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Involvement of receptor potentials and action potentials in mechano-perception in plants

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Abstract. The rapid turgor movements of *Mimosa pudica* and some carnivorous plants have long stimulated the interest of botanists. In addition, it is becoming evident that slower responses of plants to mechanical stimuli, such as coiling of tendrils and thigmomorphogenesis, are common phenomena. Electrophysiological studies on mechano-perception have been carried out in *M. pudica* and carnivorous plants, and have established that the response to mechanical stimulation is composed of three steps: perception of the stimulus, transmission of the signal, and induction of movement in motor cells. The first step is due to the receptor potential, the second and third steps are mediated by the action potential. In this article, the mechanisms of responses to mechanical stimuli of these plants are considered. Since higher plants are composed of complex tissues, detailed analysis of electrical phenomena is rather difficult, and so the mechanism for generating the receptor potential had not yet been studied. Characean cells have proved to be more amenable to the study of the electrophysiology of plant membranes because of their large cell size and the ease by which single cells can be isolated. Recent progress in studies of the receptor potential in characean cells is also discussed.

Keywords: action potential, *Aldrovanda*, *Chara*, mechano-perception, *Mimosa*, receptor potential.

Introduction

Plants are always exposed to various external stimuli, such as light, gravity, chemicals, temperature and mechanical stress. The sensitivity of plants to these stimuli is as high as that of animals, or sometimes higher (Shropshire 1979). Compared with animals, plants are quiet and do not show dynamic responses to these stimuli, except for some special plants, such as *Mimosa* and some carnivorous plants. However, all are capable of perceiving external stimuli and show responses, although these responses may not sometimes be recognised by us.

In animals, it has been established that electrical processes play a central role in perception of stimuli, transmission of signals and induction of movement, such as muscle contraction. Involvement of electrical processes has also been recognised in the above mentioned plants that show rapid movements. The sequence begins with deformation of the plasma membrane by a mechanical stimulus and conversion of the mechanical signal to an electrical signal via the generation of a receptor potential. The action

potential then transmits the signal to the motor cells which induce movement by manipulating the turgor of these cells. Involvement of Ca^{2+} and Cl^- channels in the generation of action potentials has been reported in these plants. On the other hand, information on the mechanism of generation of the receptor potential is scanty, probably due to technical difficulties in measuring the membrane potential in such small receptor cells.

Characean cells have proved to be one of the most suitable materials for analysis of electrical phenomena in plant cells, and this is also true for their suitability for studies on mechano-perception. Characean cells generate receptor potentials in response to mechanical stimuli, allowing the opportunity to make detailed analyses of the mechanism of generation of mechanically-induced receptor potentials.

In the first part of this article, an historical perspective on mechano-sensitivity in plants is presented, together with an outline of electrical signal processing in *Aldrovanda vesiculosa* and *M. pudica*. In the following section, analysis of receptor potentials in characean cells is presented.

Rapid turgor movement

Movement of plants is classified based on two criteria. The first classification is based on the relation between the direction of stimulus and that of movement of the plant organ. When the direction of movement is dependent on that of stimuli, it is called 'tropism'. When the direction of movement is anatomically fixed and is independent of the direction of the stimulus, it is called 'nasty'. The second classification is based on the motive force that induces movement of the organ. Movement caused by change of the turgor pressure of cells is called 'turgor movement'. Movement caused by the local difference of the growth rate is called 'growth movement'. The movements dealt with in this section, thigmonasty, are caused by the change of the turgor pressure of cells.

Response in *A. vesiculosa*

The response of plants to a mechanical stimulus starts from conversion of the deformation of the plasma membrane into an electrical signal, the receptor potential. In *Dionea muscipula* and *A. vesiculosa*, receptor cells have been identified. The study of *D. muscipula* has been repeatedly introduced in text books. *A. vesiculosa* is an aquatic carnivorous plant equipped with trap-lobes to catch small animals. A pair of these semicircular lobes is connected together by the midrib, and each lobe has about 20 sensory hairs (Fig. 1). Each sensory hair has four small sensory cells, 20 μm in length and 7 μm in diameter (Fig. 2A). The cell
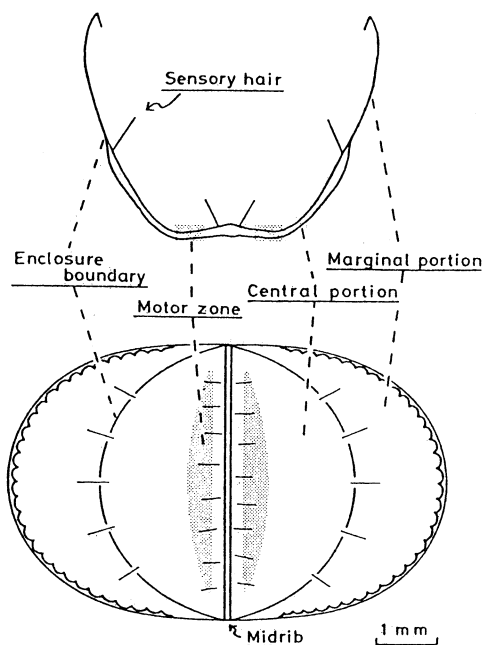

Fig. 1. Schematic drawing of cross section (upper) and upper view (lower) of the trap-lobes of *A. vesiculosa*. See text for further explanation. Cited from Iijima and Sibaoka (1981).

walls of sensory cells are thinner, and the sensory hair is easily bent at the position of the sensory cells (joint). The deformation of the sensory cells induces a series of electrical processes resulting in closure of the trap-lobes. Iijima (1981) succeeded in measuring the receptor potential from the sensory cells by inserting microelectrodes, one into a sensory cell and another into a cell of a lobe, and then generating a receptor potential by bending a sensory hair (Fig. 2). When the deformation was small, a receptor potential was recorded in the sensory cell (R), but no change in the membrane potential (E_m) of the lobe cell (L) (*1*). When deformation was increased, a larger receptor potential was induced and an action potential was recorded in the lobe cells after a delay (*2*). Further increase in deformation of sensory cells resulted in the instantaneous generation of an action potential in the lobe cell (*3*).

