Electrical Perception of “Death Message” in *Chara*: Analysis of Rapid Component and Ionic Process

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Electrical response upon wounding was analyzed in *Chara corallina*. A specimen comprising two adjoining internodal cells was prepared. One cell (victim cell) was killed by cutting and any changes in the membrane potential of the neighboring cell (the receptor cell) were measured. Upon cutting the victim cell, the receptor cell generated four kinds of depolarizations: (1) rapid component, (2) slow and long-lasting component, (3) action potential and (4) small spike. Rapid and slow components were observed in most cells. On the other hand, the action potential and small spike were not always ubiquitous among specimens. When an action potential was generated just after cutting the victim cell, the rapid component could not be observed due to masking by the action potential. It was suggested that both rapid and slow components were generated at the nodal end. On the other hand, action potentials were thought to be generated at the flank of the receptor cell. High turgor pressure of the cell was necessary for generating both rapid and slow components. Experiments under K\(^{+}\)-induced depolarization unequivocally showed that the Cl\(^{-}\) channel at the nodal end of the receptor cell was activated upon cutting the victim cell.

**Keywords**: *Chara* — Chloride channel — Death message — Membrane potential — Wounding response.

Abbreviations: APW, artificial pond water; [Ca\(^{2+}\)], concentration of free Ca\(^{2+}\) in external medium; [Cl\(^{-}\)], concentration of Cl\(^{-}\) in external medium; \(\Delta E_{\text{rap}}\), amplitude of rapid component; \(\Delta E_{\text{sl}}\), amplitude of slow component; \(E_{\text{m,ap}}\), electrode in pool A; \(E_{\text{m,sl}}\), potential difference between pools A and B; \(E_{\text{m,c}}\), electrode in pool C; \(E_{\text{m,mem}}\), membrane potential; \(E_{\text{m,rest}}\), resting membrane potential; \(E_{\text{m,peak}}\), peak potential of rapid component; \(L_{\text{c,rap}}\), length of portion of receptor cell in pool B; \(L_{\text{c,sl}}\), length of portion of receptor cell in pool C; R cell, receptor cell; V cell, victim cell.

**Introduction**

Wounding is one of the most serious stresses for plants. Generation of an electrical response upon wounding has been reported in various plants (Mertz and Higinbotham 1976, Meyer and Weisenseel 1997, Stahlberg and Cosgrove 1994, Frachisse et al. 1985, Fromm and Eschrich 1993, Julien et al. 1991, Julien and Frachisse 1992, Rhodes et al. 1996, Robin and Bonnemain 1985, Robin 1985). However, the physiological role of the electrical signal had been obscure but for some instances such as *Mimosa pudica*, in which rapid movement of the leaf is triggered by an electrical signal generated upon wounding (Sibaoka 1953). Involvement of an electrical signal in systemic synthesis of protease inhibitor upon wounding in tomato attracted much attention among botanists (Wildon et al. 1992, Stankovic and Davies 1996). It is suggested that cells neighboring the killed cells first perceive “death message”, resulting in generation of electrical signal. In higher plants, it is difficult to isolate the electrical signal of a target cell from those of surrounding cells. Characeae has been one of the most suitable materials for electrophysiology of plant cells (Shimmen et al. 1994, Tazawa and Shimmen 2001). By taking advantage of this material, we have analyzed the mechanism of receptor potential generated upon mechanical stimulation (Shimmen 1996, Shimmen 1997a, Shimmen 1997b, Shimmen 1997c, Shimmen 2001c, Shepherd et al. 2001).

In the previous paper, I reported that Characeae can be a useful material for analyzing an electrical signal upon wounding (Shimmen 2001a). I prepared a specimen comprising two adjoining internodal cells. One cell (the victim cell) was killed and changes in the membrane potential of the neighboring cell (the receptor cell) was analyzed. When the victim cell was killed by cutting, at least one of three kinds of response was induced; (1) slow and long lasting depolarization, (2) action potentials and (3) small spikes. Slow depolarization was induced in all specimens examined. Action potentials and small spikes were superimposed to the slow depolarization, but they were not always ubiquitous among specimens. Therefore, slow depolarization was a target of my study. It was found that high cell turgor pressure was necessary for generation of the response and that the response was generated at the nodal end of the receptor cell (Shimmen 2001a).

