Protoplasma (1992) 168: 141–152

PROTOPLASMA

Hydrostatic pressure mimics gravitational pressure in characean cells

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Received October 25, 1991
Accepted December 30, 1991

Summary. Hydrostatic pressure applied to one end of a horizontal *Chara* cell induces a polarity of cytoplasmic streaming, thus mimicking the effect of gravity. A positive hydrostatic pressure induces a more rapid streaming away from the applied pressure and a slower streaming toward the applied pressure. In contrast, a negative pressure induces a more rapid streaming toward and a slower streaming away from the applied pressure. Both the hydrostatic pressure-induced and gravity-induced polarity of cytoplasmic streaming respond identically to cell ligation, UV microbeam irradiation, external Ca^{2+} concentrations, osmotic pressure, neutral red, TEA Cl^-, and the Ca^{2+} channel blockers nifedipine and $LaCl_3$. In addition, hydrostatic pressure applied to the bottom of a vertically-oriented cell can abolish and even reverse the gravity-induced polarity of cytoplasmic streaming. These data indicate that both gravity and hydrostatic pressure act at the same point of the signal transduction chain leading to the induction of a polarity of cytoplasmic streaming and support the hypothesis that characean cells respond to gravity by sensing a gravity-induced pressure differential between the cell ends.

Keywords: Calcium; *Chara corallina*; Cytoplasmic streaming; Gravity perception; Hydrostatic pressure; Sensory transduction.

Introduction

The constant gravity signal is transduced by plants into a number of physiological, growth, and developmental responses. These range from a polarity of cytoplasmic streaming (Ewart 1903) to karyogamy and the onset of sporulation in certain fungi (Moore 1991) to the well-known tropistic responses of higher plants. Although the effects of gravity on plants have been studied throughout this century, the mechanisms involved in gravity sensing remain obscure.

At present the starch-statolith hypothesis, in which sedimenting amyloplasts are considered to be primary gravireceptors, is the most generally accepted model for plant gravisensing (Sack 1991). However the existence of graviresponsive, statolith-free plants (Hayashi 1957, Westing 1971) and plant tissue (Wareing and Nasr 1958, Gersani and Sachs 1990) as well as the findings that starch-deficient and starch-free mutants are gravitropic (Caspar and Pickard 1989, Kiss et al. 1989, Kiss and Sack 1989, Moore 1989) argue in favor of the existence of an alternative gravisensing system.

We have studied the gravity-induced polarity of cytoplasmic streaming in the large statolith-free internodal cells of characean algae. In a horizontal cell streaming proceeds at the same rate right and left; however, in a vertical cell the downwardly-directed endoplasm streams ca. 10% faster than the upwardly-directed stream (Wayne et al. 1990). As an outcome of this research, we have proposed a model for gravisensing in which the entire protoplast settles within the cell wall (or extracellular matrix) as a consequence of gravitational pressure (Wayne et al. 1990). Thus, as a result of gravitational pressure, the cell experiences compressive stresses between the plasma membrane and the cell wall at the bottom of the cell and tensile stresses between the plasma membrane and the cell wall at the top of the cell. In this way the cell may perceive the directionality of the gravitational vector.

We have previously shown that placing a characean cell in an external medium more dense than the protoplast causes a reversed gravity response, i.e., the endoplasm streams up faster than down. We interpret these results to indicate that in a dense medium the protoplast will become buoyant, thus inducing compressive stresses between the plasma membrane and the wall at the top of the cell and tensile stresses between the plasma membrane and the cell wall at the bottom

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of the cell. This results in a reversal of the gravity signal (Wayne et al. 1990), and provides strong support for the gravitational pressure model.

Since gravitational pressure can induce a polarity of cytoplasmic streaming, we wanted to test whether or not a hydrostatic pressure applied to one end of the cell can mimic the effect of gravity by inducing a polarity of cytoplasmic streaming. Here we report that hydrostatic pressure mimics the effect of gravity, indicating that gravity may induce a polarity of cytoplasmic streaming through its action on the plasma membrane via gravitational pressure. Moreover, we show that the requirements of hydrostatic pressure-induced polarity are identical to those of the gravity-induced polarity.