When an action potential was generated in lobe cells located at the base of a sensory hair, it was propagated over the lobe within 40 ms (Iijima and Sibaoka 1982). Upon arrival of action potentials at cells of the motor zone (Fig. 1), rapid closure of lobes was induced. When the turgor pressure was decreased by adding mannitol to the external medium of the lobes, closure of the lobes did not occur, although action potentials were normally generated (Iijima and Sibaoka 1983; Iijima 1991). This indicated that closure of lobes was a turgor movement. The motor zone has three cell layers, the inner and outer epidermis, and the middle layer. It has been suggested that cells of the inner epidermal layer of the motor zone lose their turgor to induce closure of lobes (Ashida 1934). Iijima and Sibaoka (1981) measured E_m of cells in three layers of the motor zone. The resting membrane potential and the amplitude of action potential in each of the three cells were identical. It was suggested that an action potential induces a drastic change of turgor pressure only in the inner epidermis. Iijima and Sibaoka (1983) found efflux of pre-loaded $^{86}Rb^+$, a tracer of K^+, during closure of trap-lobes.

Movement of main pulvinus of *M. pudica*

Coupling of action potentials with changes of turgor pressure of cells in localised areas of the motor organ has been studied in *M. pudica*. The motor organs of this plant are the main pulvinus, the subpulvinus and the laminar pulvinus, which induce movement of the main petiole, subpetiole and leaflet, respectively. In *M. pudica*, the receptor cells have not yet been identified. However, it has been suggested that they are localised in pulvini, based on the fact that action potentials are generated only in the pulvinus upon shaking and touching (Sibaoka 1951; Oda and Abe 1972; Abe 1980). Thus, both motor cells generating action potentials and sensory cells generating action potentials are localised in the pulvini. Another possibility is also suggested: that motor cells generate both receptor potentials and action potentials
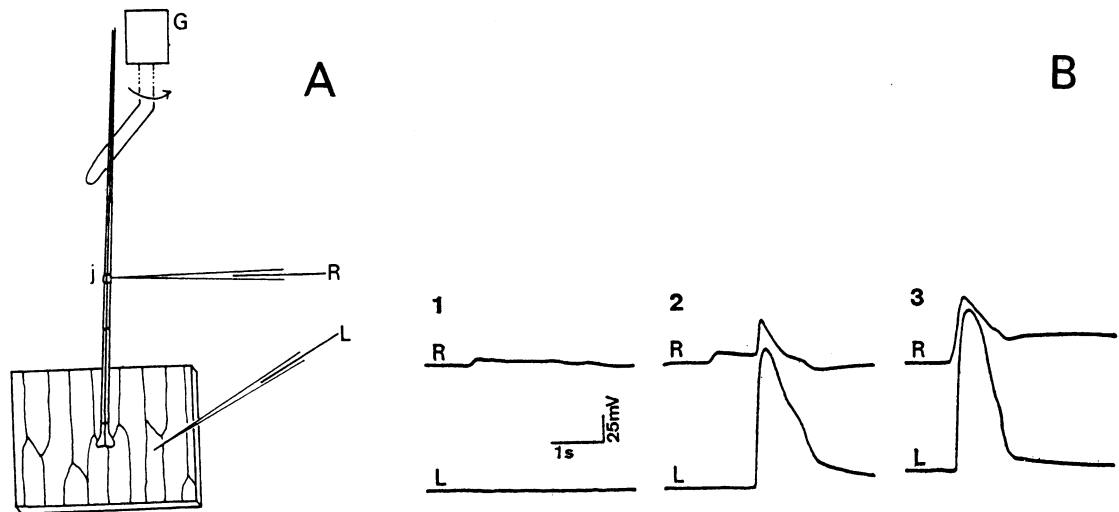
Fig. 2. (*A*) Measurement of receptor potential of sensory cell and action potential from a lobe cell of *A. vesiculosa*. Microelectrodes were introduced into a sensory cell (R) at the joint (j) and into a lobe cell (L). The distal portion of the sensory hair was pushed with a fine glass rod connected to a pen-writing galvanometer (G). (*B*) E_m recorded from a sensory cell (R) and that from a lobe cell (L). See text for further explanation. Modified from Sibaoka (1991).

Thus, studies on analysis of perception of mechanical stimuli is very scanty in *M. pudica*. However, analysis of ionic process during turgor movement has been extensively carried out in this material. In this section, flux of ion during turgor movement of main pulvinus is dealt with. Main pulvini are generally used for analysis, probably due to their large size. Allen (1969) reported an increase in K^+ efflux from pulvinus cells during rapid movement, and Oda *et al.* (1976) reported efflux of both K^+ and Cl^- during movement, supporting the idea that release of ions is responsible for turgor movement of pulvini.

