ELECTRICAL RESPONSES OF THE CARNIVOROUS CILIATE Didinium nasutum IN RELATION TO DISCHARGE OF THE EXTRUSIVE ORGANELLES

By RITSUO HARA, HIROSHI ASAI
Department of Physics, Waseda University, Tokyo 160, Japan

AND YUTAKA NAITOH
Institute of Biological Sciences, University of Tsukuba, Ibaraki 305, Japan

Accepted 28 May 1985

SUMMARY

1. The carnivorous ciliate Didinium nasutum discharged its extrusive organelles when a strong inward current was injected into the cell in the presence of external Ca²⁺ ions.
2. In the absence of external Ca²⁺ ions, the strong inward current produced fusion of the apex membrane of the proboscis.
3. External application of Ca²⁺ ions after the fusion of the apex membrane produced discharge of the organelles.
4. An increase in Ca²⁺ concentration around the organelles seems to cause discharge of the organelles.
5. Ca²⁺ concentration threshold for the discharge of the pexicysts seems to be lower than that for the toxicysts.
6. External Ca²⁺ ions were not necessary for discharge of the organelles upon contact with Paramecium.
7. Chemical interaction of the apex membrane with Paramecium membrane may cause intracellular release of Ca²⁺ ions from hypothetical Ca²⁺ storage sites around the organelles.
8. A small hyperpolarizing response seen before the discharge upon contact with Paramecium seems to correlate with the chemical interaction.
9. The depolarizing spike response associated with discharge of the organelles is caused by the depolarizing mechanoreceptor potential evoked by mechanical stimulation of the proboscis membrane by the discharging organelles.

INTRODUCTION

When a hungry carnivorous ciliate Didinium nasutum bumps against a prey, such as Paramecium and Tetrahymena, with the apex of its proboscis, it discharges the extrusive organelles, pexicysts and toxicysts, to capture and paralyse the prey. The captured prey is then drawn towards the proboscis by retracting the discharged organelles into the proboscis. Didinium then engulfs the prey through the proboscis (Balbiani, 1873; Thon, 1905; Jennings, 1906; Mast, 1909; Calkins, 1915; Reukauf,
1930; Dragesco, 1962; Schwartz, 1965). When the specimen collides with inedible ciliates, such as *Stylonychia* and *Euplotes* (Mast, 1909), or with the wall of its glass container, it does not discharge the organelles, but instead shows an avoiding reaction due to a transient reversed beating of cilia.

The importance of chemical factors of the prey in the food preference of *Didinium* has been emphasized for a long time (Seravin & Orlovskaya, 1977; Berger, 1980; Martin & Antipa, 1982). However, the chemical factors of the prey which interact with the proboscis to trigger the discharge of the extrusive organelles still remain unidentified.

Electron microscopical examinations of the proboscis and the extrusive organelles were reported first by Yagi & Shigenaka (1965). Wessenberg & Antipa (1970) demonstrated that when the organelles were discharged, the toxicysts penetrated into the cell body of *Paramecium*, while the pexicysts just attached to the cell surface. The mechanism by which the extrusive organelles are discharged still remains unsolved. Recently Hara & Asai (1980) demonstrated that *Didinium* showed a small hyperpolarization followed by a large depolarization in association with discharge of the extrusive organelles upon contact with *Paramecium*.

In order to understand the mechanisms by which *Didinium* recognizes and captures *Paramecium*, it is important to know the relationship between the electrical events in the membrane and the discharge of the extrusive organelles. We therefore examined the electrical responses of the membrane of *Didinium* to various kinds of stimuli in relation to discharge of the organelles. Parts of the present works have been reported in preliminary form elsewhere (Hara, Asai & Naitoh, 1980, 1981).

**Material and Methods**

Encysted *Didinium nasutum* obtained from Carolina Biological Supply Co. (Burlington, North Carolina) were excysted in a wheat-straw infusion. They were then fed on *Paramecium caudatum* which were cultured in a bacterized wheat-straw infusion. The specimens, which were kept in *Paramecium*-free culture medium for about 3 h, were washed in a standard saline solution (in mmol l\(^{-1}\): KCl, 1; CaCl\(_2\), 1 and Tris-HCl, 1; pH 7.2) and equilibrated in the solution for more than 5 min prior to experimentation. A single specimen of *Didinium* was impaled with two microelectrodes and examined by conventional electrophysiological techniques (Naitoh & Eckert, 1972).

