Electrical Perception of “Death Message” in *Chara*: The Role of Turgor Pressure

Teruo Shimmen^{1*}, Koreaki Ogata², Nobuyuki Yanagihara² and Tohru Yoshihisa¹

¹ Department of Life Science, Graduate School of Life Science, University of Hyōgo,
Harima Science Park City, Hyōgo 678–1297, Japan

² Department of Pharmacology, School of Medicine, University of Occupational and Environmental Health,
1–1 Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka 807–8555, Japan

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Summary Specimens consisting of two tandem internodal cells were prepared using *Chara corallina* in order to analyze the electrical response upon wounding. When one cell (victim cell) was killed by severing, another cell (receptor cell) generated a sharp spike followed by a long-lasting depolarization at the nodal end. It has been reported that K⁺ released from the victim cell is involved in generation of the long-lasting depolarization (Shimmen 2006). In the present study, the trigger to induce the sharp spike was studied. When the turgor pressure of the victim cell was decreased by adding 1 M methanol to the external medium, a sharp spike similar to that induced by wounding was generated at the nodal end of the receptor cell. It is suggested that the sudden disappearance of the turgor pressure of the victim cell due to severing is a trigger to induce the sharp spike of the receptor cell.

Key words *Chara*, Death message, Membrane potential, Turgor pressure, Wounding response.

Generation of electrical responses upon wounding is a general phenomenon in 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 1985, Robin and Bonnemain 1985). Wildon *et al.* (1992) found that the electrical signal is involved in systemic synthesis of a proteinase inhibitor in tomato. It was also reported that both variation potential and action potential induced synthesis of proteinase inhibitor in tomato (Stankovic and Davies 1996). Thus, the electrical signal seems to play a pivotal role in wounding response of plants.

It is suggested that the neighbor cells first perceive the message from the killed cells and generate the electrical signal. However, it will be hard to selectively monitor the electrical signal from the target cells in higher plants because of their complex morphology. Using *Chara corallina*, a simple model system to analyze the wound-induced electrical response was developed (Shimmen 2001). A specimen consisting of tandem two internodal cells was prepared. One cell (victim cell) was killed and the electrical response of the neighbor cell (receptor cell) was monitored. When the victim cell was severed, two kinds of electrical response were induced at the nodal end of the receptor cell: A shape spike and a following long-lasting depolarization. During the long-lasting depolarization, action potentials were generated due to

activation of the voltage-dependent ion channels (Shimmen 2002). Involvement of K⁺ released from the victim cell in generating the long-lasting depolarization was suggested (Shimmen 2005, 2006). We came to a hypothesis that the sharp spike is induced *via* disappearance of the turgor pressure of the victim cell upon cutting, based on the following experience. Some species of *Spirogyra* differentiate the terminal cell to be a rhizoid for anchoring to the substratum. When algal filaments of such species were severed, a new terminal cell recognizes its terminal position and starts the rhizoid differentiation (Nagata 1973). We noticed that the distal end of the terminal cell became convex due to its turgor pressure. Before cutting the algal filaments, the septum between cells is flat due to balancing of turgor pressure. It was indicated that the terminal cell recognizes its terminal position *via* stretching of the membrane at the distal end (Inoue *et al.* 2002). On the analogy to *Spirogyra*, we speculated that a stretching force is applied to the nodal end of the receptor cell when the turgor pressure of the victim cell was lost upon severing in *C. corallina*. However, we cannot observe stretching of the end membrane of an internodal cell, since the cell end is surrounded by nodal cells. It was suggested that the membrane depolarization is induced at the nodal end of the receptor cell by lowering the turgor pressure of the victim cell, without severing the cell. Upon addition of 0.2 M sorbitol to the external medium of the victim cell, a significant membrane depolarization was induced. However, a sharp spike was not observed (Shimmen 2001).

Recently, we came to a hypothesis that more rapid

* Corresponding author, e-mail: shimmen@sci.u-hyogo.ac.jp
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decrease in the turgor pressure of the V cell may induce a sharp spike of the R cell, since the turgor pressure should be suddenly lost upon cutting the victim cell. Hayama *et al.* (1979) applied 4% methanol to the external medium in order to induce strong transcellular osmosis in *Nitella flexilis*. In the present study, we found that addition of 1 M methanol to the external medium of the victim cell induced a sharp spike in the victim cell.

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 tandem internodal cells were prepared by removing neighbor internodal and branchlet cells with scissors (Shimmen 2001). They were kept in artificial pond water (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.