Abe (1981) found efflux of Cl^- upon stimulation from cortex excised from the lower half of the main pulvinus, but not from that excised from the upper half. Samejima and Sibaoka (1980) unequivocally demonstrated that the release of Cl^- occurred exclusively in the lower half of the main pulvinus. They excised a leaf from a plant together with a minute piece of stem, and peeled both lateral surfaces of the main pulvinus to expose the cortical tissue for insertion of the electrode. After mounting an isolated leaf on a lucite plate, they inserted microelectrodes into cortical cells to measure E_m, and a Cl^--sensitive silver electrode into the tissue to measure the extracellular (apoplastic) Cl^- concentration (Fig. 3*A*). The leaf was stimulated by dropping ice-cold solution on a pulvinus (direct stimulation) or on a petiole (indirect stimulation). The bending movement was monitored with a mechano-electric transducer. Cortical cells in both upper (adaxial) half and lower (abaxial) half generated action potentials with the same pattern, a fast rising spike followed by a long-lasting plateau potential, indicating that cortical cells in both upper and lower halves were excitable. However, Samejima and Sibaoka (1980) found big

differences in the efflux of chloride between upper and lower halves. Significant increases in the apoplastic Cl^- concentration upon stimulation were induced in the lower half of the pulvinus, but only a slight change in the upper half (Fig. 3*B*). Thus, drastic efflux of Cl^- ions was induced only in the lower half. It seems that cells of both upper and lower halves have similar excitability, but action potentials are coupled with large ion effluxes only in the lower half. For electroneutrality, efflux of the same amount of cations, such as K^+, is expected in the lower half. Kumon and Tsurumi (1984) found an increase in the extracellular K^+ concentration in the lower half of the main pulvinus during slow downward movement by photostimulation. These reports indicate that turgor movement is induced by the decrease in the turgor pressure of cells in a localised area of a motor organ.

After the rapid movement, the main pulvinus recovers its original position. It takes more than 10 min for complete recovery. This recovery should be caused by re-absorption of ions released into the apoplastic space around cells. This process seems to be an active one, since the recovery is strongly dependent on the energy metabolism of the leaf, that is, light promotes recovery and inhibition of either photosynthesis or respiration retards the recovery. As expected, the rate of decrease in the Cl^- concentration in the apoplast is accelerated by light and is inhibited by disruption of energy metabolism (Samejima and Sibaoka 1980).

Ion channels involved

It has been suggested that mechano-sensitive ion channels are involved in generation of receptor potentials, and voltage-dependent ion channels in generation of action potentials. Receptor potentials have been studied in three
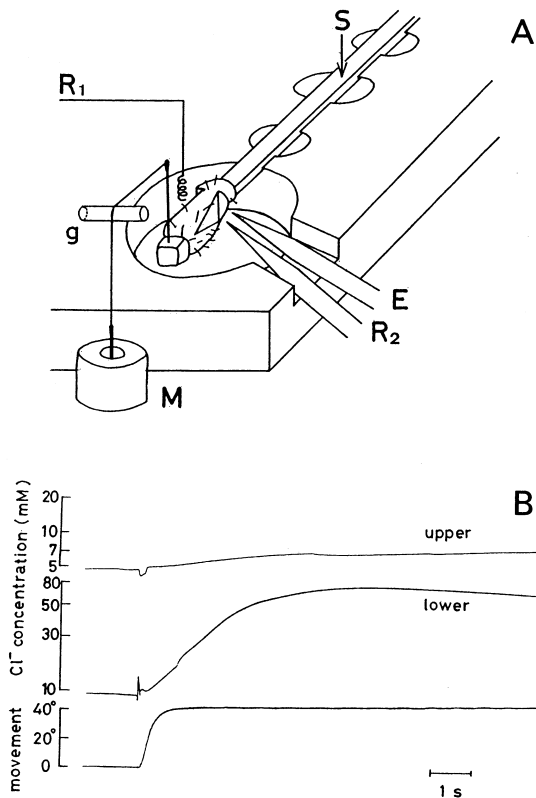
Fig. 3. Measurement of Cl⁻ efflux from cortical cells of main pulvinus of *M. pudica*. (*A*) Set-up for measurements. A leaf was stimulated by applying ice-cold solution to the petiole (S) or directly to the pulvinus. E, glass microelectrode or Cl⁻- sensitive silver electrode; R_1, reference Ag–AgCl electrode; R_2, reference glass microelectrode; g, glass rod; M, mechano-electric transducer. (*B*) Records of increase in apoplastic Cl⁻ concentration and movement of pulvinus. Cited from Samejima and Sibaoka (1980).

carnivorous plants, *D. mucscipula* (Benolken and Jacobson 1970), *A. vesiculosa* (Iijima 1981) and *Drosera intermedia* (Williams and Pickard 1972). However, the ionic mechanism for generation of receptor potentials has not been elucidated. The development of the patch-clamping technique (Sakmann *et al*. 1980) has enabled the study of single ion channels. Using this technique, stretch-activated channels have been discovered in plant membranes (Falke *et al*. 1988; Cosgrove and Hedrich 1991; Ding and Pickard 1993) and it is expected that stretch-activated channels in sensory cells will be found.