Further analysis showed the presence of an additional ubiquitous component of electrical response. Just after cutting the victim cell, a rapid depolarization and a following rapid repolarization was generated before start of the slow depolarization or action potential. This component had been occasionally recorded in my previous study (arrowhead in Fig. 3 of Shimmen (2001a)). However, I did not pay my attention to this response. In the present study, I found that this is also an ubiquitous response generated by the receptor cell upon death of the
Death message in Chara

When an action potential was generated at the very beginning of the response, this rapid component was masked. I focused my attention on characterization of this rapid component. In addition, the ionic mechanism of depolarization induced by wounding was also a target of the present study.

Results

Response of receptor cell upon cutting victim cell

Specimens consisting of two internodal cells were prepared by removing neighboring internodal cells and branchlet cells with scissors (Shimmen 2001a). They were mounted on a chamber consisting of two pools, A and B (Fig. 1A). A specimen was mounted in the chamber so that a part of the R cell

Fig. 1 Chamber for standard measurement and example of record showing three kinds of depolarizing response. (A) Chamber used for standard measurement. A specimen was mounted so that the receptor cell (R cell) was partitioned into pools A and B. Potential difference between pools A and B was measured by electrodes (E_A, E_B). The victim cell (V cell) was killed by cutting with scissors and the electrical response of receptor cell (R cell) was analyzed. The length of partition between two pools (P) was 10 mm. The length of the portion of the R cell situated in pool B (L_B) was about 2 mm in most cases, but occasionally about 20 mm. A layer of very thin nodal cells is intercalated between R and V cells. (B) Example of recording. L_B was about 2 mm. At the time shown with an arrowhead, the V cell was cut. a, start of slow component; b, an action potential generated at the cell portion in the pool B; c, an action potential generated at the cell portion in the pool A; d, small spikes; e, repetitive bipolar action potentials. For further explanation, see the text.

Fig. 2 Identification of rapid component. Experiments were carried out as shown in Fig. 1A. L_B was about 2 mm. Four examples are shown (A–D). At the time shown with an arrowhead, the V cell was cut. Downward small arrow, rapid component; downward white arrow, action potential generated at the portion of the R cell in the pool B; upward white arrow, action potential generated at the portion of the R cell in the pool A; star, shoulder due to generation of slow component. For further explanation, see the text.
Identification of cell portion generating rapid and slow components. The experiment was carried out as shown in Fig. 1A. \( L_{AB} \) was about 20 mm. At the time shown with an arrowhead, the V cell was cut. a, rapid component; b, start of slow component; c, action potential generated at the portion of the R cell in the pool B; d, action potential generated at the portion of the R cell in the pool A. For further explanation, see the text.

was situated in the groove of the partition (P) that separated pools A and B. The R cell was sealed into the groove with white Vaseline. The length of P was 10 mm. The length of the portion of the R cell in pool B (\( L_{AB} \)) was about 2 mm in most cases, occasionally 20 mm. Pools A and B were filled with artificial pond water (APW) buffered with 5 mM HEPES-Tris (pH 7.0). The potential difference between the two pools (\( E_{AB} \)) was measured with agar electrodes which were connected to Ag-AgCl wire via 3 M KCl solution (\( E_A, E_B \)).

As reported previously, three kinds of depolarizing responses were generated by the R cell upon cutting the V cell with scissors (Shimmen 2001a). The result shown in Fig. 1B was not always a typical example, but clearly showed three kinds of depolarizing responses. When the V cell was cut with scissors (arrowhead), \( E_{AB} \) changed to the positive direction, and the slow component started (a). An action potential was generated at the cell portion in the pool B (b) and it was transmitted to the cell portion in the pool A (c) (bipolar action potential). Following another bipolar action potential, a train of small spikes was generated (d). After a rest of about 1 min, repetitive firing of bipolar action potentials started again (e). As reported in the previous paper, slow and long lasting depolarization was observed in all specimens examined. On the other hand, the action potential and small spike were not always ubiquitous among specimens (Shimmen 2001a).

