Possible Involvement of Mechanosensitive Ca$^{2+}$ Channels of Plasma Membrane in Mechanoperception in Chara

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When an internodal cell of Chara corallina was stimulated with a mechanical pulse of various amplitudes lasting for 0.1 s (mechanical stimulus), the cell generated a receptor potential, which was highly dependent not only on the strength of the stimulus but also on the extracellular Cl$^-$ concentration. Extracellular Ca$^{2+}$ was indispensable for generating receptor potential, since removal of Ca$^{2+}$ reversibly inhibited generation of the receptor potential. The cytoplasmic Ca$^{2+}$ level transiently rose upon mechanical stimulation. The stronger the mechanical stimulus, the larger was the increase in the cytoplasmic level of Ca$^{2+}$. It is proposed that the first step of receptor potential is an activation of mechanosensitive Ca$^{2+}$ channels at the plasma membrane.

Keywords: Ca$^{2+}$ — Chara — Mechanical stimulus — Mechanosensitive Ca$^{2+}$ channel — Receptor potential.

Abbreviations: APW, artificial pond water; [Ca$^{2+}$], cytoplasmic level of Ca$^{2+}$; Δ[Ca$^{2+}$], increase in [Ca$^{2+}$]; PM, photomultiplier.

Introduction

Plants respond to various circumstantial stimuli such as light, temperature, water, etc. Among them, mechanical stimuli are also very important signals and enhance ethylene biosynthesis so that plants change their form from elongated to expanded (Botella et al. 1995, Goeschl et al. 1966). Mechanical bending of sensory hairs in the lobe of the Venus fly trap induces generation of an action potential at the motor cells resulting in a sudden closure of the lobe (Sibaoka 1991). Geotropism may be another example of mechanosensing in plants.

The cellular mechanism of signal perception of a mechanical stimulus has not been fully studied, probably due to the complicated structures of multicellular systems of higher plants. Taking advantage of the simple structure of characean plants, Kishimoto (1968) and Shimmen (1996) studied the ionic mechanism of mechanical signal perception at the cellular level. Shimmen (1996) dropped a weight onto an internodal cell of Chara corallina (mechanical stimulus), and demonstrated the following. (i) Upon the mechanical stimulus, a change in membrane potential ($E_m$) took place in the depolarizing direction in artificial pond water (APW), which was sometimes followed by an action potential (Shimmen 1996). (ii) The amplitude of the change in $E_m$ was dependent on the strength of the mechanical stimulus. Thus it is concluded that the response in $E_m$ to a mechanical stimulus was a type of receptor potential (Kishimoto 1968, Shimmen 1996). (iii) Electrical membrane resistance decreased during receptor potential, suggesting activation of ion channel(s) (Shimmen 1997c). (iv) The receptor potential was accounted for by an activation of Cl$^-$ channels at the plasma membrane, because the change in $E_m$ was highly dependent on the Cl$^-$ concentration in the bathing medium (Shimmen 1997b). However, it remained unclear whether the mechanical stimulus was sensed by the Cl$^-$ channel itself or not.

It is well known that characean cells generate action potential. In the case of action potential, Cl$^-$ channel activation takes place in a Ca$^{2+}$-dependent manner, i.e. the electrical stimulus first induces activation of a Ca$^{2+}$ channel probably in a voltage-dependent manner (Kikuyama and Tazawa 1998, Kikuyama 2000). The activation of Ca$^{2+}$ channels necessarily increases Ca$^{2+}$ influx and causes a transient increase in the cytoplasmic level of Ca$^{2+}$ (Δ[Ca$^{2+}$]). The high [Ca$^{2+}$]c causes activation of Cl$^-$ channels not only at the plasma membrane but also at the tonoplast (Kikuyama 1986, Kikuyama 2000, Kikuyama and Shimmen 1997) in a Ca$^{2+}$-dependent manner.

Since Cl$^-$ channel activation also takes place in the generation of receptor potential (Shimmen 1997b), we hypothesized that Cl$^-$ channel activation during receptor potential also takes place in a Ca$^{2+}$-dependent manner as is the case for the action potential. According to this hypothesis, it is expected that a transient Δ[Ca$^{2+}$]c also takes place at the moment of mechanical stimulation, and we confirmed this in the present study. Furthermore, it is suggested that the Ca$^{2+}$ channel at the plasma membrane of Chara is mechanosensitive because the Δ[Ca$^{2+}$]c is dependent on the strength of mechanical stimuli.