As shown in Fig. 1, the anterior end of a *Paramecium* which was squeezed out through a glass capillary tip (about 30 \(\mu\)m inner diameter) was brought into contact with the apex of the proboscis of the impaled *Didinium* by driving the capillary toward the *Didinium* proboscis. The driving velocity was changed from 20 to 250 \(\mu\)m s\(^{-1}\) by changing the rotation velocity of the knob of the micromanipulator.

Discharge of the extrusive organelles was monitored photoelectrically. A phototransistor was placed at the edge of the apex of the proboscis of a magnified (\(\times 200\)) image of the impaled *Didinium*. The light intensity of the position where the transistor was placed changed when the discharge occurred. All the experiments were performed at room temperature, 20–25 °C.

The surface area of *Didinium* was estimated by assuming that the surface area coincides with that of a rotation body of a certain line. The line was estimated so as
Electrical responses in Didinium

Fig. 1. Schematic representation of Didinium nasutum impaled with an intracellular electrode (e) and its prey Paramecium caudatum captured in a glass capillary (c). The anterior end (a) of the prey is squeezed out through the opening of the capillary. The capillary is moved towards the impaled Didinium so that the anterior portion of the prey makes contact with the apex of the proboscis (p) of Didinium. tox, toxicsysts; pex, pexicysts. Drawing of Didinium by courtesy of Mr C. Pape.

Fig. 2. Schematic representation of a rotation body which approximates to the shape of the cell body of Didinium. The rotation body is obtained by rotating a thick line drawn on the x-y plane around the y axis. The three equations in the figure correspond to the three different portions of the line, respectively. Numbers in the figure indicate the approximate size of different portions of the body in \( \mu m \).

to approximate the profile of a photographed Didinium (Fig. 2). The thick line drawn on the x-y plane consists of three portions: the proboscis region, the trunk region and the posterior region. The rotation body is obtained by rotating the line around the y axis.
RESULTS

Basic electrophysiological characteristics of the membrane

Resting potential

The membrane potential of *Didinium* in the standard saline solution measured \(-40.7 \pm 3.1\) mV (mean and its standard deviation obtained from 20 different measurements). As shown in Fig. 3A (open circles), the membrane was depolarized when

![Graph A](image)

![Graph B](image)

**Fig. 3.** Concentration effects of both K\(^+\) (A) and Ca\(^{2+}\) (B) ions in the external solution on both the resting potential (open circles) and the saturated peak level of the action potential (solid circles) produced by an injection of outward current (duration, 200 ms) into the cell of *Didinium*. External Ca\(^{2+}\) concentration, \([\text{Ca}^{2+}]_o\), was kept constant at 1 mmol l\(^{-1}\) in A. External K\(^+\) concentration, \([\text{K}^+]_o\), was kept constant at 1 mmol l\(^{-1}\) in B. Each open circle is a mean obtained from five measurements and each closed circle is a mean of two measurements. Each vertical line shows the range of fluctuation in the measured values.
Electrical responses in Didinium

$\left[ K^+ \right]_0$ (external $K^+$ concentration) was increased in a range from 1 to 16 mmol l$^{-1}$, while $\left[ Ca^{2+} \right]_0$ (external $Ca^{2+}$ concentration) was kept constant at 1 mmol l$^{-1}$. The maximum slope of the potential change was about 30 mV per ten-fold change in $\left[ K^+ \right]_0$. The membrane was also depolarized by increasing $\left[ Ca^{2+} \right]_0$ in a range from 0·016 to 16 mmol l$^{-1}$, while $\left[ K^+ \right]_0$ was kept constant at 1 mmol l$^{-1}$. The maximum slope of the potential change was about 20 mV per ten-fold change in $\left[ Ca^{2+} \right]_0$ (Fig. 3B; open circles).