Identification of rapid component

During the study, I noticed that a rapid component was generated just after cutting the V cell in most specimens. In the previous study, a rapid component was occasionally recorded (arrowhead of Fig. 3 and 7 in Shimmen (2001a)). However, this rapid component was not recorded in most cases, because a main target for analysis was a slow component and slower chart speed of the pen-writing recorder was employed (Fig. 1B). When the electrical response was recorded with a higher chart speed, rapid change was recorded. \( E_{AB} \) rapidly changed to the positive direction upon cutting the V cell (Fig. 2A downward small arrow). After a peak, \( E_{AB} \) quickly came back to the negative direction. During a repolarizing phase of the rapid component, the slow component was started and then an action potential (downward white arrow) was generated at the cell portion in the pool B. After a slow component continued for a while (star), an action potential was generated at the cell portion in the pool A (upward white arrow). Cytoplasmic streaming of the R cell at the flank near the node did not stop at the moment of generation of the rapid component (downward white arrow) but stopped upon generation of an action potential (downward white arrow). In Fig. 2B, a rapid component (downward small arrow) was successively followed by an action potential at the cell portion in the pool B (downward white arrow) and that in the pool A (upward white arrow). The slow component could be observed as a shoulder between action potentials (star). In Fig. 2C, the time gap between rapid component (downward small arrow) and action potential (downward white arrow) was very short. In Fig. 2D, the rapid component was completely masked by an action potential (downward white arrow). Thus, this rapid component seemed to be an ubiquitous response, although it was sometimes masked by an action potential (Fig. 2D).

### Site of depolarization

The amplitudes of rapid component (\( \Delta E_{rap} \)) and that of slow component (\( \Delta E_{sl} \)) were analyzed by changing the configuration of mounting the specimen in a measuring chamber (Fig. 1A). The measurement of the amplitude of the rapid component and that of the slow component was disturbed by generation of an action potential. When an action potential was not generated, the amplitudes of both rapid and slow components could be easily measured. As shown in Fig. 2A and B, the amplitude of both components could be also measured. The amplitude of the slow component was measured after the end of an action potential (star). As shown in Fig. 2C, the ampli-

### Table 1 Effect of length of cell portion in pool B (\( L_{AB} \)) on amplitude of rapid (\( \Delta E_{rap} \)) and slow (\( \Delta E_{sl} \)) components

<table>
<thead>
<tr>
<th>Exp.</th>
<th>( L_{AB} ) (mm)</th>
<th>( \Delta E_{rap} ) (mV)</th>
<th>( \Delta E_{sl} ) (mV)</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>121±6</td>
<td>143±6</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>59±5</td>
<td>75±2</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>-5</td>
<td>194±9</td>
<td>225±8</td>
<td>11</td>
</tr>
</tbody>
</table>

\( n \): number of specimens examined. Experiments 1 and 2 were carried out as shown in Fig. 1A and Experiment 3 as shown in Fig. 4A.
The amplitude of the rapid component could be measured but that of slow component could not. The responses as shown in Fig. 2C and D were not used for the analysis.

Specimens were mounted as shown in Fig. 1A and the effect of changing the length of the portion of the R cell in the pool B ($L_B$) was analyzed. An example of results obtained with $L_B$ of 20 mm is shown in Fig. 3. Upon cutting the V cell (arrowhead), a rapid component was generated (a), followed by a slow component (b). Superimposed to the slow component, action potentials were generated first at the cell portion in the pool B (c) and then at the pool A (d). Amplitudes of both rapid and slow components in Fig. 3 ($L_B = 20$ mm) seem small compared with those in Fig. 2 ($L_B = 2$ mm). Statistical results are summarized in Table 1. As is evident, the amplitude of both components was significantly decreased by increasing $L_B$ (compare Exp. 1 and 2). A specimen was mounted in the chamber so that the node between two cells was located in the grooved partition (Fig. 4A). The node was completely embedded in white Vaseline. I made the assumption that the electrical signal measured upon cutting the V cell mainly reflected the response at the nodal end of the R cell (Shimmen 2001a). The amplitude of both rapid and slow components was significantly increased (Fig. 4B and Table 1 Exp. 3). In most experiments, an action potential was not generated.