Results

Re-examination of the effect of Cl$^-$ outside the cell

We first studied whether similar results to those of the previous work (Shimmen 1996, Shimmen 1997a, Shimmen...
Mechanosensitive Ca\textsuperscript{2+} channel in Chara plasma membrane are also observed with our system, because a rotary solenoid was used to apply mechanical stimuli in the present study. All mechanical stimuli were applied onto the cell for 0.1 s, i.e. electric current was applied to the solenoid for 0.1 s. The cells were partitioned into two pools: pool A and pool B. Then both pools A and B were filled with 55 mM KCl supplemented with 0.1 mM CaCl\textsubscript{2} (simply shown as 55KCl) and with APW supplemented with 100 mM sorbitol (simply shown as APW), respectively (Fig. 6). Since the membrane potential of the cells in pool A is very close to zero, any potential difference between pools A and B can be regarded as the membrane potential of the cells in pool B (Shimmen et al. 1976) and will be simply shown as $E_{m}$. When a cell in pool B was mechanically compressed for 0.1 s, the cell showed a small but significant response in $E_{m}$ and a typical example is shown in Fig. 1. The amplitude of the response was dependent on the strength of the mechanical stimuli. In the case of Fig. 1a, a mechanical stimulus of about 53 g caused the response of about 0.5 mV, while a stimulus of 63 g caused a response of about 2.5 mV (Fig. 1a). When the cell was mechanically stimulated in 55KCl in which $E_{m}$ is highly depolarized, the response changed its direction to a negative one ($-1.5$ mV, Fig. 1b), indicating that activation of Cl\textsuperscript{–} channels at the plasma membrane is involved in the response. These results indicate that the amplitude of the response is dependent not only on the mechanical stimuli but also on the Cl\textsuperscript{–} concentration outside the cell, and are completely consistent with the report by Shimmen (1997b).

Effect of Ca\textsuperscript{2+}

The possible contribution of Ca\textsuperscript{2+} in generation of a receptor potential was studied by removing Ca\textsuperscript{2+} from the extracellular bathing medium as follows. First, receptor potential was measured in a condition in which both pools of the measuring vessel were filled with a medium containing 55 mM KCl, 0.1 mM CaCl\textsubscript{2} and 23 mM sorbitol (simply shown as 0.1Ca-KCl), because the cell loses excitability in a medium containing 55 mM KCl. It must be noted that 10 mM CaCl\textsubscript{2} and MgCl\textsubscript{2} are isotonic to 23 mM sorbitol. A representative example is shown in Fig. 2, in which the strength of the mechanical stimulus was kept constant at 37 g for 0.1 s. Upon the first stimulus, a receptor potential was observed with an amplitude of

![Fig. 1](image1.png)  
**Fig. 1** Dependence of the amplitude of receptor potential ($E_{m}$) on the strength of mechanical stimuli and Cl\textsuperscript{–} concentration outside the cell. The cell was mechanically stimulated at time zero for 0.1 s. (a) A cell was stimulated in APW ($[\text{Cl}^{-}]_{o} = 0.4$ mM). (b) A cell was stimulated with 67 g in 55KCl ($[\text{Cl}^{-}]_{o} = 55$ mM).

![Fig. 2](image2.png)  
**Fig. 2** Effect of extracellular Ca\textsuperscript{2+} on the amplitude of the receptor potential ($\Delta E_{m}$). Mechanical stimuli of 37 g for 0.1 s were applied to the same cell at time zero. Since measurements were carried out in a medium containing 55 mM KCl, the $\Delta E_{m}$ was negative.
Mechanosensitive Ca$^{2+}$ channel in Chara plasma membrane

About 10 min after replacing the 0.1Ca-KCl with a medium containing 55 mM KCl and 10 mM MgCl$_2$ but no Ca$^{2+}$ (simply shown as 0Ca-KCl), a receptor potential was scarcely observed (0Ca-KCl in Fig. 2). The amplitude of the receptor potential, once highly attenuated in 0Ca-KCl, was recovered (about –4.4 mV) when the medium was exchanged again with a medium containing 55 mM KCl and 10 mM CaCl$_2$ (10Ca-KCl in Fig. 2). Similar experiments showed that the receptor potential was significantly inhibited in 0Ca-KCl in all 14 cells tested.

Although Shimmen (1997b) already reported that the Ca$^{2+}$ channel inhibitors La$^{3+}$ and nifedipine showed only a small effect on the receptor potential, the effect of the inhibitors Gd$^{3+}$ and La$^{3+}$ was also studied with much higher concentrations.

Ca$^{2+}$ channel inhibitor

Although Shimmen (1997b) already reported that the Ca$^{2+}$ channel inhibitors La$^{3+}$ and nifedipine showed only a small effect on the receptor potential, the effect of the inhibitors Gd$^{3+}$ and La$^{3+}$ was also studied with much higher concentrations.