Membrane potential response to an electric current

When a weak inward current (less than 0·4 nA, duration 200 ms) was injected into the cell, the membrane potential level shifted exponentially with time as if it were a passive parallel (RC) network (Fig. 4 A, B). The input resistance of a typical cell was calculated from the steady value of the potential shift and the current intensity to be $4 \times 10^7$ Ω, which corresponds to the membrane resistance of $1 \times 10^8$ Ω cm$^2$. The input capacitance was calculated from the time constant of the passive exponential change (21 ms) to be $5 \times 10^{-10}$ F, which corresponds to a membrane capacitance of $1 \times 10^{-6}$ F cm$^{-2}$. The initial exponential response was followed by a time-dependent decrease in the potential shift, when the current intensity was higher (Fig. 4C).

When an outward current was injected into the cell, the initial passive exponential potential change was followed by an active spike-like depolarizing response (Fig. 4D, F). The peak level and the rate of rise of the response increased with increasing current intensity, approaching their saturated levels. As shown in Fig. 3B (closed circles), the peak level of the spike response shifted towards the depolarizing direction in accordance with increasing $\left[ Ca^{2+} \right]_0$ in a range from 0·016 to 16 mmol l$^{-1}$ at a constant $\left[ K^+ \right]_0$ (1 mmol l$^{-1}$). The slope of the shift was about 30 mV per ten-fold increase in $\left[ Ca^{2+} \right]_0$. In contrast, change in $\left[ K^+ \right]_0$ (1–16 mmol l$^{-1}$) at a constant $\left[ Ca^{2+} \right]_0$ (1 mmol l$^{-1}$) had little effect on the peak level (Fig. 3A; closed circles). Ciliary

Fig. 4. Electrical responses of Didinium nasutum (Vm) to an injection of electric current pulse (I) (duration, 200 ms). (A)–(C) The responses to an inward current; (D)–(F) the responses to an outward current. The baseline of trace I corresponds to the reference potential level. Vm, the first-order time derivative of Vm.
reversal was always accompanied by the spike response. More prolonged ciliary reversal was observed when the response was larger.

The spike response was usually followed by a rather slow depolarizing response. The slow response subsided slowly after the outward current was turned off (Fig. 4E,F).

Membrane potential response to mechanical stimulus

A specimen of Didinium was mechanically stimulated by hitting its surface with the tip (about 2 μm in diameter) of a glass microneedle, which was driven perpendicularly to the cell surface by electrical activation of a piezoelectric transducer upon which the needle was mounted. A weak mechanical stimulus produced a small, slow depolarizing mechanoreceptor potential (Fig. 5A). The amplitude of the receptor potential increased and was eventually followed by a rapid, spike-like depolarizing response when the stimulus intensity was increased. The peak of the rapid response increased with increasing stimulus intensity, approaching its saturation level (Fig. 5B–D).

The depolarizing mechanoreceptor potential was always evoked when a suprathreshold mechanical stimulus was applied anywhere to the cell surface. However the response was smaller when the stimulus was applied to the posterior surface than to the rest of the cell surface (Fig. 6).

Membrane potential responses and discharge of the extrusive organelles upon contact with Paramecium

Hyperpolarizing response

As shown in Fig. 7, an impaled Didinium showed a small hyperpolarization when the apex of its proboscis was touched by a captured Paramecium. The approximate time of the touch is shown as a black dot on each potential trace (Vm) in the figure. The hyperpolarization increased as Paramecium was pressed against the proboscis. The rate of the potential change became higher when Paramecium was pressed faster (approximate speed of touch was 20 μm s⁻¹ in A, 50 μm s⁻¹ in B and 250 μm s⁻¹ in C). The hyperpolarization was not seen when Paramecium was pressed against any part of the cell surface other than the apex.