Analysis by guillotine method

When the victim cell was killed by cutting with scissors, small artificial noise due to insertion of the tip of scissors into...
the bathing solution was occasionally recorded just before start of the electrical response of the R cell. It was suspected that a rapid component analyzed in the present study might be a noise due to insertion of scissors into the bathing medium. To exclude this possibility, a guillotine method was developed (Fig. 5A). A specimen was mounted in a same configuration as shown in Fig. 1A. A razor blade (Ra) was attached to a sheet of polyacrylate resin (weight 5.6 g) (Pl). The polyacrylate sheet was connected to a strip of polyester thread (T) and suspended over the V cell. The position to drop the razor blade was fixed with a guide made of polyacrylate (not shown). Before dropping, the edge of the razor blade was dipped into the bathing medium and held in place using the thread. The polyacrylate sheet with the razor blade was dropped by releasing the thread. Thus, possible noise due to insertion of a metal into the bathing medium upon cutting the V cell was avoided. An example of the measurement is shown in Fig. 5B. Upon cutting the V cell (arrowhead), a rapid component was generated (a). During the repolarizing phase of the rapid component, a slow component started (b) and \( E_{AB} \) gradually changed to the positive direction. Superimposed to the slow depolarization, an action potential was generated at the cell portion in the pool B (c) and then at the cell portion in the pool A (d). Various types of response as shown in Fig. 2 were also observed by the guillotine method (data not shown).

**K-anesthesia method**

The effect of ion channel inhibitors was analyzed. The amplitude of the slow depolarization was dependent on the level of the resting membrane potential (\( E_{r} \)) (Shimmen 2001a). When an ion channel inhibitor was added to the bathing medium of *Chara corallina*, \( E_{m} \), sometimes depolarized (Shimmen 1997a). Therefore, the possibility arose that an inhibitor decreases the amplitude of the depolarizing responses by affecting \( E_{m} \). In addition, it is necessary to measure \( E_{m} \), not the amplitude of the responses, in order to analyze the results based on equilibrium potential for ions across the plasma membrane. Although, a microelectrode is generally used to measure \( E_{m} \), it is impractical in the present experiments, since the R cell moves or vibrates to some extent upon cutting the V cell. Therefore, \( E_{m} \) was measured using the K-anesthesia method (Shimmen et al. 1976). For the measurement, a chamber composed of three pools, A, B and C, was prepared (Fig. 6A). The length of pool B was 3 mm. A specimen was mounted so that the free node of the R cell was in the pool A and the node sandwiched between R and V cells was in pool C. Pools A and C were filled with APW (pH 7.0). Pool B was filled with 100 mM KCl. In the presence of \( K^{+} \) of a high concentration, \( E_{m} \) is close to 0 mV (Shimmen 2001b). Therefore, the potential difference measured between pools B and C (\( E_{BC} \)) reflects \( E_{m} \) of the cell portion in the pool C (K-anesthesia method, Shimmen et al. 1976).

A control measurement using the K-anesthesia method is shown in Fig. 6B. \( E_{m} = -212 \text{ mV} \) before cutting the V cell was.\( E_{m} \) was measured using the K-anesthesia method (Shimmen et al. 1976). The potential difference measured between pools B and C (\( E_{BC} \)) reflects \( E_{m} \) of the cell portion in the pool C (K-anesthesia method, Shimmen et al. 1976).

Upon cutting the V cell (arrowhead), a rapid component was induced (a), followed by a slow component (b) and an action potential (c). Various types of responses as shown in Fig. 2 were also obtained by this method (data not shown). Thus, the mode of response measured by the K-anesthesia method was fundamentally the same as that obtained by the experiments to bath a specimen in APW.