Material and methods

Culture conditions

Chara corallina Klein ex Wild., em. R.D.W. (= *Chara australis* R. Brown) was cultured in aquaria containing soil/water medium under continuous white fluorescent light. The photon fluence rate of photosynthetically active radiation was 33 μmoles/m^2/s as measured with a Quantum/Radiometer/Photometer with a quantum sensor (Li-Cor Inc., Lincoln, NB, U.S.A.). The cells were grown at 27–28 °C (300–301 K). Under these conditions $CaCO_3$ is not deposited on the cell walls. Details of the culture conditions, and the ionic composition of the medium can be found in Wayne and Staves (1991).

Induction of hydrostatic pressure

A positive or negative hydrostatic pressure was applied to one end of *Chara* cells with the apparatus shown in Fig. 1. This apparatus was modified from one described by Wayne and Tazawa (1988) and originally designed by Kamiya (1940). One end of a single internodal cell, typically 35–45 mm long, was inserted into the apparatus. Silicone grease and a plexiglass block effectively isolated the medium surrounding this end of the cell, forming a double chamber (Fig. 1). The free end of the cell was bathed in the appropriate medium and covered with a cover glass. Thus, the inserted cell end was exposed to the hydrostatic pressure inside the apparatus whereas most of the cell was exposed to ambient atmospheric pressure. The inserted cell end typically extended 1–2 mm beyond the silicone grease. The apparatus was connected to a bottle containing artifical pond water (APW; 0.1 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$ buffered to pH 7.2 with 2 mM Hepes-NaOH) or a buffered Ca^{2+} solution (Wayne 1985) with Nalgene 180 lab/food grade tubing. When the level of the solution in the bottle was at the same height as the cell, the pressure applied to the inserted cell end was equal to that of the remainder of the cell. However, when the bottle was raised or lowered from this position, a positive or negative hydrostatic pressure was applied to the cell end inserted into the apparatus. A hydrostatic pressure change of 98 Pa, above the 0.1 MPa supplied by atmospheric pressure alone, is induced for each centimeter the bottle of APW is raised or lowered from the equilibrium position. Experiments were carried out at 25–26 °C (298–299 K).

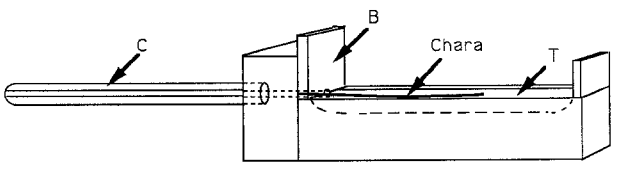

Fig. 1. Diagram of the double chamber used to introduce a hydrostatic pressure to one end of the cell. *T* Trough in which most of the cell lies, *B* plexiglas block, *C* capillary, which is connected by a tube to a water bottle. The cell is inserted so that the majority of the cell remains in the trough and approximately 1–2 mm is inserted in the chamber

Observations of cells

The apparatus was placed on the stage of a vertically- or horizontally-oriented Olympus (Tokyo, Japan) CH-2 microscope equipped with a × 20 (A 20 PL, NA = 0.4) objective lens and × 10/18 L CWHK oculars. Cytoplasmic streaming was observed with the chloroplasts in focus. Streaming velocity was determined by measuring the time required for individual cytoplasmic particles to stream past a 242 μm ocular micrometer. Twenty-five measurements were made in each direction with the microscope focused at the plane of the actin cables. The polar ratio was determined for vertically-oriented cells by calculating the ratio of the velocity of the downwardly-moving stream to that of the upwardly-moving stream. For horizontally oriented cells, the polar ratio is defined as the ratio of the velocity of the streaming away from the applied hydrostatic pressure to that of the streaming toward the applied pressure.

