Short Communication

Electrical Perception of the ‘Death Message’ in Chara: Characterization of K+-induced Depolarization

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When the nodal end of an internodal cell of Chara corallina was subjected to a pulse treatment with artificial cell sap, a depolarization lasted in artificial pond water. This depolarization could also be induced by pulse treatment with KCl solution, indicating that K+ in the artificial cell sap is responsible (K+-induced depolarization). The depolarization was prolonged in the presence of 2 mM KCl and the prolonged depolarization was terminated by supplementing with either CaCl2, MgCl2, or NaCl. These results supported the hypothesis that K+ released from the killed cell is responsible for generation of the wound-induced membrane depolarization.

Keywords: Chara — Depolarization — K+ — Membrane potential — Wounding response.

Abbreviations: ACS, artificial cell sap; APW, artificial pond water; BSA, bovine serum albumin.

Wounding induces significant membrane depolarization in plants (summarized in Shimmen 2005). However, the mechanism of induction of the depolarization is still obscure. By taking advantage of characean cells, I started the analysis in order to elucidate the mechanism of wound-induced depolarization. I prepared a specimen comprising two adjoining internodal cells. One internodal cell (victim cell) was killed by cutting and a change of the membrane potential in another internodal cell (receptor cell) was analyzed (Shimmen 2001). Upon cutting a victim cell, a receptor cell generated four kinds of depolarizing response: (i) rapid depolarization; (ii) long-lasting depolarization; (iii) action potential; and (iv) a small spike. The rapid depolarization, action potential and small spike were not always ubiquitous among the specimens. On the other hand, the long-lasting depolarization was observed in all specimens. This depolarization was found to be generated at the nodal end of the receptor cell (Shimmen 2002). Hereafter, this long-lasting depolarization is called wound-induced depolarization.

In the previous study, I hypothesized that the wound-induced depolarization is triggered by K+ released from the victim cell, based on an observation that pulse treatment of the nodal end with artificial cell sap (ACS) could induce a depolarization similar to the wound-induced depolarization (Shimmen 2005). The wound-induced depolarization was prolonged by adding K+ to the external medium and the prolonged depolarization was stopped by adding either Ca2+, Mg2+ or Na+ to the medium containing K+. In the present study, the effect of cations on the depolarization induced by the pulse treatment with the ACS was studied in order to examine the K+ hypothesis for generation of the wound-induced depolarization.

In the previous study (Shimmen 2005), I showed that addition of ACS containing 80 mM KCl, 30 mM NaCl, 10 mM CaCl2, and 10 mM MgCl2 (Moriyasu et al. 1984) to the external medium of the nodal end induced a depolarization, which slowly repolarized in artificial pond water (APW). In the previous study (Shimmen 2005), ACS was added to the nodal end of the receptor cell just after the end of the wound-induced depolarization generated by cutting the victim cell. In the present study, single internodal cells had been separated from neighboring cells and kept for 2–14 d in APW before use.

A depolarization was induced by a pulse treatment with ACS, as reported previously (Shimmen 2005). A typical example is shown in Fig. 1 (left). First, the external medium of the nodal end was APW. After an ACS pulse of 2 min (ACS), the potential change in APW was chased. The membrane potential gradually repolarized in APW. When the membrane potential stabilized in APW, the nodal end in the pool B was subjected to treatment with 80 mM KCl solution for 2 min and the change of the membrane potential was chased in APW. To quantify the duration of the depolarization, the time needed for repolarization to the level of half peak potential was analyzed. Hereafter, this time period is simply referred to as the duration of the depolarization. The depolarization induced by 80 mM KCl was longer than that induced by ACS. The same results were obtained for all specimens examined: 5.1±0.8 min for ACS and 23±3.8 min for 80 mM KCl (n = 6). In the previous study, it was shown that Ca2+, Mg2+ and Na+ have a tendency to bring the membrane potential to the negative direction (Shimmen 2005). This is probably the reason why treatment of the nodal end with ACS induced a depolarization shorter than that induced by KCl solution. It is suggested that K+ in ACS is responsible for induction of the depolarization (K+-induced
depolarization). In the following analyses, depolarization was induced by the ACS pulse.

The effect of the length of the ACS pulse on the duration of the depolarization in APW was studied. The nodal ends were treated with an ACS pulse of various lengths and then the change of the membrane potential was chased in APW. As seen in Fig. 2, the duration of the depolarization increased with the increase in length of the ACS pulse.

