discharge could be induced by applying the lyotropic anions SCN^- and I^- in conjunction with either K^+ or Na^+ and even choline, provided that Ca^{2+} was present in the medium. On the other hand, in the absence of Ca^{2+}, or when the tissue had been treated with some Ca-channel inhibitors, the discharge was completely prevented. Since Ca^{2+} is generally recognized as a powerful inhibitor of the discharge of isolated nematocysts [5–7], the effect of Ca^{2+} was presumably exerted on some cell structure other than the nematocyst. It was concluded that the activation of nematocytes, either directly or through supporting cells, is a Ca^{2+}-induced phenomenon. It is worth pointing out that unlike nematocysts of *Aiptasia mutabilis* [8], nematocysts from acontia of *Calliactis parasitica* do not release measurable amounts of Ca^{2+} [9]. Therefore we investigated the role of Ca^{2+} in activating the acontial nematocytes of *Calliactis parasitica.*

Acontial filaments, approximately 15 mm in length, were removed from specimens of *Calliactis parasitica* maintained in an aquarium at 20–25 °C and fed weekly with prawn meat. The observations were performed using an inverted microscope at magnifications ranging between 32x and 80x. The tissue was placed in a glass channel (4 mm wide, 75 mm long), on the bottom of which was a reticle that allowed an easy evaluation of the transmission speed of discharge by measuring the time interval between the discharge at the first and at the last line of the grid (3 mm apart). The acontial filament was straightened and fixed by penetrating its cut ends with two sharpened glass rods moved by micromanipulators (fig.). The tissue was neither stretched nor slackened. The channel was filled with artificial seawater (ASW) with the following composition [10] (in mmol $\cdot$ l^{-1}): NaCl 466, KCl 9.7 $CaCl_2$ 10, $MgCl_2$ 24, $MgSO_4$ 28. A constant flow pump (Sage mod. 351) was used to keep the experimental solution, which contained 553 mM NaSCN plus 10 mM $CaCl_2$, flowing at a speed of 16 mm s^{-1}. When a Ca^{2+}-free experimental solution was used, the latter was preceded by Ca^{2+}-free ASW containing 0.5 mM EGTA, in which Ca^{2+} was replaced by an osmotically equivalent amount of Na^+. All solutions were buffered with imidazole at pH 7.8.

In the presence of Ca^{2+} the SCN^- solution induced mass discharge in all tests within 4 min. The first response of the tissue to the discharging solution was the protrusion of nematocytes at the surface of the acontial filament. It should be pointed out that such a response differs from extrusion [11], that is the expulsion of nematocysts from the tissue. Usually protrusion started at one cut end of the acontium, and proceeded along its length toward the other end. Thereafter, the nematocytes began to fire at the end where protrusion had started, and discharge of all nematocytes proceeded sequentially in the same direction without interruption. Occasionally, both protrusion and discharge started from both ends. In these cases they proceeded in opposite directions towards the center of the filament. The discharge never started at the center of the acontial filament. Furthermore, when the center of the acontium was isolated from the external medium by



A Experimental set up; M: micromanipulators. *B* Detail of the transverse channel. Both channels are shown as wider than they actually are.

covering it completely in vaseline paste for a length of 1 mm, the transmission was blocked at that level.

The transmission speed of protrusion and discharge observed in various experiments is shown in the table. The average speed of transmission of both responses was about three orders of magnitude lower than both propagation speed in nerve nets and cell-to-cell transmission through the myoepithelium of Coeleterates[12, 13].

In Ca^{2+}-free ASW the spontaneous contractile activity of the acontia disappeared soon. No discharge was observed when the Ca^{2+}-free SCN^- solution was applied. This result confirms the requirement of Ca^{2+} for inducing the discharge of in situ nematocysts. When the Ca^{2+} concentration in the NaSCN solution was lowered to 0.1 mM l^{-1}, the discharge invaded short segments of tissue along the acontia without a regular sequence. At a Ca^{2+} concentration of 0.01 mM l^{-1} no discharge was elicited.

The inhibitory effect of La^{3+}, a well-known Ca^{2+}-channel blocker[14], was tested by preincubating the tissue in ASW containing 2 mM l^{-1} $LaCl_3$. When the discharging solution (NaSCN plus Ca^{2+}) was flowing, no discharge was elicited. For further evidence of the role of Ca^{2+} in the discharge process, the acontial filament was treated in its central part with ASW containing also 2 mM l^{-1} $LaCl_3$,. To prevent diffusion of this solution along the acontium, another channel, 2 mm wide, crossed at right angles the center of the main channel (fig.) in which the acontial filament was placed. ASW containing La^{3+} was dropped for 5 min to the center of the acontial filament so that it flowed down through the transverse channel without reaching other parts of the filament. When the NaSCN plus Ca^{2+} solution was allowed to flow through the main channel, both protrusion and discharge started as usual from one end of the acontium and stopped near the La^{3+}-treated segment. Thereafter, the response also started at the opposite end of the acontium and reached the other edge of the treated segment. In addition, an attempt was made to test whether the spread of discharge depended on the presence of either Ca^{2+} or SCN^- in the suspension medium. The tissue was treated with NaSCN plus Ca^{2+}. As soon as the discharge started at one end, ASW was passed through the channel at a speed of 24 mm s^{-1}. The discharge spread as usual along the entire length of the filament. On the other hand, when Ca^{2+}-free ASW containing also 2 mM l^{-1} of EGTA was applied after the start of the discharge, the spread was blocked within 15 s. The same effect was obtained within 7 s by using ASW containing 2 mM l^{-1} of $LaCl_3$. These results suggest that SCN^- acts by triggering at the cut end of the acontium the discharge response that, in turn, can spread provided that Ca^{2+} is present in the medium.