To determine whether the results we obtain by eye using a stopwatch can be reproduced by electronic data gathering and analysis, we used the following protocols. Gravity- or pressure-stimulated cells were observed on an Olympus BH-2 microscope equipped with Nomarski differential interference contrast optics, and the streaming rate was determined by eye as described above. Simultaneously, a video image of the streaming cytoplasm was collected by a video camera (Model CCD 72, Dage/MTI, Michigan City, IN, U.S.A.). The video image was processed using the Motion Enhancement mode of an Image-1 (Universal Imaging Corporation, West Chester, PA, U.S.A.) image processing and analysis system and viewed on a video monitor so that the endoplasm appeared as white particles moving over a black background. The processed image was recorded on video tape using a video recorder, digitized by a video processor (Model VP-110, Motion Analysis Corp., Santa Rosa, CA, U.S.A.) and viewed on a video monitor. The digitized data were collected by a 386 based computer and analyzed with a custom-made program using the ExpertVision Software. A computer program (copies available by request) was used to determine the speeds of the particles. In the process, it eliminated artifactual nonlinear paths as well as paths having unrealistically high or low speeds (i.e., $<$ or $>$ 2 × SD). The results obtained by electronic data gathering and analysis were not significantly different from those obtained by eye using a stopwatch. Since the results obtained with the stopwatch method are as reliable and objective as those obtained with the image processing and motion analysis systems, and the former method is much more convenient and faster, the stopwatch method is our method of choice. Another advantage of the ease and speed afforded by the stopwatch method is that it has enabled visiting scientists to repeat key experiments to their satisfaction in a short time.

Statistics were calculated with the aid of MINITAB (Minitab Inc., State College, PA). The streaming rates were analyzed statistically using *t* tests or paired *t* tests.

Ligation of cells

Internodal cells were covered with 100 mM sorbitol for 1 min to reduce turgor pressure. One or both ends of the cells were ligated with Singer polyester thread. The ligated ends were then cut off and the cells were allowed to recover for at least 30 min before further manipulation.

Irradiation of cells with a UV microbeam

Cells were irradiated through a number 1 cover glass with an ultraviolet microbeam (with a peak at 365 nm) that was constructed from a Zeiss HBO 50 bulb and lamp housing, including a condenser and a field diaphragm. The irradiated area was ca. 1 cm in diameter. The energy fluence rate through the cover glass was 239 ± 0.3 W/m^{-2} (n = 4) as measured with a Radiometer (Model IL 1700, International Light, Newburyport, MA, U.S.A). Cells were irradiated for 2 min, providing an energy fluence of 28,680 J/m^2. The effect of hydrostatic pressure on the polar ratio was analyzed, then the middle of the cell, where the streaming rates are measured, was irradiated and the polar ratio was determined again. Subsequently, the cell was irradiated at the single node that was exposed to atmospheric pressure and the polar ratio was measured again. In some cases, cells were exposed to only a single irradiation. All irradiations took place while the cells were mounted in the double chamber. Cells were irradiated with visible light by using the light passing through the substage condenser of an Olympus CH-2 microscope. The energy fluence rate was 92 W/m^2 and the cells were irradiated for 360 s giving an energy fluence of 33,120 J/m^2.

Preparation of calcium buffers and other solutions

Calcium buffers were prepared using the apparent association constants that were calculated with the aid of a computer program (Wayne 1985). The Ca^{2+} buffers included 0.1 mM KCl, 0.1 mM NaCl, 1 mM EGTA, 2 mM Hepes and various concentrations of $CaCl_2$. The buffers were titrated to pH 7.2 with NaOH.

In order to prepare solutions having various osmotic pressures, APW (7.4 kPa = 3 mOsm) was brought to 310 kPa (= 125 mOsm) and 636 kPa (= 257 mOsm) by the addition of ethylene glycol. The osmotic pressures of the solutions were measured with a freezing point depression osmometer (Model One-Ten Fiske Associates, Needham Heights, MA, U.S.A.). A 1% w/v (= 34.6 mM) aqueous stock solution of neutral red (N^8,N^8,3-trimethyl-2,8-phenazinediamine monohydrochloride; Allied Chemical Corporation) was added to APW to obtain a final concentration of 0.001% (34.6 µM). Tetraethylammonium chloride (TEA Cl^-; Aldrich) was added as a dry powder to the neutral red-containing APW. A 30 mM stock solution of nifedipine (Sigma Chemical Company, St. Louis, MO, U.S.A.) in DMSO was diluted with a calcium buffer containing 10 µM free Ca^{2+}.

$LaCl_3$ was added to an unbuffered APW solution which contained only 0.1 mM KCl, 0.1 mM NaCl and 0.1 mM $CaCl_2$. The pH of this solution is 6.2 and it has an osmotic pressure of 2.48 kPa.