Although La$^{3+}$ at 1 mM inhibited generation of the action potential, it did not inhibit the receptor potential in 20 cells tested (data not shown). Furthermore, simultaneous measurement of the receptor potential and light emission of aequorin [photomultiplier (PM) current] demonstrated that no inhibitory effect of 1 mM La$^{3+}$ was observed on the generation of a receptor potential or on a transient increase in PM current (Fig. 3), although the measurement was carried out on only two cells. Gd$^{3+}$ also showed no inhibitory effect on generation of a receptor potential in all four cells tested. Fig. 4 is one typical example showing that the cell generated receptor potentials whose amplitude was clearly dependent on the strength of mechanical stimuli, as was the case in Fig. 1.

Fig. 3 An example of simultaneous measurements of receptor potential ($E_m$) and PM current (=[$Ca^{2+}$]) of a cell in 55KCl supplemented with 1 mM LaCl$_3$. Since measurement was carried out in 55KCl, the $\Delta E_m$ was negative. Stimulation was at time zero.

Fig. 4 A typical result showing the effect of 1 mM Gd$^{3+}$ on receptor potential ($E_m$). The measurement was carried out in the APW supplemented with 1 mM GdCl$_3$. The strength of the mechanical stimulus was 6.8, 11, 18 and 31 g for 0.1 s. Each stimulation was at time zero.

Fig. 5 A typical example of simultaneous measurements of $\Delta E_m$ and PM current (=[$Ca^{2+}$]) upon mechanical stimuli on the same cell. Since measurements were carried out in 55KCl, the $\Delta E_m$ was negative. Each stimulus induced not only $\Delta E_m$ but also $\Delta [Ca^{2+}]$. (a) The mechanical stimulus was 55 g for 0.1 s. (b) The mechanical stimulus was 72 g for 0.1 s. Each stimulation was at time zero.
Table 1  Relationship between the strength of mechanical stimuli and ∆[Ca^{2+}]

<table>
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<th>Cell no.</th>
<th>50 g for 0.1 s</th>
<th>106 g for 0.1 s</th>
<th>160 g for 0.1 s</th>
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<tr>
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<td>5.7</td>
<td>61.6</td>
<td>876</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>7.1</td>
<td>60</td>
</tr>
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<td>3</td>
<td>0</td>
<td>0</td>
<td>277</td>
</tr>
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<td>21.7</td>
<td>49.5</td>
<td>&gt;2,000</td>
</tr>
<tr>
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<td>3.5</td>
<td>670</td>
<td>617</td>
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<tr>
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</tr>
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</tr>
<tr>
<td>9</td>
<td>0</td>
<td>3.5</td>
<td>258</td>
</tr>
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</table>

Transient ∆[Ca^{2+}]

The first experiments above (Fig. 2), in which extracellular Ca^{2+} was removed, suggest the possible contribution of Ca^{2+} to generation of receptor potential. In contrast, the second inhibitor studies do not (Fig. 4). Thus, we tried to obtain more direct evidence for the possible contribution of Ca^{2+} to the receptor potential. If Ca^{2+} channel activation also has an important role in generation of receptor potential as is the case for the action potential, transient ∆[Ca^{2+}] should take place upon mechanical stimuli. The photoprotein aequorin was used as an indicator of the [Ca^{2+}]. An internodal cell of Chara loaded with aequorin was mechanically stimulated, and changes in both E_{m} (receptor potential) and light emission of aequorin (∆[Ca^{2+}]) were measured simultaneously in 55KCl and recorded. Fig. 5 shows one representative example. Both parameters, light emission of aequorin and membrane potential, started to change at the moment of the stimulus, and this was the case in all trials on >10 cells. Fig. 5 also shows that the larger stimulus induced not only the larger receptor potentials but also the larger increase in the PM current. Table 1 is a summary showing the relationship between the strength of mechanical stimuli and the increase in the PM current. Since the experiment shown in Table 1 was carried out at a different time and on different cultures from those of other experiments including those in Fig. 5, the strength of the mechanical stimuli sufficient to cause a ∆[Ca^{2+}] was much larger than that in Fig. 5. Thus, Table 1 does not contain the data shown in Fig. 5. Although increases in the PM current were largely different from cell to cell, there was a tendency for the stronger stimulus to cause a larger increase in the PM current, suggesting that activation of Ca^{2+} channels at the plasma membrane is dependent on the strength of the mechanical stimuli (Table 1).