The electrical measurements were carried out using a chamber composed of three pools (Fig. 1). A specimen consisting of two tandem internodal cells was mounted on the chamber so that the node between internodal cells was located in pool B. Partitions (P) between pools were electrically sealed with white Vaseline. The length of P was about 10 mm. The node was attached to P separating pools A and B. Hereafter, an internodal cell separated between pools A and B and that separated between pools B and C were called receptor cell (R cell) and victim cell (V cell), respectively. The length of pool B was about 3 mm. All pools were filled with APW buffered with 5 mM HEPES–NaOH (pH 7.0). Hereafter, buffered APW was simply called APW. The potential difference between pools A and B was measured with agar electrodes which were connected to Ag–AgCl wire *via* 3 M KCl solution (E_A , E_B). Under such measuring configuration, the electrical response generated at the nodal end of the R cell was selectively monitored (Shimmen 2001,

Fig. 1. Setup for analysis of methanol-induced depolarization. A specimen consisting of two tandem internodal cells was mounted on a chamber having three pools, A, B and C. The node was attached to the partition separating pools A and B (P). An internodal cell separated between pools A and B and another separated between pools B and C were receptor cell (R) and victim cell (V), respectively. The potential difference between two pools, A and B, was measured with agar electrodes (E_A , E_B). Under such measuring configuration, response generated at the nodal end of the R cell was selectively monitored.

2002, 2003).

The change of turgor pressure of internodal cells upon addition of either 1 M methanol or 0.2 M sorbitol to the external medium was measured according to Ogata (2010).

Electrical signals were amplified with an amplifier (Microelectrode Amplifier MEZ7101, Nihon Kohden, Tokyo, Japan) and recorded using a pen-writing recorder (VP-6358 A, National Panasonic, Tokyo, Japan).

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

Results

Some organic solvents, permeable to the biological membrane, transiently decrease the turgor pressure of *Chara* cells (Steudle and Tyerman 1983). The effect of methanol was confirmed according to Ogata (2010). At the beginning, the external medium was APW (Fig. 2 upper). Upon replacement of the external medium with APW supplemented with 1 M methanol (meth), the turgor pressure began to decrease and attained a minimum value after about 5 s. Then the turgor pressure started to recover. After about 2 min of methanol application, turgor pressure recovered the original level (data not shown). Thus, decrease in turgor pressure upon addition of 1 M methanol was quick and transient. The external medium was replaced with APW lacking methanol. The effect of 0.2 M sorbitol on the turgor pressure was examined using the same cell (Fig. 2 lower). Upon replacement of the external medium with APW supplemented with 0.2 M sorbitol (sorb), the turgor pressure slowly decreased. It stayed at a low level and recovered upon replacement of the external medium with APW lacking sorbitol (data not shown). It must be noticed that the initial speed of turgor pressure decrease induced by 1 M methanol was significantly higher than that induced by 0.2 M sorbitol (Fig. 2).

Since 1 M methanol is significantly hypertonic to the intracellular osmolarity of *Chara* cells: 247 ± 3 mOsm (Shimmen 2001), any toxic effect on the cell was suspected. This was examined by a microscopic observation of cells (Fig. 3). First, an internodal cell was bathed in APW (0 s). The external medium was replaced with APW supplemented with 1 M methanol. However, morphology of the cell did not change at all (Fig. 3 left, 10, 60 s) and cytoplasmic streaming continued without cessation (not shown). When 5 internodal cells were incubated in APW supplemented with 1 M methanol for 2 d, all cells maintained a normal morphology and active cytoplasmic streaming (data not shown). Thus, 1 M methanol did not exert severe ill effects on internodal cells with respect to morphology, cytoplasmic streaming and survival. Next, the effect of 1 M sorbitol was examined (Fig. 3, right). Just after replacement of the external medium with APW supplemented with 1 M sorbitol

Fig. 2. Change of turgor pressure upon addition of either methanol or sorbitol to the external medium of an internodal cell. Measurement was carried out according to Ogata (2010). First, the external medium was APW. Upon replacement of the external medium with APW supplemented with 1 M methanol (meth), turgor pressure gradually decreased and then started to recover after about 5 s. After the end of measurement, the external medium was replaced with APW lacking methanol. When the turgor pressure restored an original level (lower trace), the external medium was replaced with APW supplemented with 0.2 M sorbitol (sorb). Turgor pressure gradually decreased. For further explanation, see the text. Vertical bar represents 0.1 MPa.

Fig. 3. Effect of methanol and sorbitol on morphology of internodal cell. Internodal cells were treated with APW supplemented with either 1 M methanol or 1 M sorbitol. After 10 and 60 s, photographs were taken. For further explanation, see the text.

(10 s), plasmolysis started and cytoplasmic streaming stopped (not shown). After 60 s, morphology of the cell was miserable due to severe plasmolysis (60 s).

The effect of 1 M methanol on membrane potential was examined. In the experiment to analyze the wounding response, the V cell was severed and the electrical

response of the R cell was monitored (Shimmen 2001). In Fig. 1, left cell corresponds to R cell and right one V cell. The external medium was first APW. When the external medium of pool C was replaced with APW supplemented with 1 M methanol, two types of depolarization were generated after a delay. Hereafter, replacement of the external medium with APW supplemented with methanol is described as "addition of methanol". In some specimens, addition of 1 M methanol to pool C induced a sharp spike followed by a slow repolarization (Fig. 4a). In other specimens, a slow and transient depolarization without sharp spike was generated upon addition of methanol to pool C (Fig. 4b). A similar response lacking a sharp spike was generated when the turgor pressure of the V cell was moderately decreased by adding 0.2 M sorbitol (Shimmen 2001). Among 21 specimens examined, a sharp spike was generated in 19 cells. However, the number of specimens generating a sharp spike was variable between culture batches. In some culture batches, all specimens generated only a slow depolarization without sharp spike.