Measurement of hydraulic conductivity and reflection coefficients

The hydraulic conductivity (L_p) of *Chara* was measured using the method of transcellular osmosis (Dainty and Ginzburg 1964, Kamiya and Tazawa 1956, Wayne and Tazawa 1990). Briefly, a cell was placed symmetrically into the transcellular osmosis chamber and equilibrated in APW (7.4 kPa). The APW in one chamber was replaced with APW containing 50 mM sorbitol (133.7 kPa) and the initial rate of transcellular water flow (Q, in m^3/s) was measured by following the movement of an air bubble in a capillary from 12 s to 30 s after the onset of transcellular osmosis. See Wayne and Tazawa (1988) for details of the method and the calculations used to determine L_p. The osmotic permeabilty coefficient (P_{os}, in m/s) can be calculated from the hydraulic conductivity since $P_{os} = L_p RT/\bar{V}_w$, where $\bar{V}_w$ is the partial molar volume of water (in m^3/mol), R is the gas constant (8.31 Pa m^3/mol/K) and T is the temperature (in K).

The reflection coefficient (σ) for ethylene glycol is operationally defined as the ratio of the volume flow of water induced by ethylene glycol (133.7 kPa) to the volume flow of water induced by sorbitol. All transcellular osmosis experiments were performed at 25 °C (298 K).

The osmotic pressures of single internodal cells were measured with a freezing point depression osmometer.

Conversion factors

We express all our data in S. I. units. However, Pascals can be readily converted to other units by using the following conversion factors: 1 atm = 101,325 Pa; 1 bar = 10^5 Pa; 1 Torr = 133.3 Pa; 1 mm Hg = 133.3 Pa and 1 cm H_2O = 98 Pa. Osmotic pressure (π) can be calculated from the osmotic concentration (c, in mol/m^3 = mOsm) using the following relation:

$$\pi = RTc \quad (1)$$

where R is the gas constant (8.31 Pa · m^3/mol/K) and T is the temperature in Kelvin.

Results

Effect of applying hydrostatic pressure to horizontal cells

Application of hydrostatic pressure induces a polarity of cytoplasmic streaming in horizontal *Chara* cells (Fig. 2). Horizontal cells which experience no pressure differential between the cell ends (i.e., the height of the solution in the bottle is identical to that of the cell)

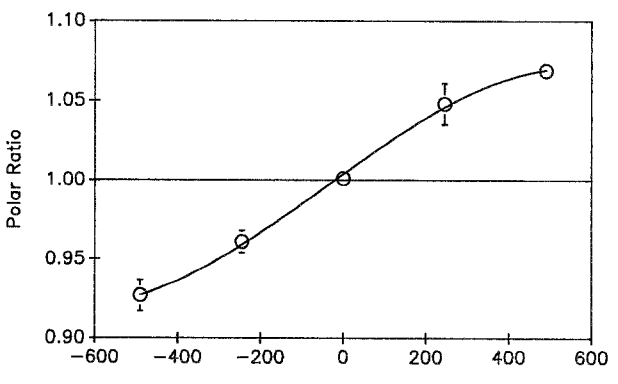

Fig. 2. The effect of hydrostatic pressure on the polar ratio of a horizontal cell. The polar ratio is defined as the velocity of streaming away from the applied pressure divided by the velocity toward the applied pressure. The data presented represent mean values ± S.E. for 3 cells exposed to each pressure

exhibit identical streaming rates toward and away from the inserted cell end. This is expressed as a polar ratio of 1.0. By contrast, as the bottle is raised, the polar ratio becomes greater than 1.0 and as it is lowered, the polar ratio becomes less than 1.0. Note that the greatest applied hydrostatic pressure (490 Pa) is equivalent to the osmotic pressure induced by only a 0.198 mOsm solution.

A comparison of streaming rates and polar ratios determined by the stopwatch method and the image processor/motion analysis method shows that the electronic method produces slightly faster streaming rates (0.93 μm/s) and slightly higher polar ratios (0.004) (data not shown). However, paired *t* tests indicate that the results are not significantly different ($p = 0.23$ for streaming rates, $n = 30$; and $p = 0.39$ for polar ratios, $n = 15$).