In nerve or skeletal muscle, Na⁺ channels are involved in the generation of action potentials, since the equilibrium potential for Na⁺ across the plasma membrane is inner positive. However, this is not the case in plants, where Ca²⁺ and Cl⁻ channels play a key role in propagation of action potentials. Iijima and Sibaoka (1985) analysed the response of the membrane potential to various ions in lobe cells of *A. vesiculosa*. The resting membrane potential was very sensitive to changes in the K⁺ concentration, but not to Ca²⁺

and Cl⁻, indicating involvement of K⁺ channels in generation of the resting membrane potential. The peak value of the action potential was independent of K⁺ and Cl⁻, but strongly dependent on Ca²⁺. Together with the fact that action potential was blocked by a Ca²⁺-channel blocker, La³⁺, Iijima and Sibaoka (1985) concluded that the action potential was generated via Ca²⁺ influx in lobe cells of *A. vesiculosa*.

Samejima and Sibaoka (1982) studied the effect of extracellular ions on the generation of action potentials by protoxylem cells of the petiole, and that by the upper-half cells of the main pulvinus of *M. pudica*. Since the dependence of the peak value of the action potential on the Cl⁻ concentration was larger than that of the resting membrane potential, they suggested that these action potentials were due to a Cl⁻ spike. In the upper half of the main pulvinus, only a small amount of Cl⁻ was released during the action potential (Fig. 3), but this small amount of Cl⁻ efflux might be enough to bring the membrane potential from the resting level to the peak level of an action potential. Campbell and Thomos (1977) reported that a Ca²⁺ chelator and La³⁺ inhibited movement in *M. pudica*, suggesting possible involvement of Ca²⁺ channels. Involvement of both Ca²⁺ and Cl⁻ channels in the generation of action potentials has been reported in characean cells. The increase in cytoplasmic Ca²⁺ caused by the Ca²⁺ spike of the plasma membrane has been linked to the activation of Cl⁻ channels in the plasma membrane (Shiina and Tazawa 1987, 1988; Mimura and Shimmen 1994). A similar situation may apply in *M. pudica*, where a large efflux of Cl⁻ in the lower half of the main pulvinus may be induced by action potentials via some form of intracellular signal processing, which will be dealt with below.

Mechanism of turgor movement

When plant cells generate action potentials they shrink due to the efflux of water together with ions (Oda and Linstead 1975). However, it has been suggested that efflux of ions from motor cells is much larger. Iijima and Sibaoka (1983) estimated that about 20% of K⁺ in the motor cells would move from the cell interior to the outside during the closure of lobes of *A. vesiculosa*. When a trap was stimulated in artificial pond water (APW) supplemented with 200 mM mannitol (almost isotonic with the osmolarity of trap cells), they did not close, but normal action potentials were generated. When the trap which had been stimulated in an isotonic solution was transferred into hypotonic APW, it remained open, indicating that drastic leakage of ions was not induced during the generation of action potentials in the isotonic medium. From this observation, Iijima and Sibaoka (1983) suggested that the turgor pressure is necessary for solute leakage and that it is induced by bulk flow, not by diffusional flow.

Possible involvement of the actin cytoskeleton in turgor movement has been suggested. Cytochalasin B and phal-

loidin, inhibitors of actin filament (Cooper 1987), inhibited seismonastic movement of *M. pudica* (Fleurat-Lessarad *et al*. 1988). Kameyama *et al*. (2000) reported that actin filaments in the motor cells at the lower side of the pulvinus, but not at the upper side, became more peripheral after bending in *M. pudica*. They also showed that actin was dephosphorylated during movement. The structure of cytoplasm supported by actin filaments is significantly modified by treatment with an inhibitor of protein phosphatase in root hair cells of *Hydrocharis* (Yokota *et al*. 2000*a*). Thus, phosphorylation–dephosphorylation of actin or related proteins may induce drastic changes in the cytoplasmic structure of motor cells. In *A. vesiculosa*, action potentials were found to be a Ca^{2+} spike (Iijima and Sibaoka 1985). Hodick and Sievers (1988) also reported that the action potential was strictly dependent on the external Ca^{2+} in trap-lobes of *D. muscipula*. Thus, an increase in the Ca^{2+} concentration in the cytoplasm of motor cells upon generation of action potentials was expected. Ca^{2+} is a regulator of actin-associated proteins, such as myosin (Yokota *et al*. 1999) and villin (Yokota *et al*. 2000*b*), and it is possible that inflow of Ca^{2+} may initiate signal processing, resulting in the bulk flow of ions via the actin cytoskeleton system. Ca^{2+}-dependent protein kinase is associated with plant actin filaments (McCurdy and Harmon 1992) and induction of protein kinase by mechanical stimulation has been reported in alfalfa leaves (Williams and Holland 1996).

Studies in Characeae

Characean cells have been one of the most useful materials for studies on plant membranes (Shimmen *et al*. 1994; Tazawa and Shimmen 2001). Since the native habitat of Characeae is aquatic, experiments in solution represent the natural condition. The large cylindrical morphology of internodal cells is a great advantage for electrophysiology, where several microelectrodes can be inserted into both the cytoplasm and the vacuole without significant damage to the cell. Intracellular perfusion, whereby the composition of the vacuole and cytoplasm can be experimentally manipulated, has further increased the usefulness of this material for electrophysiology (Shimmen *et al*. 1994; Tazawa and Shimmen 2001). The characteristics of characean internodal cells, that is, cylindrical shape, large size and the ability to generate action potentials, has led these cells to be called 'green axons', while the regulation of actin-based cytoplasmic streaming by action potentials (Tazawa and Kishimoto 1968) suggests that 'green muscles' might also be an appropriate term. Thus, characean cells occupy a special niche in the physiological investigation of many aspects of plant function.