After the methanol-induced depolarization, a wound-induced depolarization was induced by severing the V cell in order to compare the mode of these responses. This experiment was carried out in 13 specimens, which generated a sharp spike upon methanol-treatment. In some specimens, the initial part of the methanol-induced depolarization was quite similar to that of the wound-induced depolarization. Figure 5 shows a typical ex-

Fig. 4. Methanol-induced depolarization. Experiments were carried out using a chamber shown in Fig. 1. The external medium was first APW. When the external medium of pool C was replaced with APW supplemented with 1 M methanol (arrows), two types of response were generated at the end of the receptor cell (a and b). For further explanation, see the text.

ample. Upon addition of 1 M methanol (meth), a sharp spike was generated (1) and then an action potential was (2). The action potential was transmitted to the cell part in pool A (3), resulting in generation of a bipolar action potential. After the membrane potential recovered to the level before the methanol addition, the V cell was served in pool C. After a sharp spike (4), a bipolar action potential (5, 6) and a long-lasting depolarization (7) were generated. When an action potential was not generated in the methanol-stimulation as shown in Fig. 4b, it was also absent in the wound-induced depolarization (data not shown).

The effect of 1 M sorbitol on membrane potential was examined (Fig. 6). Upon addition of 1 M sorbitol to the

Fig. 5. Comparison of methanol- and wound-induced responses. A specimen was mounted as shown in Fig. 1. When the external medium of pool C was replaced with APW supplemented with 1 M methanol (meth), a rapid spike was generated (1). After generation of a bi-polar action potential (2, 3), the membrane repolarized. When the victim cell was cut in pool C (cut), an initial spike (4), a bi-polar action potential (5, 6) and a long-lasting depolarization (7) were successively generated. For further explanation, see the text.

Fig. 6. Sorbitol-induced depolarization. Experiments were carried out using a chamber shown in Fig. 1. The external medium was first APW. When the external medium of pool C was replaced with APW supplemented with 1 M sorbitol (arrow), a rapid spike (1) and then a long-lasting depolarization were generated. Irregular spikes were superimposed on the long-lasting depolarization. For further explanation, see the text.

external medium of the V cell (arrow), a sharp spike (1) was generated after a delay and then a long-lasting depolarization with irregular spikes was induced. The mode of the response generated by 1 M sorbitol was similar to that generated upon severing the V cell.

Discussion

A sharp spike was generated by decreasing the turgor pressure of the V cell with 1 M methanol (Fig. 4a), supporting our hypothesis that generation of the sharp spike is triggered by the sudden disappearance of the turgor pressure of the V cell upon cutting. The sharp spike generated upon methanol-treatment was quite similar to that induced by wounding (Fig. 5). As shown in Fig. 2, the initial rate of turgor decrease upon treatment of the V cell with 1 M methanol was higher than that induced by treatment with 0.2 M sorbitol. Treatment with 0.2 M sorbitol never induced a sharp spike (Shimmen 2001), indicating that the initial rate of turgor decrease must be higher to induce the sharp spike. In some specimens, however, the sharp spike was not induced by treatment with 1 M methanol (Fig. 4b). In these specimens, however, the R cell generated a sharp spike upon severing the V cell (data not shown). In methanol stimulation, turgor gradually decreases, depending on the water efflux across the plasma membrane of the V cell. On the other hand, upon cutting the V cell, turgor pressure is expected to suddenly be lost. It is suggested that susceptibility of the nodal end of the R cell to the decrease in turgor pressure of the V cell might be different among specimens. At present, the reason remains unsolved.

In the previous paper (Shimmen 2002), activation of chloride channels at the beginning of the wound-induced depolarization was reported in *Chara*. In mesophyll cells of *Arabidopsis thaliana*, it was suggested that activation of the mechano-sensitive anion channel is mediated by convex curvature of the plasma membrane (Qi *et al.* 2004). The nodal end of *Chara* is equipped with a function to transduce the pressure signal into an electrical signal (Shimmen 2003). Upon decrease or loss of turgor pressure of the V cell, the nodal end might become convex, resulting in activation of chloride channel. Transduction of pressure signal into electrical one upon wounding has been suggested in tomato (Stankovic and Davies 1998) and sunflower (Stankovic *et al.* 1997). When the flank region of internodal cells was mechanically stimulated, a depolarization is induced due to activation of Cl^- channels (Shimmen 1997). Kaneko *et al.* (2005) showed increase in cytoplasmic Ca^{2+} upon mechanical stimulation of flank region, suggesting possible activation of the Ca^{2+} channel in addition to Cl^- channel upon mechanical stimulation at the flank region of the internodal cell. Amplitude of the rapid spike upon severing the V cell decreased to some extent, when the extracellular Ca^{2+} was decreased (Shimmen 2002). Thus, pos-

sible activation of Ca^{2+} channel at the nodal region upon wounding is a target of the future study.

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