The above results confirm that the discharge of in situ acontial nematocytes of *Calliactis parasitica* is a Ca^{2+}-induced phenomenon, as previously observed in *Aiptasia mutabilis*[4]. The main result is that the activation of these cells can be transmitted bidirectionally through the tissue. The block of transmission caused by the removal of Ca^{2+} from the suspension medium, as well as the blocking effect of La^{3+}, suggest that a Ca^{2+} influx may be associated with the transmission. An increase in Ca^{2+} permeability could be induced by the lyotropic anion SCN^-[15]. In our experiments, SCN^- reaches the tissue through the cut ends. The transmission speed is about 3 orders of magnitude lower than impulse propagation through nerve nets and myoepithelial cell-to-cell transmission. This suggests that it is not directly mediated by action potentials. Nevertheless, it is possible that the activation of discharge spreads slowly owing to the duration of excitation-discharge coupling. Another feature encountered in the present investigation is that discharge is preceded by the protrusion of nematocytes towards the external medium. Such a response has been interpreted[16] as a 'ready to fire' position of the nematocyte that, in turn, is brought about by a system of fibers. In our preparation, this response is transmitted with a speed slightly higher than that of discharge.

The cell-to-cell transmission through the acontial tissue, which had not been described up to now, raises new questions about the nature of the transmitted signal and the physiological significance of intercellular junctions in this tissue.

Transmission speed of protrusion and discharge along the acontial filaments of *Calliactis parasitica*.

Transmission speed 10^{-3} cm · s^{-1}	
Protrusion	Discharge
13.0	11.5
13.9	9.0
13.9	7.7
12.0	7.9
9.7	7.6
15.8	10.3
13.6	10.7
10.5	9.1
15.0	12.8
13.3	9.1
Mean 13.1	9.6
SD (± 1.79)	(± 1.64)

Student's t-test showed that the difference between the mean values was significant at $p < 0.001$.

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Endothelium-dependent relaxation induced by sodium fluoride in the rabbit ear artery

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Summary. Sodium fluoride (NaF) produced concentration-dependent relaxation of isolated rabbit ear artery precontracted with norepinephrine. In contrast, an arterial preparation with the endothelium rubbed off did not relax, but contracted in response to NaF. NaF-induced relaxation was not influenced by indomethacin but was inhibited by methylene blue or N^G-monomethyl-L-arginine. The results indicate that NaF relaxes the artery by releasing a so-called EDRF.
Key words. Sodium fluoride; endothelium-dependent vasorelaxation; rabbit ear artery.

It is well known that a number of endogenous as well as exogenous substances produce relaxation of blood vessels through the mediation of an increase in the level of intracellular cyclic AMP or cyclic GMP[1]. For instance, agonists for β-adrenoceptors, stimulators of adenylate cyclase such as forskolin, and inhibitors of phosphodiesterase including methylxanthines, are known to relax smooth muscles, including those of blood vessels, by increasing the intracellular cyclic AMP level[2,3]. So-called endothelium-derived relaxing factor (EDRF)[4,5], and a number of nitrogen oxide-containing substances such as nitroprusside and nitroglycerin, relax vascular smooth muscle cells by the mediation of cyclic GMP[6].

Sodium fluoride (NaF) has long been known to influence the activity of the adenylate cyclase system and to produce an increase in cyclic AMP[7]. NaF has therefore been frequently used for analysis of the functioning of the β-adrenoceptor-adenylate cyclase system in in vitro experiments, in both homogenized cell-free preparations and isolated tissue preparations.

It has recently been demonstrated in vascular tissues that NaF produces contraction[8] and that NaF stimulates synthesis of prostaglandin (PG) I_2, a potent vasodilator, in both vascular smooth muscles[9] and endothelial cells[10]. Very recently, Cushing et al.[11] reported that NaF produces endothelium-dependent relaxation by releasing both EDRF and prostanoid in the coronary artery in a number of mammals.

It was observed in the present study that NaF produces relaxation of the rabbit ear artery, one of the most popular preparations for pharmacological and physiological studies[12], primarily by releasing EDRF.

Methods

Male Japanese White rabbits, weighing 2 kg, were anesthetized with pentobarbital sodium at 30 mg/kg i.v. and exsanguinated from the common carotid artery. The ear artery was dissected out and placed in chilled Krebs' bicarbonate solution. The composition of the solution (in mM) was: NaCl 119, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and glucose 11.1. The solution was previously aerated with a gas mixture of 95% O_2 and 5% CO_2.

Isolated arterial segments were cleaned of connective tissues under a dissecting microscope and made into ring preparations 4 mm in length. In some preparations, the endothelium was removed by gentle rubbing with a thin wooden stick. The absence of endothelium was confirmed by the absence of relaxation in response to acetylcholine- or calcium ionophore A23 187[13]. Each preparation was suspended in a tissue bath of 10 ml which contained Krebs' solution aerated with the gas mixture and maintained at 37 °C as described previously[3,13]. Preparations were given an optimum load of 2 g and equilibrated for 1.5 h before starting the experiments. Isometric tension was recorded on an ink-writing oscillograph (Nihon Kohden Kogyo, Tokyo, Japan, model WI-641G) via force-displacement transducers (Nihon Kohden Kogyo, Tokyo, Japan, model TB-611T).

Relaxation responses of blood vessels were examined after moderate contraction with 0.3 μM, which was about EC_{50}, of norepinephrine. The maximum relaxation of each preparation was obtained by adding 0.1 mM papaverine at the end of the experiment.