Measurement of the velocity gradient of cytoplasmic streaming across an optical section of the cell reveals that equal volumes flow at equal rates in both directions of a horizontal cell when no differential pressure is applied. When a positive pressure is applied, streaming near the actin bundles is faster away from the applied pressure than toward it. However, a larger volume of cytoplasm flows toward the applied pressure than away from it (Fig. 3). Thus, equal volumes flow in each direction and the law of conservation of volume (i.e., the continuity law) is obeyed.

In contrast to the effect of water flow during vacuolar perfusion (Tazawa 1968), the volume flow of water induced by 490 Pa is too small to have any measurable effect on the polar ratio of intact cells. The hydraulic conductivity (L_p) in *Chara*, as measured by transcellular osmosis, is $1.18 \pm 0.06 \times 10^{-12}$ m/s/Pa ($P_{os} = 1.6 \times 10^{-4}$ m/s) and the volume flow of water moving through the cell (Q) is only 9.1×10^{-15} m^3/s when there is an applied pressure (P_a) of 490 Pa as calculated from the following equation:

$$Q = {}^1/_2 L_p S P_a \quad (2)$$

where S is the surface area of half of the cell (3.14×10^{-5} m^2).

We can calculate the average velocity of water movement through the cell (J_v, in m^3/m^2/s = m/s) using the following equation:

$$J_v = Q/A \quad (3)$$

where A is the cross sectional area of the cell (1.96×10^{-7} m^2). The average velocity is 4.63×10^{-8} m/s. Then we can use the Hagen-Poiseuille law to determine the velocity distribution of the water moving through the cell. Accordingly, the velocity profile is parabolic, and the maximal velocity is in the center of the vacuole (Fig. 4). The maximal velocity is twice as large as the average velocity and is thus 9.27×10^{-8} m/s (Nobel 1983). The velocity (V_x) at any distance from the center of the cell (r_x) is described by the following equation:

$$V_x = J_{v\,max}(1 - r_x^2/r^2) \quad (4)$$

where r is the radius of the cell (0.25×10^{-3} m).

Let's assume that the cytoplasm is 20×10^{-6} m in thickness. We can then calculate the velocity of water movement at the tonoplast, where $r_x = 0.23 \times 10^{-3}$ m, to be 7.41×10^{-9} m/s. If the streaming rate is

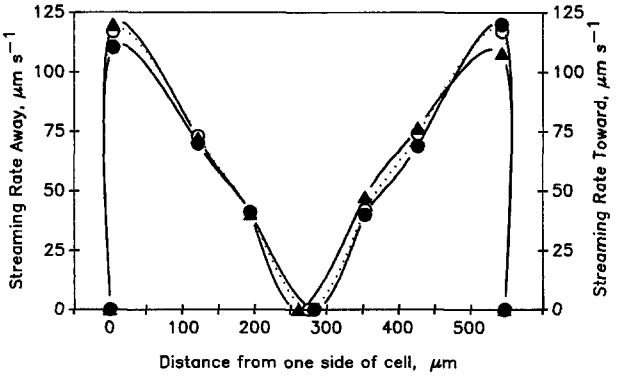

Fig. 3. Velocity gradients of cytoplasmic streaming across an optical section of *Chara*. Cells under positive pressure, + 490 Pa, (▲) stream more quickly away from the site of applied pressure but have a larger volume flowing toward the pressure. An applied negative pressure, − 490 Pa, (●) induces a reversed response, while no applied pressure (○) results in equal rates and volumes of streaming in both directions. Mean values ± S.E. (smaller than the symbols) are presented (n = 3)

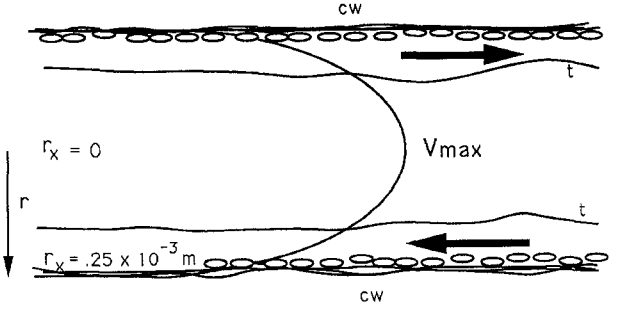

Fig. 4. A diagram of a longitudinal section through a *Chara* internodal cell showing the velocity distribution of water flow induced by hydrostatic pressure from the left. The greatest velocity (V_{max}) is in the center of the cell. The bold arrows indicate the direction of cytoplasmic streaming. *r* Radius of the cell, r_x distance from the center of the cell, *cw* cell wall, and *t* tonoplast (note its undulating nature which allows the motive force generated at the interface between the ectoplasm and endoplasm to be transmitted to the particles in the vacuole, causing them to stream). The plasma membrane is closely appressed to the cell wall