**Ion channel inhibitors**

Using the K-anesthesia method, the effects of inhibitors of stretch-activated channel (100 \( \mu M \) Ga\(^{3+} \)), Ca\(^{2+} \) channel (100 \( \mu M \) LaCl\(_3\), 100 \( \mu M \) nifedipine), and Cl\(^− \) channel (1 mM anthracene-9-carboxilic acid, 100 \( \mu M \) ethacrynic acid) were examined. Pools A, B and C of the chamber (Fig. 6A) were filled with APW (pH 7.0), 100 mM KCl and APW (pH 7.0) supplemented with one of inhibitors, respectively. After \( E_{m} \) attained a stable value (after about 20 min), the V cell was cut with scissors. Anthracene-9-carboxilic acid and nifedipine slightly depolarized \( E_{m} \). The effects of all inhibitors on peak
Effect of extracellular Ca\(^{2+}\)

In the next experiment, the effect of Ca\(^{2+}\) concentration in the external medium ([Ca\(^{2+}\)]) was examined by the K-anesthesia method (Fig. 7). At first, pools A and C were filled with APW (pH 7.0), and pool B with 100 mM KCl (Fig. 6A). After \((E_m)_{\text{rap}}\), attained a stable value, the medium in the pool C was replaced with APW of various [Ca\(^{2+}\)]. \((E_m)_{\text{rap}}\) depolarized progressively with time after bathing cells in the medium of low [Ca\(^{2+}\)]. Therefore, experiments were carried out about 5 min after replacement of the external medium of the pool C. \((E_m)_{\text{rap}}\) was lower than [Ca\(^{2+}\)] that of pCa 7 (Fig. 7). The peak potentials of both components significantly decreased due to depolarization of \((E_m)_{\text{rap}}\) and change of the peak potentials of responses to the negative direction.

### Effect of extracellular Cl\(^{-}\)

The effect of Cl\(^{-}\) concentration in the external medium ([Cl\(^{-}\)]) was examined (Table 2). When choline chloride of high concentration was added to the external medium, \((E_m)_{\text{rap}}\) gradually depolarized with time. To minimize this depolarization, specimens were pre-incubated in APW supplemented with 1 mM CaCl\(_2\) at least overnight, and 1 mM CaCl\(_2\) was added to the bathing medium for the measurement. Pools A and C of the chamber (Fig. 6A) were filled with APW (pH 7) supplemented with 1 mM CaCl\(_2\) and 90 mM sorbitol, and the pool B with 100 mM KCl. After \((E_m)_{\text{rap}}\), attained a stable value, the bathing medium of the pools A and C was replaced with APW (pH 7) supplemented with 1 mM CaCl\(_2\) and 50 mM choline chloride. After about 5 min, the value of \((E_m)_{\text{rap}}\) was recorded and the V cell was cut. In the experiments to analyze the effect of 100 mM choline chloride, 180 mM sorbitol was used instead of 90 mM sorbitol. The value of \((E_m)_{\text{rap}}\), \((E_m)_{\text{sl}}\), and \((E_m)_{\text{sl}}\) in the presence of 50 mM choline chloride were close to those of the control experiments (Table 2 Exp. 1, 2). However, \((E_m)_{\text{rap}}\) was depolarized after incubation in the medium containing 100 mM choline chloride. The peak values of both components shifted to the negative direction in the presence of 100 mM choline chloride (Table 2 Exp. 3).

### Effect of cell turgor pressure

The effect of sorbitol was also examined. Pools A and C of the chamber (Fig. 1A) were filled with APW (pH 7) supplemented with 1 mM CaCl\(_2\) and sorbitol of various concentrations. Pool B was filled with 100 mM KCl. After \((E_m)_{\text{rap}}\), attained a stable level, the V cell was cut. Addition of either 180 or 230 mM sorbitol to the bathing medium rather shifted \((E_m)_{\text{rap}}\) to the negative direction (Table 2 Exp. 5, 6). The peak potentials of both components significantly shifted to the negative direction in the presence of 230 mM sorbitol (Table 2 Exp. 6).