Fig. 5. Electrical responses (Vm) of Didinium nasutum to a mechanical stimulation of the central portion of the cell. Sm, relative voltage of the electric pulse (duration, 3-2 ms) applied to a piezoelectric element for driving the stimulation microneedle against the cell surface. The baseline of the Sm trace corresponds to the reference potential level. Vm, the first-order time derivative of Vm. Intensity of the mechanical stimulation was progressively increased from A to D.
Fig. 6. Electrical responses (Vm) of *Didinium nasutum* to a mechanical stimulation. A mechanical stimulation was applied to the basal region of the proboscis (A), to the central portion of the cell body (B) and to the posterior portion of the cell body (C). Sm, relative voltage of the electric pulse (duration, 3-2 ms) applied to the piezoelectric driver of the stimulation needle. The baseline of the Sm trace corresponds to the reference potential level. Vm, the first-order time derivative of Vm.

Fig. 7. The electrical responses (Vm) of *Didinium nasutum* upon contact with *Paramedum* at three different contact speeds: (A) about 20 μm s⁻¹; (B) about 50 μm s⁻¹; (C) about 250 μm s⁻¹. The dotted line (O) shows the reference level for Vm. A black dot on each Vm trace indicates an approximate moment when the anterior end of *Paramedum* touch the apex of the proboscis of *Didinium*.

When the hyperpolarization reached a certain level, *Didinium* discharged the extrusive organelles, showing a large depolarizing response (Fig. 7). It is noteworthy that *Didinium* discharged only pexicysts when the contact with *Paramecium* was very slow and slight (Fig. 7A). *Paramecium* was not paralysed by bombardment with the pexicysts, but was paralysed by the toxicysts.

*Didinium* showed a similar hyperpolarizing response and discharge of the organelles upon contact with a nickel-paralysed, non-beating *Paramecium* (Kuzunicki, 1963) or with an ethanol-deciliated *Paramecium* (Machemer & Ogura, 1979) as it did upon contact with a normal *Paramecium*. *Didinium* scarcely discharged the organelles upon contact with a Triton X-100-extracted (concentration of Triton X-100, 0.01% vol; extraction time, 30 min at 0°C; Naitoh & Kaneko, 1972) or a heat-killed (1 min at 50°C) *Paramecium*. The hyperpolarizing response of *Didinium* to these dead *Paramecium*, if it occurred, was very small and was followed by incomplete discharge of the pexicysts. *Didinium* showed neither hyperpolarization nor discharge of the organelles upon contact with the tip of a glass rod similar in size to the anterior end of *Paramecium*. 
Fig. 8. Simultaneous recording of the electrical response (Vm) of *Didinium nasutum* and the electrical signal (D) corresponding to the discharge of the extrusive organelles produced by the contact with *Paramecium*.

*Didinium* also showed the hyperpolarization and the following discharge of the extrusive organelles upon contact with *Tetrahymena*, another well known prey item of *Didinium*, in a manner similar to that shown for *Paramecium*.

**Depolarizing response**

As described in the preceding section, discharge of the extrusive organelles was accompanied by a membrane depolarization. This response consisted of two components, an initial rapid, spike-like component and a long-lasting component.

The time relationship between the discharge and the potential response is clearly demonstrated in Fig. 8, where the potential response (Vm) and the electrical signal corresponding to the discharge of the organelles (D) are displayed together on a CRO screen and recorded. As shown in the figure, the membrane depolarization occurred almost simultaneously with the start of the discharge of the extrusive organelles, which is shown as an upward deflection in trace D. The discharge was never preceded by the depolarization.

The rapid depolarization seen in association with discharge of only pexicysts (Fig. 7A) was identical with that seen in association with a complete discharge of the extrusive organelles (Fig. 7B,C). However, the following long-lasting component of the depolarizing response was different in these two cases. When both pexicysts and toxicysts were discharged, the membrane depolarization lasted for several seconds, showing a small perturbation in the potential level (Fig. 7B,C). When only the pexicysts were discharged, the depolarization subsided within 500–800 ms, followed by repetitive depolarizations. The amplitude and duration of the repetitive depolarizations decreased gradually with time (Fig. 7A).