100×10^{-6} m/s, then the streaming towards and away from an applied pressure of 490 Pa will be 99.9925×10^{-6} m/s and 100.0074×10^{-6} m/s, respectively. This will be reflected in a polar ratio of only 1.0001 due to the hydrostatic pressure-induced movement of water. This represents the maximal polar ratio induced by water flow; it would be even smaller at the actin bundles (where our measurements are made) since both r_x^2 and the streaming velocity of the endoplasm are greater there.

Effect of ligation and UV microbeam irradiation

We carried out ligation experiments to determine whether the ends of the cells are required for the hydrostatic pressure-induced polarity of cytoplasmic streaming as they are for the gravity-induced polarity (Wayne et al. 1990). Ligation of either or both cell ends prevents hydrostatic pressure sensing in *Chara*, regardless of the magnitude and sign of the applied pressure (Fig. 5) without reducing the streaming rate (mean of ligated cells = 157 ± 1 μm/s, mean of intact cells = 135 ± 5 μm/s). The loss of polarity of cytoplasmic streaming is independent of which cell end is ligated or which end is inserted onto the apparatus. Thus, both cell ends must be intact for the perception of hydrostatic pressure, as is the case for the perception of gravity.

When a single node is irradiated with a UV microbeam (energy fluence = 28,680 J/m^2) the internodal cell loses its ability to sense both gravity and hydrostatic pressure

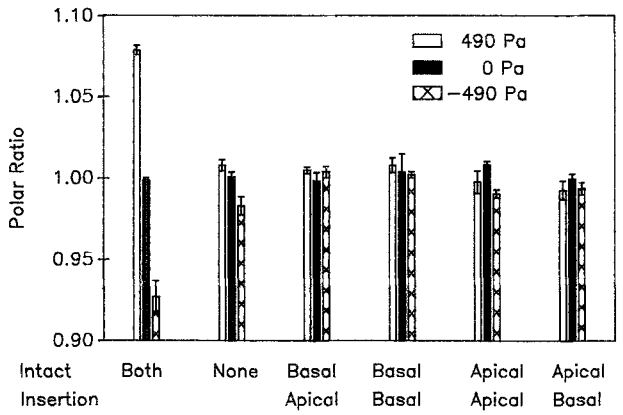

Fig. 5. The effect of cell ligation on the ability of horizontal *Chara* cells to respond to hydrostatic pressure. Cells were ligated at the morphologically apical or basal end or at both ends. Only intact cells can respond to applied pressure. Ligation of either or both ends abolishes the response. The response is independent of which cell end is inserted into the double chamber. Each bar represents mean values ± S.E. for 3 different cells

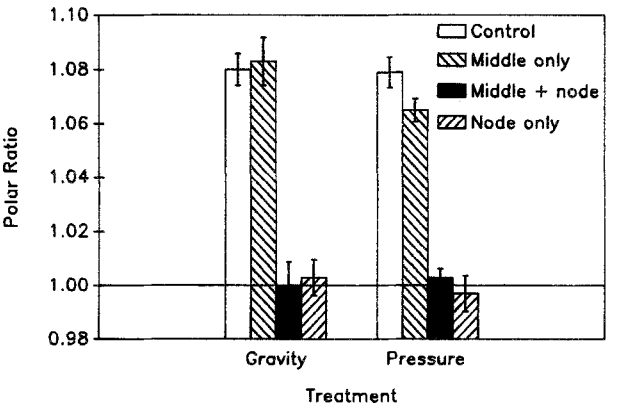

Fig. 6. The effect of UV microbeam irradiation on the ability of *Chara* cells to respond to gravity and 490 Pa applied hydrostatic pressure. Cells were irradiated for 2 min with a UV microbeam. UV irradiation in the middle of the cell had no significant effect on the gravity-induced or the pressure-induced polar ratio, wheras irradiation of the node abolished the response in both treatments regardless of whether or not the center of the cell had been previously irradiated. Mean values ± S.E. are presented (n = 3 for node only, n = 6 for others)