### Table 2  Effects of choline chloride and sorbitol on resting membrane potential \((E_m)_{\text{rap}}\) and slow components \((E_m)_{\text{sl}}\)

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Sorbitol (mM)</th>
<th>Choline chloride (mM)</th>
<th>((E_m)_{\text{rap}}) (mV)</th>
<th>((E_m)_{\text{sl}}) (mV)</th>
<th>((E_m)_{\text{sl}}) (mV)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>-210±3</td>
<td>-75±3</td>
<td>-59±3</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>50</td>
<td>-203±3</td>
<td>-77±5</td>
<td>-64±5</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>100</td>
<td>-174±4</td>
<td>-87±6</td>
<td>-85±7</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>0</td>
<td>-206±3</td>
<td>-69±8</td>
<td>-59±7</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>180</td>
<td>0</td>
<td>-223±4</td>
<td>-95±11</td>
<td>-89±7</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>230</td>
<td>0</td>
<td>-214±6</td>
<td>-126±2</td>
<td>-124±10</td>
<td>6</td>
</tr>
</tbody>
</table>

\(n\): number of cells examined. Experiments were carried out by the K-anesthesia method (Fig. 6A).
Response under K⁺-induced depolarization

The response under K⁺-induced depolarization was analyzed. Specimens were mounted as shown in Fig. 1A. The pools A and B were filled with APW (pH 7) supplemented with 100 mM KCl and APW (pH 7) supplemented with 180 mM sorbitol, respectively. After 20 min, the medium of the pool B was replaced with APW (pH 7) supplemented with 100 mM KCl. Before cutting the V cell, Eₘ changed to the negative direction (Fig. 8A). The average amplitude was 13.9±4.9 mV (n = 9). Some cells did not generate the response. It is supposed that the cell was damaged during transfer from the Petri dish for pre-incubation to the measuring chamber under the severe bathing medium.

Discussion

Site of depolarization

The present study showed that the R cell generated four kinds of depolarization, (1) rapid component, (2) slow and long-lasting component, (3) action potential, and (4) small spikes (Fig. 1B, 2). In the measurement as shown in Fig. 1A, E_AB should reflect the sum of the response at the nodal end and that at the flank of the R cell in the pool B. When Lₙ was longer (20 mm), the amplitude of both components was smaller (Fig. 3a, b, Table 1 Exp. 2), indicating that these were generated at the nodal end but not at the flank of the R cell. On the other hand, the amplitude of the action potential was larger (Fig. 3c), indicating that an action potential was generated at the flank of the R cell in the pool B. When Lₙ was 2 mm, the amplitude of the action potential seemed to be smaller (Fig. 2A downward white arrow). It was suggested that the amplitude of an action potential generated at the flank of the R cell became apparently smaller due to the large slow component generated at the nodal end. When experiments were carried out as shown in Fig. 4A the amplitude of both rapid and slow components significantly increased. The contribution of the response generated at the nodal end should be large in the electrical signal measured (Fig. 4B, Table 1 Exp. 3).

One layer of nodal cells are intercalated between internodal cells and these cells are connected by plasmodesmata (Spanswick and Costerton 1967, Franceschi et al. 1994, Ogata 2000). When the V cell was killed, one surface of the nodal cells should be exposed to the external medium. Therefore, a possibility is suggested that responses were generated by the nodal cell, but not by the internodal cell. Since this problem remains unsolved, I want to conclude that two components were generated at the nodal complex including the nodal end of the internodal cell and node cells.

Involvement of cell turgor pressure

In the previous paper, I reported that high turgor pressure was necessary for generation of the slow component (Shimmen 2001a). This was re-confirmed in the present study (Table 2 Exp. 4–6). In addition, it was found that the cell tur-
gor pressure was also concerned with generation of the rapid component. By increasing the sorbitol concentration to 230 mM, the peak potentials of rapid and slow components significantly changed to the negative direction (Table 2 Exp. 6). Before cutting the V cell, the turgor pressure of both cells should be balanced at the node between R and V cells. When the V cell was killed, strong stress should be applied to the nodal end (or nodal complex) of the R cell due to its turgor pressure. When the cell turgor pressure was decreased by adding sorbitol to the external medium, the stress applied to the nodal end of the R cell upon cutting the V cell should be smaller. Thus, it is suggested that the mechanical stress applied to the nodal end is concerned with generation of these components. The intracellular osmolarity of the present material is 247±3 mOsM (Shimmen 2001a). The turgor pressure of cells in the presence of 230 mM sorbitol in the external medium would be equivalent to about 20 mOsM. This small turgor pressure of the R cell might be enough to generate the two components of smaller amplitude (Table 2, Exp. 6). It has been suggested that wound-induced hydraulic signals are the trigger for generation of variation potentials in higher plants (Malone and Stammlpeoc 1991, Malone 1992, Stahlberg and Cosgrove 1997). Zimmermann and Beckers (1978) reported that action potentials were generated by turgor pressure changes in C. corallina. The presence of a stretch-activated ion channel has been reported in various plant materials (Cosgrove and Hedrich 1991, Ding et al. 1993, Ding and Pickard 1993a, Ding and Pickard 1993b, Falk et al. 1988). Thus, a possibility is suggested that a stretch-activated ion channel(s) is concerned with depolarization at the nodal end of the R cell upon cutting the V cell.