It is interesting to note that ciliary beating of *Didinium* had been inactivated during the long-lasting depolarization after complete discharge of the organelles. The beating amplitude was small and the beating direction had been kept slightly reversed. In contrast, conspicuous ciliary reversal took place in association with each depolarization of the repetitive response seen after discharge of pexicysts alone.
Electrical responses in Didinium

Fig. 9. Electrical responses (Vm) of Didinium nasutum upon contact with Paramecium in various solutions with different [Ca\(^{2+}\)]\(_o\): (A) 10\(^{-3}\) mol l\(^{-1}\); (B) 10\(^{-4}\) mol l\(^{-1}\); (C) 10\(^{-6}\) mol l\(^{-1}\); (D) less than 10\(^{-8}\) mol l\(^{-1}\). [K\(^+\)]\(_o\) was kept constant at 1 mmol l\(^{-1}\) throughout. [Ca\(^{2+}\)]\(_o\) was controlled by 5 mmol l\(^{-1}\) EGTA-Ca buffer in solution B, C and D. O, reference level for Vm; Vm, first-order time derivative of Vm. A black dot on each Vm trace shows approximate moment when Paramecium touched the proboscis.

Fig. 10. Electrical responses (Vm) of Didinium nasutum upon contact with Paramecium in various solutions with different [K\(^+\)]\(_o\): (A) 1 mmol l\(^{-1}\); (B) 4 mmol l\(^{-1}\); (C) 8 mmol l\(^{-1}\); (D) 16 mmol l\(^{-1}\); (E) 32 mmol l\(^{-1}\); (F) 50 mmol l\(^{-1}\). [Ca\(^{2+}\)]\(_o\) was kept constant at 1 mmol l\(^{-1}\) throughout. O, reference level for Vm; Vm, first-order time derivative of Vm.

Effects of [Ca\(^{2+}\)]\(_o\) and [K\(^+\)]\(_o\)

As shown in Fig. 9, both the initial spike response and the following long-lasting depolarization were markedly depressed by lowering [Ca\(^{2+}\)]\(_o\) (10\(^{-6}\)–10\(^{-8}\) mmol l\(^{-1}\) or less) at constant [K\(^+\)]\(_o\) (1 mmol l\(^{-1}\)), whereas the preceding hyperpolarizing response was little affected. The initial spike response was followed by a long-lasting hyperpolarization, instead of a depolarization, when [Ca\(^{2+}\)]\(_o\) was lower than 10\(^{-6}\) mol l\(^{-1}\) (Fig. 9C, D). It should be noted that the discharge of extrusive organelles upon contact with Paramecium was not affected by lowering [Ca\(^{2+}\)]\(_o\).

When [K\(^+\)]\(_o\) was increased (1–50 mmol l\(^{-1}\)), the electrical responses seen upon contact with Paramecium were greatly diminished (Fig. 10). However, the discharge of the extrusive organelles still occurred in high-K\(^+\) solutions. In fact, the membrane showed a small hyperpolarization instead of a depolarization in association with the
Fig. 11. Effects of the membrane depolarization and hyperpolarization produced by injection of electric currents on the electrical responses of Didinium nasutum in association with the discharge of the extrusive organelles upon contact with Paramecium. (A) Membrane potential response (Vm) to a depolarizing (outward) conditioning current (I) (duration, 900 ms). (B) Electrical response in the depolarized specimen associated with the discharge. Approximate moment of the discharge is indicated by an arrow. (C) membrane potential response to a hyperpolarizing (inward) conditioning current (I) (duration, 900 ms). (D) Electrical response in the hyperpolarized specimen associated with the discharge. O, reference level for Vm; Vm, first-order time derivative of Vm.

Table 1: Discharge of the extrusive organelles of Didinium nasutum in response to an injection of strong inward electric current

<table>
<thead>
<tr>
<th>Duration (ms)</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
<td>Ca</td>
<td>-</td>
<td>Ca</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>100</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>200</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>300</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>500</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1000</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

-, Discharge did not take place; +, only pexicysts discharged; ++, both pexicysts and toxicysts discharged. At least four different specimens were examined and the results were always consistent.
discharge of the organelles, when \([K^+]_o\) was as high as 50 mmol l\(^{-1}\), hence the resting potential level was slightly positive (Fig. 10F). No initial spike response was observed in such a high-\(K^+\) solution (Fig. 10E,F).