The response of characean cells to mechanical stimuli has been recognised for a long time (Ewart 1903). Cessation of cytoplasmic streaming is induced by pinching, pulling or bending the internodal cells of *Nitella* or *Chara* (Lauterbach

1921 cited in Kishimoto 1968). Osterhout, a pioneer in this field, analysed electrical responses of characean cells to mechanical stimulation, such as bending, pinching and cutting. He found that when the stimulus was not severe, the cell generated a reversible change of the electrical potential, termed the variation potential, whereas when the stimulus was severe, it induced an irreversible change, the death wave (Osterhout and Hill 1931).

Kishimoto (1968) applied quantitative mechanical stimulation to *Chara* internodal cells by hitting with a bar driven by an electromagnet, and succeeded in recording a change of membrane potentials. The amplitude of the response was dependent on the strength of stimulus and the potential change could be summated. These are criteria for a receptor potential. Although an internodal cell of Characeae is not a differentiated receptor cell, this potential change induced by mechanical stimuli is hereafter called receptor potential. Staves and Wayne (1993) stimulated an internodal cell with a glass rod which was manipulated with micromanipulator. They analysed action potentials, but not the receptor potential.

Development of measuring apparatus

To advance our understanding of electrical events associated with mechano-stimulation, I developed an apparatus to apply quantitative stimuli to internodal cells of Characeae in a more simple way (Shimmen 1996). As shown in Fig. 4, an
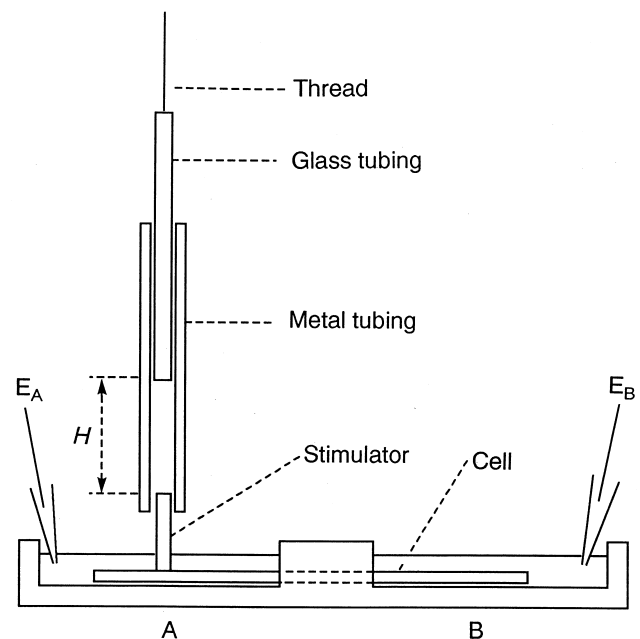

Fig. 4. Set-up for measurement of electrical responses of internodal cells of Characeae to mechanical stimulation. An internodal cell of Characeae is partitioned into two halves in pools A and B. The cell is mechanically stimulated by dropping a piece of glass tubing onto a stimulator. E_A, E_B, electrodes; H, height from which the glass tubing is dropped. See text for further explanation. Cited from Shimmen (1996).

internodal cell is partitioned into two compartments using a polyacrylate vessel composed of two pools (A and B). The two cell parts are insulated with Vaseline. Both pools are filled with APW and the potential difference between the two pools is recorded via agar electrodes (E_A, E_B). A block of polyacrylate or small glass rod (stimulator) is placed on the cell part in pool A and the cell is stimulated by dropping a piece of glass tubing onto it. A metal tubing which works as a guide for dropping the glass tubing is fixed to the vessel. The intensity of the stimulus is controlled either by changing the weight of the glass tubing or by changing the height (H) from which it is dropped. The position of the glass tubing before it is dropped is controlled with a length of polyester thread and the glass tubing is dropped by releasing the thread. In most cases, the intensity of the stimulus is controlled by changing H. For *Chara* cells, glass tubing of about 1 g is used. When the cell is thinner, the weight of the glass tubing must be reduced. After reporting this apparatus (Shimmen 1996), I found the following description by Ewart (1903): 'the simpler mode of producing a shock-stoppage is to lay a small cover-slip over the object, and then to allow a thin metal rod to fall through glass tubes of various lengths, which are held over the cover-slip, but do not touch it. Or rods of different weight may be used and allowed to fall from the same height, the force of impact being then directly proportional to the mass'. As it turned out, my apparatus was simply a modification of the method of Ewart (1903). However, unlike Ewart, I was able to take advantage of nearly a century of new information on mechano-sensing in Characeae, as well as the modern insights in the plant plasma membrane.

In electrophysiology, E_m is usually measured by inserting a microelectrode into a cell. However, it is almost impossible to monitor E_m with a microelectrode during mechanical stimulation, due to impairment of the seal around the inserted microelectrode. However because of the large size of characean cells, it is possible to measure E_m without actually inserting a microelectrode, because part of the cell can be isolated and used as the electrical reference. This is made possible by applying high concentrations of KCl, which forces the membrane potential towards zero. Using a chamber, shown in Fig. 4, pool B is filled with a solution containing 100 mM KCl, and pool A is filled with APW supplemented with 180 mM sorbitol, which is osmotically isotonic to 100 mM KCl. In the presence of 100 mM KCl in the external medium, E_m is close to 0 mV. Therefore, the potential difference measured between pools A and B represents E_m of the cell part in pool A (K-anesthesia method; Shimmen *et al.* 1976).