**Involvement of Cl⁻ channel**

Ca²⁺ and Cl⁻ are candidates for generation of depolarizing responses at the nodal end, since the equilibrium potentials across the plasma membrane for these ions are positive inside (Tazawa et al. 1974, Williamson and Ashley 1982). Therefore, the targets of the present study were these ion channels. A significant effect on the peak potentials of two components was not observed for inhibitors examined (data not shown). Another strategy to identify ion species involved in electrogenesis is modification of extracellular concentrations of the target ions. The peak potential of both components seemed to be changed to the negative direction to some extent with decrease in [Ca²⁺] (Fig. 7). However, the possibility remains that the response was inhibited due to the ill effect of very low [Ca²⁺] in the external medium. When the concentration of choline chloride was increased to 100 mM, the peak potentials of both components changed to the negative direction to some extent (Table 2 Exp. 3). However, the effect of 100 mM choline chloride must be compared with that of 180 mM sorbitol, which is isotonic to 100 mM choline chloride (Table 2 Exp. 5). Thus, the main effect of choline chloride seems to be an osmotic one.

A voltage-clamping experiment is also one of the strategies to identify ion species involved. However, it seems difficult to apply this technique, since the configuration of the specimen drastically changes upon cutting the V cell. I have applied analysis under K⁺-induced depolarization and succeeded in demonstrating that a chloride channel is activate by mechanical stimulation in C. corallina (Shimmen 1997b). When the K⁺ concentration of the external medium was increased to 100 mM, $E_m$ is almost 0 mV (Shimmen 2001b). In the presence of 100 mM KCl in the external medium, the equilibrium potential for Cl⁻ across the plasma membrane is calculated to be –39 mV, since the Cl⁻ concentration in the cytoplasm is 21 mM (Tazawa et al. 1974). Under such circumstances, $E_m$ changed to the negative direction upon cutting the V cell (Fig. 8A). The Cl⁻ concentration of APW supplemented with 50 mM K₂SO₄ was 1.3 mM and the equilibrium potential for Cl⁻ was calculated to be 70 mV. In this case, $E_m$ changed to the positive direction upon cutting the V cell (Fig. 8B). It seems reasonable to conclude that Cl⁻ channel was activated upon cutting the V cell, although the external medium was not physiological. The amplitude of the electrical response under the K⁺-induced depolarization significantly increased in the measurement to embed the node in the groove of the partition (described in Results). This indicates that the Cl⁻ channel activated under K⁺-induced depolarization is mainly located at the nodal end, but not at the flank of the R cell.

Both rapid and slow components were generated in APW supplemented with 100 mM choline chloride. The peak potentials of rapid and slow components was –87 mV and –85 mV, respectively, in the presence of 100 mM choline chloride in the external medium (Table 2 Exp. 3). These peak potentials are more negative than the equilibrium potential for Cl⁻ across the plasma membrane, –39 mV. Thus, it is possible that depolarization could be induced by activation of Cl⁻ channel in the presence of 100 mM choline chloride in the external medium. However, I could not obtain evidence to show that rapid and slow components were generated by activation of Cl⁻ channel. The peak value of both components shifted to the negative direction in the presence of EGTA (Fig. 7). Further analysis in relation to Ca²⁺ channel is also needed.