**Effect of transmembrane current**

Neither an inward current, to hyperpolarize the membrane of *Didinium* beyond the peak level of the initial hyperpolarizing response, nor an outward current, to depolarize the membrane beyond the peak level of the depolarizing response, caused the discharge of the extrusive organelles (Fig. 11A,C). A specimen of *Didinium* which was depolarized or hyperpolarized by sustained current injection discharged its extrusive organelles upon contact with *Paramecium* as did the normal specimen. In the depolarized specimen the discharge was associated with a small, spike-like depolarizing response which is indicated by an arrow in Fig. 11B. The spike response was followed by a gradual decrease in the level of membrane depolarization. In the hyperpolarized specimen, the discharge was associated with a marked depolarizing spike (Fig. 11D). The membrane had been kept somewhat depolarized after the discharge, even though the cell had been subjected to a sustained inward current.

**Discharge of the extrusive organelles in response to an extraordinarily strong stimulus**

When the apex of the *Didinium* proboscis was strongly hit by the tip of a glass needle driven in a direction parallel to the longitudinal axis of the specimen at a speed of more than 4 mm s\(^{-1}\), which was about 40 times as fast as the normal speed at which *Paramecium* captured in a capillary were driven against the proboscis, the pexicysts were discharged in association with a depolarizing response without showing the initial hyperpolarizing response. Sometimes the discharge of the pexicysts was followed by a partial, incomplete discharge of the toxicysts.

When a very strong inward current (up to 50 nA) was injected into the cell of *Didinium* in the standard saline solution, the specimen discharged the extrusive organelles. In contrast, an outward current injection did not produce discharge of the organelles. When the electric charge brought into the cell was relatively small (2.5 \(\times\) \(10^{-10}\)-4 \(\times\) \(10^{-9}\) C), only pexicysts were discharged (Table 1). The discharge of both pexicysts and toxicysts took place when the electric charge was greater than \(10^{-8}\) C. The threshold charge tended to decrease with increasing current intensity. When the current intensity was less than about 15 nA, a sustained injection of inward current did not cause the discharge of the organelles.

When \([Ca^{2+}]_o\) was lowered to less than \(10^{-8}\) mol l\(^{-1}\) by adding 5 mmol l\(^{-1}\) EGTA to the external solution, a strong inward current injection did not cause organelle discharge but did produce fusion of the apex membrane. The threshold charge for the membrane fusion was almost identical with that for the discharge of pexicysts in the presence of \(Ca^{2+}\). When the intensity of the inward current was increased, the degree of fusion increased and the proboscis moved as though to engulf a *Paramecium*. It is interesting to note here that discharge of the organelles occurred when 1 mmol l\(^{-1}\) \(Ca^{2+}\) was introduced into the external solution after the apex membrane had been fused by inward current injection. Only pexicysts were discharged when the degree of fusion was small, whereas both pexicysts and toxicysts were discharged when the fusion was large.
DISCUSSION

Basic electrophysiological properties

The electrophysiological properties of the membrane of Didinium are similar to those of other freshwater ciliates (Naitoh, 1982, 1984). The resting membrane is depolarized by increasing \([K^+]_o\) or \([Ca^{2+}]_o\) (Fig. 3). A membrane depolarization by an outward current injection produces a \(Ca^{2+}\)-dependent depolarizing spike response which is followed by a slow depolarizing response (Fig. 4). The depolarizing response is accompanied by a transient ciliary reversal. These membrane properties of Didinium are consistent with those reported recently by Pape (1982) and Pape & Machemer (1982).

A mechanical stimulation of any portion of the cell body of Didinium produces a depolarizing mechanoreceptor potential which causes a \(Ca^{2+}\)-dependent depolarizing spike response (Fig. 5). A hyperpolarizing mechanoreceptor potential, such as that seen in the posterior membrane of Paramecium (Naitoh & Eckert, 1969), was not observed in Didinium. The ions which carry the receptor current have not been identified.