A typical recording is shown in Fig. 5. A glass tubing of 1.3 g was used for stimulation. When it was dropped from a height of 1 cm, a small receptor potential was generated. By increasing H, the amplitude of the receptor potential

increased. At the time shown with an arrowhead, E_m reached a threshold and an action potential was generated.

In addition to E_m, membrane resistance (R_m) is also an important parameter in electrophysiology. In the apparatus shown in Fig. 4, the intracellular resistance at the partition between two pools is high and it is therefore difficult to exactly measure R_m of the cell. Development of the apparatus shown in Fig. 6 made it possible to exactly
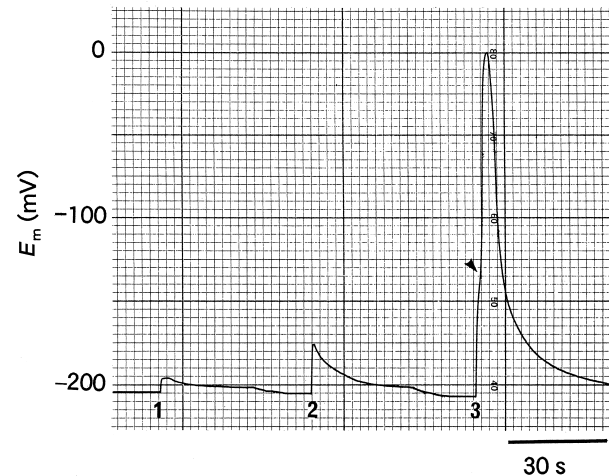

Fig. 5. A typical record in an internodal cell of *C. corallina* following mechanical stimulation. The cell was stimulated using the apparatus shown in Fig. 4. Numbers below the records represent the height (cm) from which the glass tubing (1.3 g) for stimulation was dropped. Modified from Shimmen (1997*b*).
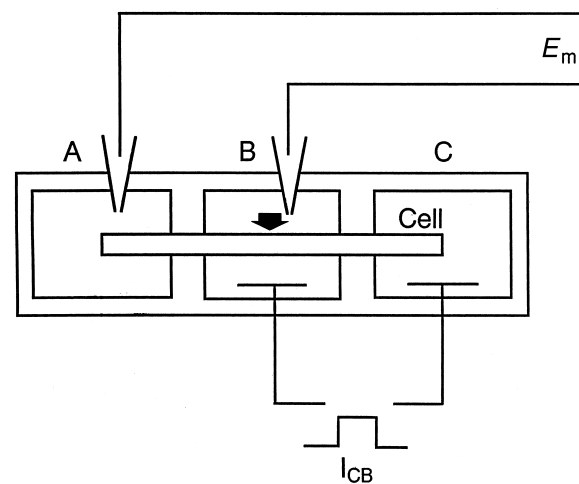

Fig. 6. Apparatus for measurement of R_m during mechanical stimulation in internodal cells of Characeae. An internodal cell is partitioned into three parts (A, B and C). Pool A is filled with 100 mM KCl, pools B and C are filled with APW supplemented with 180 mM sorbitol. E_m of the cell part in pool B is monitored by measuring the potential difference between pools A and B. Current pulses for measurement of R_m are applied between pools B and C (I_{CB}). Modified from Shimmen (1997*c*).

measure R_m (Shimmen 1997c). An internodal cell is partitioned into three pools, A, B and C. Pool A is filled with 100 mM KCl, and pools B and C are filled with APW supplemented with 180 mM sorbitol. The potential difference between pools A and B represents the E_m of the cell part in pool B. Electrical current pulses are applied between pools B and C. Thus, R_m of the cell part in pool B is measured without disturbance by the intracellular resistance at the partition between pools. The cell part in pool B is stimulated in the same way as shown in Fig. 4 (black arrow). An example of the measurement is shown in Fig. 7, where the cell was sequentially stimulated by dropping a glass tube from a height of 1, 2 and 4 cm. When the cell was stimulated from a height of 4 cm, an action potential was generated. At the peak of each receptor potential, deflection of E_m due to current pulses was smaller than that before stimulation. In most cases, an action potential started at the peak of a receptor potential as shown in Fig. 5. Occasionally, an action potential was generated after a delay from the peak of a receptor potential (Fig. 7). In such cells, decrease in R_m at the peak of a receptor potential of larger size can be clearly recognised. This technique allowed for the first time, the measurement of R_m during the mechanical induction of a receptor potential in plants (Shimmen 1997c).

Ionic mechanism of receptor potential

An intriguing question that has occupied plant physiologists is, what is the ionic mechanism for generation of receptor potentials? A possibility was that the depolarization of the receptor potential was induced due to inhibition of the activity of the electrogenic proton pump by mechanical

stimulation. However, this possibility was excluded, since the normal receptor potential could be induced even when the proton pump was inhibited (Shimmen 1997a). Other candidates included Cl^- and Ca^{2+} channels because the equilibrium potential for these ions across the plasma membrane is inner positive. Activation of ion channels is supported by the fact that R_m decreases during generation of receptor potentials (Fig. 7). By voltage-clamping the cells, it should be possible to obtain the reversal potential (equilibrium potential) across the membrane for the ion involved in generation of the receptor potential. This technique using a chamber illustrated in Fig. 6 proved to be unsuccessful because prolonged clamping of the electrical potential at the very positive level severely damaged the electrogenesis at the plasma membrane (Shimmen, unpublished result).