In the previous study, I reported that the wound-induced depolarization is significantly prolonged by adding 2 mM KCl to the external medium (Shimmen 2005). Therefore, I examined whether 2 mM KCl prolongs the ACS-induced depolarization (Fig. 3A). First, the external medium was APW. The ACS pulse of 2 min initiated a depolarization in APW (Fig. 3A, left). After the repolarization, the nodal end was again subjected to an ACS pulse of 2 min. In this case, ACS was replaced with APW supplemented with 2 mM KCl. The membrane potential stayed at the depolarized level even after 100 min. When the external medium was replaced with APW lacking 2 mM KCl, the membrane repolarized. Thus, ACS-induced depolarization was significantly prolonged in the presence of 2 mM KCl. In another 10 specimens, it was confirmed that the membrane potential stayed at the depolarized level longer than 30 min and was repolarized by removal of 2 mM KCl.

The wound-induced depolarization prolonged in the presence of 2 mM KCl was terminated when CaCl₂ was added (Shimmen 2005). This effect of CaCl₂ was examined for ACS-induced depolarization (Fig. 3B). After an ACS pulse of 2 min, a prolonged depolarization was induced in APW supplemented...
with 2 mM KCl. When the external medium was replaced with APW supplemented with both 2 mM KCl and 5 mM CaCl₂, the membrane potential soon repolarized. Thus, CaCl₂ could terminate the prolonged depolarization induced by 2 mM KCl. The same results were obtained for all specimens examined (n = 6). The prolonged depolarization in the presence of 2 mM KCl was also terminated by either 5 mM MgCl₂ (n = 7) or 10 mM NaCl (n = 7). It has been reported that these cations also terminated the wound-induced depolarization in the presence of 2 mM KCl (Shimmen 2005).

One layer of very thin cells is intercalated between internodal cells and a node is surrounded by many cells (Fig. 1 in Shibak and Tabata 1981). At the end of an isolated internodal cell, cells surrounding a node remained attached and showed active cytoplasmic streaming. Therefore, it must be noted that the depolarization was generated at the end of the internodal cell and/or these nodal cells. I could not verify whether cells intercalating between internodal cells are alive after preparation of intermodal cells.

When an internodal cell having a high turgor pressure is cut, it is expected that intracellular structures are severely destroyed and that soluble components of the cytoplasm and those of the vacuole are mixed. Since the K⁺ concentrations of both compartments are similar (Tazawa et al. 1974), it seems reasonable to induce a depolarization using an ACS. Just after cutting the victim cell, the protein concentration in the space close to the distal end of the receptor cell may be higher. Moriyasu and Malek (2004) reported that 80 g of Chara corallina cells (wet weight) contained 26.6 mg of proteins. Based on the assumption that the cytoplasm occupies about 10% of the total cell volume (Tazawa et al. 1974) and that the protein concentration of the vacuole is <1/100 of that of the cytoplasm (Moriyasu 1995), the protein concentration of the cytoplasm is estimated to be about 0.33% (w/w). To examine the effect of protein, the following experiments were carried out. First, depolarization was induced by the pulse treatment with ACS supplemented with 0.33% (w/w) bovine serum albumin (BSA), and then depolarization was chased in APW lacking BSA. However, an appreciable difference was not observed with respect to amplitude and duration of the depolarization.

The present study showed that the effect of cations on ACS-induced depolarization was the same as that on wound-induced depolarization: (i) prolongation in the presence of 2 mM KCl; and (ii) termination of the prolonged depolarization with Ca²⁺, Mg²⁺ and Na⁺. In combination with the previous report (Shimmen 2005), it is concluded that the ‘death message’ from the victim cell to induce a long-lasting depolarization of the receptor cell is released by K⁺. In the wounding response, a rapid component is generated at the very beginning (Shimmen 2005). However, it was not observed in K⁺-induced depolarization. It is suggested that a message other than K⁺ is concerned with generation of the rapid component.

Materials and Methods

Chara corallina was cultured in an air-conditioned room (25°C) as described previously (Mimura and Shimmen 1994). Internodal cells were prepared by removing neighboring internodal cells and branchlets. They were kept in APW containing 0.1 mM KCl, 1 mM NaCl and 0.1 mM CaCl₂ (pH about 5.6) at least for 2 d before use.

Measurement of the membrane potential at the nodal end was carried out as reported previously with a slight modification (Shimmen 2003). An internodal cell was mounted on a chamber having two pools, A and B (Fig. 4). The internodal cell was sealed into the groove of the partition (P) with white vaseline. The length of P was 10 mm. Pools A and B were filled with APW buffered with 5 mM HEPES-NaOH (pH 7.0). The buffered APW was simply called APW. To examine the effect of salts, they were added to the APW. The potential difference between the two pools was measured with agar electrodes connected to an Ag–AgCl wire via 3 M KCl solution (E₂ and E₀). Since a target of the present analysis is a depolarization generated at the nodal end, it is necessary to increase the contribution of a signal from the nodal end for the electrical measurement. For this purpose, the flank region of the cell in pool B was made as short as possible, i.e. the node (n) was attached to P. Electrical potential 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).

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

References


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