Discharge of the extrusive organelles and \(Ca^{2+}\) ions

The presence of \(Ca^{2+}\) ions in the external solution was essential for the discharge of the extrusive organelles by a very strong inward current injection. Part of the current must be carried by the external \(Ca^{2+}\) ions, and therefore causes an increase in \(Ca^{2+}\) concentration in the proboscis. The discharge was also produced by applying \(Ca^{2+}\) ions externally after the apex membrane of the proboscis had been fused by a strong inward current in the absence of \(Ca^{2+}\) ions. These results strongly suggest that an increase in \(Ca^{2+}\) concentration in the proboscis causes the discharge of the extrusive organelles.

On the other hand, external \(Ca^{2+}\) ions were not necessary for the discharge upon contact with Paramecium. Therefore, it is conjectured that contact of Paramecium with the apex membrane causes an increase in \(Ca^{2+}\) concentration in the proboscis, probably by the release of \(Ca^{2+}\) ions from a hypothetical \(Ca^{2+}\) storage site in the vicinity of the extrusive organelles. Yagiu & Shigenaka (1965) reported the presence of a vacuolar system near the extrusive organelles, which is a possible candidate for the \(Ca^{2+}\) storage site.

The membrane potential responses associated with discharge of the extrusive organelles

The membrane potential shift by an electric current injection in both the depolarizing and hyperpolarizing direction did not produce discharge of the extrusive organelles, even though the shift exceeded the peak level of the membrane potential responses associated with the Paramecium-induced discharge of the extrusive organelles. Moreover, the depolarizing response was simultaneous with or preceded by discharge of the extrusive organelles (Fig. 8). These facts suggest that the membrane potential responses seen upon contact with Paramecium do not act as triggers for
discharge of the extrusive organelles. The initial rapid part of the discharge-associated depolarizing response consists of first - rather slow - and subsequent - faster - components, which are clearly seen as two consecutive peaks in its Vm trace, similar to the depolarizing response to a mechanical stimulation (compare Fig. 7 with Fig. 5). In the depolarizing response to a mechanical stimulus, the first component corresponds to the mechanoreceptor potential and the second component to the Ca\(^{2+}\)-dependent spike response. These facts strongly suggest that the initial rapid part of the discharge-associated depolarizing response is caused by a mechanical stimulation of the proboscis membrane by the extruding organelles.

The hyperpolarizing response seen upon contact with *Paramecium* before discharge of the organelles became ambiguous and, in most cases, the discharge did not take place, when *Paramecium* was extracted in Triton X-100 or killed by heat before the contact. Therefore, the hyperpolarization seems to be correlated with the interaction of the apex membrane with some chemical factors in the *Paramecium* membrane, which are easily extracted by Triton X-100 or denatured by heat. When the speed of contact of *Paramecium* with the apex membrane was higher, the rate of the hyperpolarization was higher (Fig. 7). This fact also supports the idea that the hyperpolarization is caused by the interaction of the two membranes.

**Separation of the discharge of pexicysts from the discharge of toxicysts**

When the amount of positive charge, hence the amount of Ca\(^{2+}\), driven into the cell by a strong current was relatively small, only pexicysts were discharged. Therefore, the Ca\(^{2+}\) concentration threshold for the discharge of pexicysts seems to be lower than that for toxicysts. Very slight contact of *Paramecium* with the apex membrane produced discharge of pexicysts alone. This seems to suggest that the degree of contact of *Paramecium* with the apex membrane determines the amount of Ca\(^{2+}\) released from the hypothetical Ca\(^{2+}\) storage site.

Our previous view that Ca\(^{2+}\) entry into the proboscis in association with the Ca-dependent depolarizing response causes the discharge of the organelles (Hara & Asai, 1980) is no longer credible.

This work was supported by grants from the Ministry of Education of Japan to Dr H. Asai and Dr Y. Naitoh (411802, 510902, 56105002) and from Mitsubishi Foundation to Dr Y. Naitoh. The first draft of the present paper was prepared during Dr Naitoh’s stay in Ruhr University, Bochum under the sponsorship of Deutsche Forschungsgemeinschaft (SFB 114, TP A5). We thank Professor H. Machemer and Mr C. Pape of Ruhr University, and Dr B. Martinac of the University of Wisconsin for their critical reading of the manuscript.

**REFERENCES**