(Fig. 6). By contrast, when the middle of the internodal cell, where the streaming is observed, is irradiated, the UV microbeam has no effect on the ability of the cell to sense either gravity or hydrostatic pressure. UV irradiation has no effect on the motile machinery since the streaming velocity is 114.4 ± 2.2 and 114.1 ± 1.2 μm/s before and after irradiation, respectively ($p = 0.89$). Irradiation of the nodes with a similar energy fluence (33,120 J/m^2) of visible light from a microscope lamp had no effect on either the gravity- or the hydrostatic pressure-induced response. These observations strengthen the conclusion that the cell ends are required for hydrostatic pressure sensing as well as for gravisensing and indicate that the hydrostatic pressure sensor, like the gravisensor, absorbs UV radiation and is probably a protein.

The observation that both intact cell ends are required for the induction of a polarity leads us to consider the possibility that communication between the cell ends is required for the hydrostatic pressure-induced and gravity-induced responses. While we do not know the nature of the communication, we can eliminate the bulk extracellular fluid pathway for the movement of extracellular currents, since in the present experiments this pathway is restricted by a silicone grease seal.

Effect of osmotic pressure

In order to determine whether the osmotic pressure of the external medium affects the hydrostatically-induced

polar streaming of horizontal cells as it does the gravitationally-induced polar streaming (Staves et al. unpubl. data), we measured streaming rates of cells in APW, varying the osmotic pressure by the addition of ethylene glycol. Increasing the osmotic pressure of APW results in a reduction of the hydrostatic pressure-induced polarity of cytoplasmic streaming (Fig. 7). Bringing APW to 636 kPa completely inhibits the pressure-induced response, while having little effect on the rate of cytoplasmic streaming (mean streaming rates = 123 ± 1.0 μm/s, 121 ± 2.5 μm/s and 118 ± 1.6 μm/s at 7.4 kPa, 310 kPa and 636 kPa, respectively). The polarity of cytoplasmic streaming induced by the applied + 490 Pa hydrostatic pressure varies with the osmotic pressure of the bathing solution in a manner similar to the polarity induced by gravity. A change in the osmotic pressure of the external medium (π_o, in Pa) results in a change in the turgor pressure of the cell (P, in Pa) according to the following relation:

$$P = \sigma(\pi_i - \pi_o) \quad (5)$$

where σ is the reflection coefficient of the external solute (dimensionless) and π_i is the cellular osmotic pressure of these *Chara* cells (0.635 ± 0.008 MPa, n = 4). In order to characterize the relationship between the turgor pressure and hydrostatically-induced polar streaming of horizontal cells as well as gravity-induced polar streaming of vertical cells, we determined the reflection coefficient of *Chara corallina* for ethylene glycol using the method of transcellular osmosis. The reflection coefficient is 1.00 ± 0.01 (n = 3) and consequently, the turgor pressure decreases from 0.608 MPa to 0.325 MPa or 0 MPa as the external concentration of ethylene glycol increases from 0 mM to 125 mM or 257 mM, respectively. We interpreted the results as showing that both the gravity-induced and hydrostatic pressure-induced polarity of cytoplasmic streaming are inhibited by increasing the osmotic pressure of the external solution, and conclude that a turgid cell as well as two intact cell ends are causae sine qua non for the induction of polarity.

Effect of external Ca^{2+}

In order to determine whether there is a relationship between external [Ca^{2+}] and hydrostatic pressure-induced polarity of cytoplasmic streaming, as is the case for the gravity-induced polarity of cytoplasmic streaming (Wayne et al. 1990), horizontal cells were bathed in various calcium buffers and the polar ratio induced by a pressure of + 490 Pa was determined. At external [Ca^{2+}] greater than 1 μM, the streaming velocity is greater away from the applied pressure (polar ratio > 1). At 1 μM external Ca^{2+}, the streaming velocities are equal in both directions (polar ratio = 1), and when the external [Ca^{2+}] is below 1 μM the streaming velocity is greater toward the applied pressure (polar ratio < 1; Fig. 8). Varying the external [Ca^{2+}] does not induce a polarity of cytoplasmic streaming in cells not subjected to an applied hydrostatic pressure. External Ca^{2+} thus regulates both the sign and the magnitude of the response. These results show that the effect of external [Ca^{2+}] on hydrostatic pressure-induced