Channel inhibitors are also a commonly used to identify ion channels involved in electrical phenomena. However, a range of inhibitors for Cl^- and Ca^{2+} channels failed to inhibit the generation of receptor potentials (Shimmen 1997a). A completely different strategy eventually provided evidence concerning the identity of the ion channel involved (Shimmen 1997b). As mentioned above, E_m depolarises to a level close to 0 mV in the presence of 100 mM KCl in the external medium. By filling both pool A and pool B in Fig. 4 with 100 mM KCl, the whole cell was depolarised. When the cell was stimulated in such a depolarised state, E_m changed in the negative direction (Fig. 8; 100 KCl). However, when the 100 mM KCl solution was replaced with a solution of 50 mM K_2SO_4, E_m changed to the positive direction upon stimulation. By changing the external medium to 100 mM KCl solution, E_m again changed to the negative direction upon mechanical stimulation. Assuming the cytoplasmic Cl^- concentration to be 21 mM (Tazawa et al. 1974), the
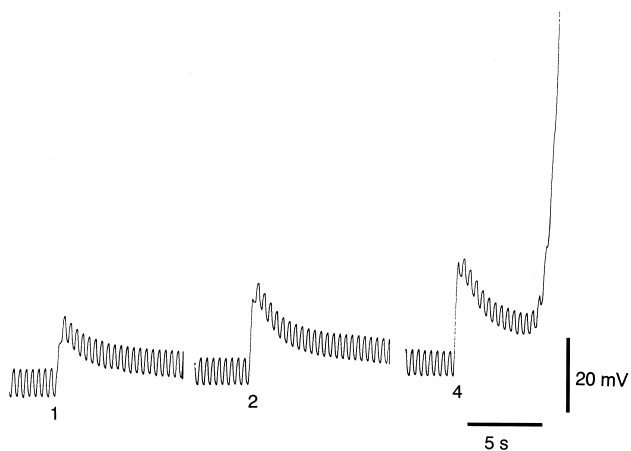

Fig. 7. Measurement of R_m during generation of a receptor potential in an internodal cell of *C. corallina*. Measurement was carried out, using the chamber shown in Fig. 6. An internodal cell of *C. corallina* was successively stimulated by dropping a piece of glass tubing from a height of 1, 2 and 4 cm. When the cell was stimulated from a height of 4 cm, an action potential was induced. Numbers below the records represent the height (cm). During the measurement, current pulses of 1 μA were applied.
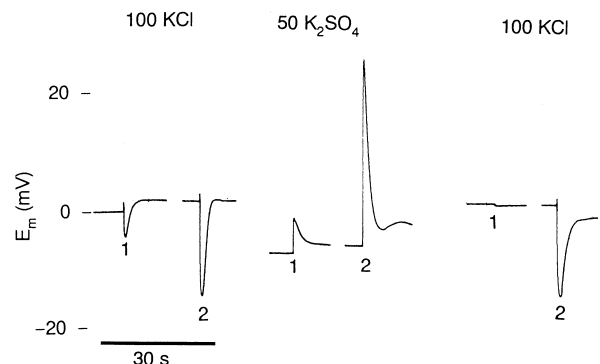

Fig. 8. Change of E_m in K^+-depolarised cell of *C. corallina* following mechanical stimulation. An internodal cell was mounted in the apparatus shown in Fig. 4. Pool B was filled with 100 mM KCl throughout the measurement. Experiments were successively carried out by applying 100 mM KCl, 50 mM K_2SO_4 and 100 mM KCl to pool A. Numbers below the records represent the height (cm) from which a piece of glass tubing was dropped. Cited from Shimmen (1997b).

equilibrium potential for Cl⁻ across the plasma membrane was calculated to be –39 mV and +70 mV in 100 mM KCl and 50 mM K_2SO_4, respectively (APW contains 1.2 mM Cl⁻). Thus, the results shown in Fig. 8 indicated that Cl⁻ channels were activated upon mechanical stimulation, albeit under non-physiological conditions (100 mM K⁺ in the external medium). Using the apparatus shown in Fig. 6, the change of R_m during mechanical stimulation in the K⁺-depolarised state was studied. E_m was depolarised in the presence of 50 mM K_2SO_4, and the cell was stimulated by dropping a piece of glass tubing. As is evident from Fig. 9, R_m significantly decreased during the electrical response, supporting the belief that mechanical stimulation under K⁺-induced depolarization is also generated by activation of an ion channel, probably Cl⁻. R_m also significantly decreased during the potential change to the negative direction upon mechanical stimulation in the presence of 100 mM KCl in the external medium (data not shown).