Discussion

Shimmen (1997b) demonstrated that the ionic mechanism of Chara receptor potential is an activation of Cl− channels at the plasma membrane because it is highly dependent on the Cl− concentration in the bathing medium, and this is also confirmed in the present study (Fig. 1a, b). In the case of the action potential in characean plants, the major process of its depolarizing phase is also activation of Cl− channels, which takes place in a Ca^{2+}-dependent manner (e.g. Kikuyama 2000) as already shown in the Introduction. Thus, we hypothesized that a similar ionic process to that in generation of the action potential is also working in generation of the receptor potential. According to this hypothesis, the sensor of the mechanical stimulus may be the Ca^{2+} channel, and the first step may be an activation of Ca^{2+} channels at the plasma membrane in a mechanosensitive manner. Then ∆[Ca^{2+}] may occur through increased influx of Ca^{2+} from the outside, resulting in activation of Cl− channels in a Ca^{2+}-dependent manner, as is the case for the action potential. We tested the hypothesis in the present study.
In the present study, an apparatus for applying a mechanical stimulus was constructed by modifying a rotary solenoid. With this apparatus, we could easily control both the strength and the duration of the mechanical stimulus though only 0.1 s duration was adopted in the present study. The solenoid method also opened up a way to stimulate the cell in a dark box (cf. Fig. 7) because the stimulator works in a remote-controlled manner. Furthermore, the most important point was that the method gave us similar results to those reported by Shimmen (Shimmen 1996, Shimmen 1997a, Shimmen 1997b) who stimulated the cell by simply dropping a glass weight onto the cell.

The possible contribution of Ca\(^{2+}\) to the generation of receptor potential was first suggested by Shimmen (1997b) because the receptor potential was significantly attenuated when [Ca\(^{2+}\)] outside the cell was extremely lowered by EGTA. In spite of this, he did not claim the result to be sufficient evidence of the indispensable importance of Ca\(^{2+}\) for generation of receptor potential, because EGTA in the bathing medium often modulates the membrane character through eliminating Ca\(^{2+}\) from the plasma membrane (Shimmen 1996). In the present study, however, [Ca\(^{2+}\)] outside the cell was decreased by changing the bathing medium from one containing 0.1 mM Ca\(^{2+}\) to another containing no Ca\(^{2+}\) but 10 mM Mg\(^{2+}\), instead of by adding EGTA to the bathing medium (cf. Kikuyama 1986). This treatment significantly attenuated the receptor potential (0Ca-KCl in Fig. 2). Since the receptor potential once attenuated was never restored by further decreasing [Ca\(^{2+}\)] outside the cell by adding Ca\(^{2+}\) to the bathing medium (Shimmen 1997b) may be excluded in the present study. Tsutui et al. (1987) reported that the internodal cell of Chara corallina remained excitable even after removal of external Ca\(^{2+}\) by EGTA, although the amplitude of the action potential was highly attenuated. The internodal cell of Nitella axilliforins remained excitable in the medium containing no Ca\(^{2+}\) but 10 mM Mg\(^{2+}\), though the action potential changed its shape to rectangular (Kikuyama 1986). These results also may not suggest the possible damage to the plasma membrane of the Chara internode in 0Ca-KCl.

The possible contribution of Ca\(^{2+}\) channel activation to the receptor potential is shown more directly by the simultaneous measurement of receptor potential and PM current. A rapid increase in PM current (\(\Delta[Ca^{2+}]\)) always took place at the same time as the receptor potential, as shown in Fig. 5. Furthermore, \(\Delta[Ca^{2+}]\) was dependent on the strength of the stimulus, as shown in Fig. 5 and Table 1. These results strongly suggest that activation of Ca\(^{2+}\) channels occurs at the plasma membrane probably in a mechanosensitive manner. Involvement of a mechanosensitive Ca\(^{2+}\) channel was also suggested in intracellular Ca\(^{2+}\) store(s) of characean plants, especially in Nitella flexilis (Kikuyama and Tazawa 2001). In this case, Ca\(^{2+}\) release from the store occurred upon stretching the store membrane, which was evoked by osmotic swelling of cytoplasm or mechanical agitation of an isolated cytoplasmic drop. A similar phenomenon was observed in insulin-secreting cells: hypotonic swelling of RINm5F insulinoma cells caused a marked increase in cytoplasmic [Ca\(^{2+}\)] (Sheader et al. 2001). Mechanosensitive Ca\(^{2+}\) channels have been reported in epithelial cells isolated from the endolymphatic sac of guinea pigs (Miyaishi et al. 2001), human smooth muscle (Holm et al. 2001) and yeast (Kanzaki et al. 1999, Maruoka et al. 2002). Channel activation by mechanical stresses in the stress fibers from rat and frog was also examined (Hayakawa et al. 2001). Involvement of the mechanosensitive channel was also reported in plants (Garrill et al. 1992, Ding and Pickard 1993).