When the V cell was cut, the symplast would become open to the external medium through plasmodesmata of the nodel cells. A possibility is suggested that the exposure of the symplast to the external medium through plasmodesmata induced the depolarization at the nodal end. Reid and Overall (1992) found rapid increase of electrical resistance of the node upon excision of one cell from a whorl cell pair, suggesting rapid closure of the plasmodesmata. The response at the plasmodesmata may be also involved in depolarizing responses observed in the present study.

To analyze the effect of ion channel inhibitor, the K-anesthesia method was employed (Fig. 6A). In K-anesthesia method, APW in pools A and C should be supplemented with 180 mM sorbitol, isotonic to 100 mM KCl, to avoid transcellular osmosis between pools A–C and B (Shimmen et al. 1976). However, lowering the turgor pressure caused decrease in the
amplitude of electrical response of the R cell upon cutting the V cell. To minimize the effect of transcellular osmosis, the length of the cell part in pool B containing 100 mM KCl was made shorter, 3 mm. I cannot exclude the possibility that transcellular osmosis affected the results to some extent. However, normal resting membrane potential and reasonable responses were generated (Fig. 6B).

It has been almost established that a Cl\(^{-}\) channel is involved in generation of action potentials in Characeae (cited in Shimmen et al. 1994, Tazawa and Shimmen 2001). A Cl\(^{-}\) channel at the flank of the internodal cell is also activated by mechanical stimulation in C. corallina (Shimmen 1997b). In higher plants, involvement of a Cl\(^{-}\) channel in inhibition of elongation growth of the hypocotyl by blue light has been reported (Cho and Spalding 1996, Lewis et al. 1997). It seems that a Cl\(^{-}\) channel plays important role in perception of the external stimuli and propagation of signals in plants. Characean cells can be a suitable material for analysis of plant Cl\(^{-}\) channels because of various advantages for electrophysiology (Shimmen et al. 1994).

Materials and Methods

Chara corallina was cultured in an air conditioned room (25°C) as described previously (Mimura and Shimmen 1994). Specimens consisting of two internodal cells were prepared by removing neighboring internodal cells and branchlet cells with scissors (Shimmen 2001a). They were kept in APW containing 0.1 mM KCl, 1 mM NaCl and 0.1 mM CaCl\(_2\) (pH about 5.6) at least 2 d before use. In experiments, one internodal cell (V cell) was killed by cutting and the electrical response of the other internodal cell (R cell) was analyzed. 

E\(_{\text{m}}\) was amplified with an amplifier (Microelectrode Amplifier MEZ7101, Nihon Kohden, Tokyo Japan) and recorded using a pen-writing recorder (VP-6358A, National Panasonic, Tokyo Japan).

Stock solutions of nifedipine (Sigma, St. Louis, MI, U.S.A.) (50 mM) prepared in dimethylsulfoxide was added to APW before use at 100 μM. Dimethylsulfoxide per se did not affect the responses at the concentration used. Anthracene-9-carboxilic acid (Nacalai Tesque Inc, Kyoto, Japan) was dissolved in APW at 1 mM, according to Shiina et al. (1987). Ethacrynic acid (Sigma, St. Louis, MI, U.S.A.) was dissolved in APW at 100 μM and the pH was adjusted at 7.0 with Tris. Stock solutions of LaCl\(_3\) (1 mM) and GaCl\(_3\) (10 mM) were prepared by dissolving them in pure water. They were added to APW at 100 μM.

To analyze the effect of extracellular Ca\(^{2+}\) concentration, EGTA buffer was used. EGTA at 1 mM was added to APW supplemented with 5 mM HEPES and the pH1 was adjusted to 7.0 with Tris. To prepare a medium of pCa 3 (–log[Ca\(^{2+}\)]), 2 mM CaCl\(_2\) was added to APW containing 1 mM EGTA. Media of pCa 6 and 7 were prepared based on the association constant between EGTA and Ca\(^{2+}\) (Jewell and Ruegg 1966).

Experiments were carried out at room temperature (23–27°C) under dim light (about 90 lux).

References


Death message in Chara


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