When an internodal cell was stimulated by dropping a piece of glass tubing from a threshold height, the duration of mechanically-induced action potentials was the same as that of electrically-induced action potentials, indicating that the mechanically-induced action potential was generated by activation of voltage-sensitive channels. When H was increased, the duration of action potential was increased. Further increase in H, resulted in very prolonged depolarization, similar to the variation potential (Shimmen 1996). It is suggested that both mechano-sensitive and voltage-
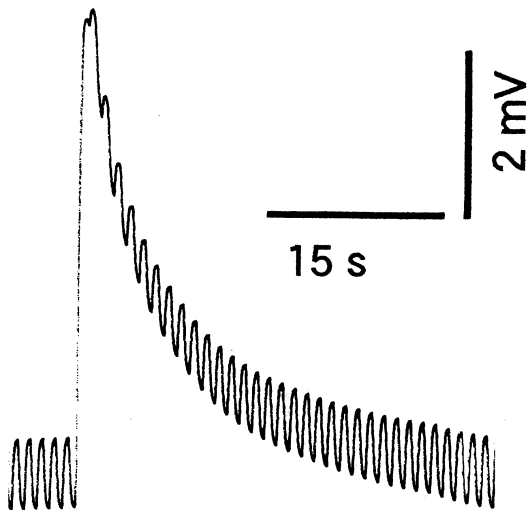
dependent channels are involved in the prolonged action potential or variation potential. Staves and Wayne (1993) described various features of touch-induced action potentials in *C. corallina*, which were consistent with the operation of both voltage-dependent and mechano-sensitive channels.

Application of the apparatus

A brief investigation was made into whether the apparatus developed for characean cells could be applied to higher plants. A seedling of broccoli (*Brassica oleracea* var. bolrytis) was mounted on the apparatus shown in Fig. 6. Upon dropping the glass tubing onto the hypocotyl, a significant change in the electrical potential was induced, the amplitude of which increased with increase in H. (Fig. 10). Thus, this apparatus developed for internodal cells of Characeae is also useful for analysis of electrical responses of higher plants to mechanical stimuli.

Conclusion

In *M. pudica* and in some carnivorous plants, the electrical signal plays a central role in signal processing in responses to mechanical stimulation. Since the plasma membrane is the first to encounter the mechanical stimulus, it will logically be the site for perception. In animal cells, it is generally accepted that receptor potential plays a central role in perception of various stimuli and it is expected that this will also be the case in plants, although information is scanty. The very early steps of mechano-sensing in plants can be investigated by monitoring the electrical signals, and this is conveniently done by exploiting various features of characean algal cells (Figs 5 and 7). Chemical changes are also associated with the electrical signals. It has been established in characean cells injected with aequorin, that
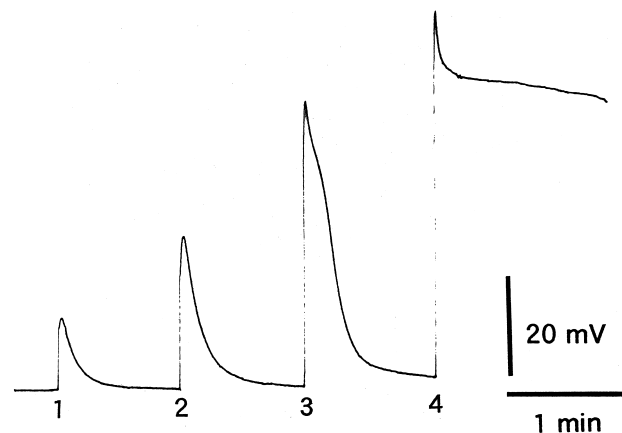
Fig. 9. Change of E_m and R_m in K⁺-depolarised cell of *C. corallina* following mechanical stimulation. An internodal cell was mounted in the apparatus shown in Fig. 6. Pools A, B and C were filled with 100 mM KCl, 50 mM K_2SO_4 and APW supplemented with 180 mM sorbitol, respectively. The cell part in pool B was stimulated by dropping a piece of glass tubing (1.3 g) from a height of 2 cm. During the measurement, current pulses of 1 µA were applied between pools B and C.

Fig. 10. Electrical response of hypocotyl of broccoli to mechanical stimulation. A seedling was mounted in the apparatus shown in Fig. 6. The hypocotyl was stimulated at pool B by dropping a piece of glass tubing (1.3 g). Numbers below the records represent the height (cm) from which the glass tubing was dropped.

generation of action potentials induces significant increases in the concentration of Ca^{2+} in the cytoplasm (Williamson and Ashley 1982; Kikuyama and Shimmen 1997). The increase in cytoplasmic Ca^{2+} initiates intracellular signal processing, resulting in cessation of cytoplasmic streaming (Tazawa and Kishimoto 1968) and activation of Cl^- channels in the tonoplast (Kikuyama 1986, 1989; Shimmen and Nishikawa 1988; Kikuyama and Shimmen 1997). The development of transgenic plants expressing aequorin has also made it possible to monitor changes in Ca^{2+} concentration following mechanical stimulation in higher plants. Increase in cytoplasmic Ca^{2+} upon mechanical stimulation (Knight *et al*. 1991, 1992; Haley *et al*. 1995) may start signal processing, resulting in various responses.

In *M. pudica* and carnivorous plants, the action potential seems to have two roles: the transmission of signals along organs and the induction of turgor movement. It is suggested that the action potential is coupled with bulk flow of ions in motor cells (Fig. 3). In muscle and characean cells, motility based on the actin system is controlled by action potentials. Although the motive force *per se* is generated by loss of turgor pressure, control of the actin-system by action potentials may also play a role in turgor movement.

The physiological role of transmission of electrical signals has been rather obscure in higher plants, but it has become evident that systemic expression of genes in wounding responses seems to be mediated by electrical signals (Wildon *et al*. 1992). Thus, electrical phenomena seem to play essential roles in the life of plants. As repeatedly stressed in the present article, characean cells can be a useful material for analysis of electrical responses of plants to various stimuli.

Acknowledgment

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