Calcium channel inhibitors did not affect the receptor potential but did affect the action potential (Shimmen 1997a).

In conclusion, we first demonstrated that \(\Delta[Ca^{2+}]\) takes place at the moment that a mechanical stimulus is applied to Chara internodal cells, and that the amplitude of \(\Delta[Ca^{2+}]\) is dependent on the strength of the mechanical stimulus. We assume that the basic process of mechanon-perception is an activation of mechanosensitive Ca\(^{2+}\) channels at the plasma membrane, though a major part of receptor potential is accounted for by activation of Cl channels. Thus, we propose the ionic mechanism of generation of receptor potential as occurring in the following order: (i) activation of mechanosensitive Ca\(^{2+}\) channels at the plasma membrane; (ii) increase in [Ca\(^{2+}\)] as a result of, probably, increased influx of Ca\(^{2+}\) from the outside; and (ii) activation of Cl channels at the plasma membrane in a Ca\(^{2+}\)-dependent manner.

**Materials and Methods**

**Plant material**

Chara corallina cultured in the laboratory was used throughout the experiment. After isolating single internodes from neighboring leaflets and internodes, they are kept in APW (0.1 mM each of KCl, NaCl and CaCl\(_2\)) for >1 d before use.

**Electrical measurements**

\(E_{m}\) of C. corallina was measured in the same manner as in Shimmen (Shimmen 1997a, Shimmen 1997b). Briefly, an internode was placed in a measuring chamber with two pools, A and B, as shown in Fig. 6a. Two kinds of medium were mainly used. One was 55 mM KCl supplemented with 0.1 mM CaCl\(_2\) (simply shown as 55KCl) and the other was APW made isotonic to 55KCl by adding 100 mM sorbitol, and this is simply shown as APW. When other media were used, the compositions are shown in the Results.

After electrically insulating two pools at the partition wall with Vaseline, pool A was filled with 55KCl and pool B with APW. Since the membrane potential of the cells in 55KCl was very close to zero, the potential difference between pools A and B was very close to the \(E_{m}\) of the cells in pool B (‘K-anesthesia method’, Shimmen et al.
whose turgor was reduced by immersing the cell in a medium containing 5 mM CaCl$_2$, was used as a Ca$^{2+}$ indicator. An aequorin solution, composed of 100 mM KCl, 6 mM MgCl$_2$, 0.1 mM EGTA and about 1 mg ml$^{-1}$ aequorin, was microinjected into the cytoplasm of an internodal cell of *C. corallina* whose turgor was reduced by immersing the cell in a medium containing 10 mM MgCl$_2$ and 100 mM sorbitol. After the injection, the injury accompanying the insertion of a micropipette was repaired by immersing the cell in a medium containing 5 mM CaCl$_2$ and 50 mM sorbitol for several seconds.

The internodal cell loaded with aequorin was put in the measuring chamber and the chamber was placed over a PM tube (R1924P, Hamamatsu Photonics, Hamamatsu, Japan) in a dark box (Fig. 7). Light emission from aequorin was measured by the PM. The PM current, which reflects [Ca$^{2+}$], was also recorded in the same manner as the $E_m$.

**Mechanical stimulation**

For applying mechanical stimuli, one of the most simple and reliable methods is to drop a weight onto the cell (Shimmen 1996). In spite of this, we adopted another method because we had to stimulate the cell in a dark box in which simultaneous measurements were carried out on [Ca$^{2+}$] and on $E_m$ (Fig. 3, 5).

An apparatus for applying mechanical stimuli was constructed as follows. A polyacrylic plate (2 mm thickness) was attached at one end of a polyacrylic rod, then the rod was attached at the other end to the axis of a rotary solenoid (439–997, RS Components, Yokohama, Japan). The apparatus was placed just beside the measuring chamber. For applying mechanical stimuli on the cell in a dark box in which simultaneous measurements were carried out. The magnetic force was added to the original weight. The increase in the apparent weight was linearly dependent on the amplitude of electric current applied to the solenoid (data not shown). In the Results, the net increases in the apparent weight of the rod was also measured directly with an electric balance. By applying electric current to the solenoid, the apparent weight of the rod increased because the magnetic force was added to the original weight. The apparent weight of the rod was also measured directly with the balance. The increase in the apparent weight was linearly dependent on the amplitude of electric current applied to the solenoid (data not shown). In the Results, the net increases in the apparent weight of the rod were shown as the strength of the mechanical stimulus.

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**References**


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