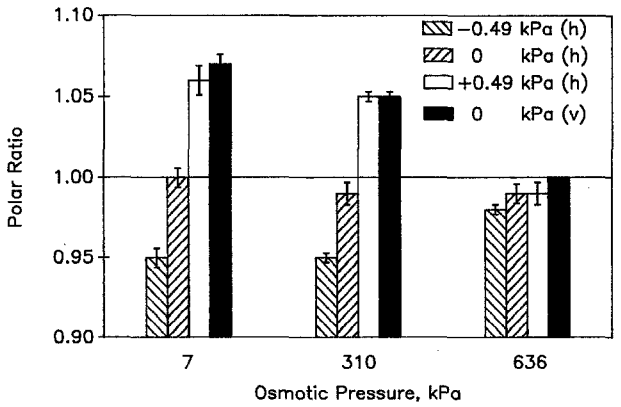

Fig. 7. The effect of external osmotic pressure on the ability of *Chara* cells to respond to gravitational and hydrostatic pressure. Horizontal (*h*) cells were subjected to the indicated applied pressure and vertical (*v*) cells were subjected to gravitational pressure. The data represent mean values ± S.E. for: 3 horizontal cells, each of which were subjected to all 3 hydrostatic pressures at each of the 3 osmotic pressures; and 3 vertical cells which were subjected to each osmotic pressure

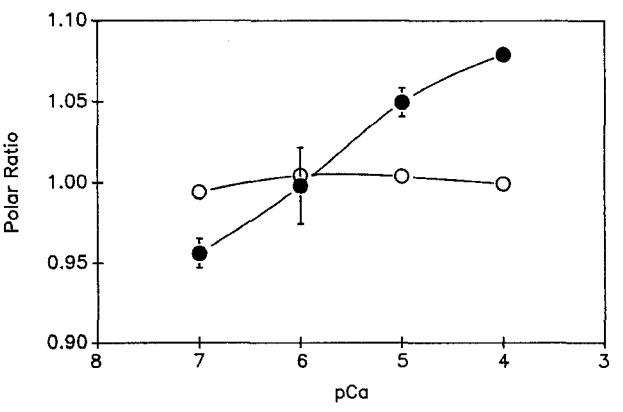

Fig. 8. The effect of external Ca^{2+} on the ability of horizontal *Chara* cells to respond to hydrostatic pressure. Internodal cells were placed sequentially in the indicated calcium buffers beginning with pCa 4. In each buffer the cells were exposed sequentially to either + 490 Pa applied pressure (●) and 0 Pa applied pressure (○). The data presented are the mean values ± S.E. (n = 3)

polarity of cytoplasmic streaming exactly parallels that for gravity-induced polarity.

Effect of Ca^{2+} channel blockers

Ca^{2+} influx through dihydropyridine-sensitive Ca^{2+} channels is necessary for gravisensing in *Nitellopsis* and we wanted to test whether or not the same class of channels is involved in hydrostatic pressure sensing. Since 100 µM nifedipine eliminates the gravity-induced polar streaming in *Nitellopsis* (Wayne et al. 1990), we treated *Chara* internodal cells with 100 µM nifedipine in order to determine whether it abolishes the hydrostatic pressure-induced polarity of cytoplasmic streaming. The gravity-induced and hydrostatic pressure-induced (+ 490 Pa and − 490 Pa) polar ratios of internodal cells are normal in 10 µM Ca^{2+} buffer containing 0.33% DMSO. However, when the cells are incubated in 100 µM nifedipine for 1 h, both the gravity-induced and pressure-induced polarity of cytoplasmic streaming are lost (Fig. 9).

$LaCl_3$ (100 µM, 1 h) reverses the gravity-induced polarity of cytoplasmic streaming in *Chara* as it does in *Nitellopsis*. Similarly, $LaCl_3$ treatment induces a reversal of the hydrostatic pressure- and gravity-induced polarity of cytoplasmic streaming in *Chara* (Fig. 10). These data indicate that at least two classes of Ca^{2+} channels may be involved in hydrostatic pressure sensing as we have suggested may be the case for gravisensing (Wayne et al. 1990).

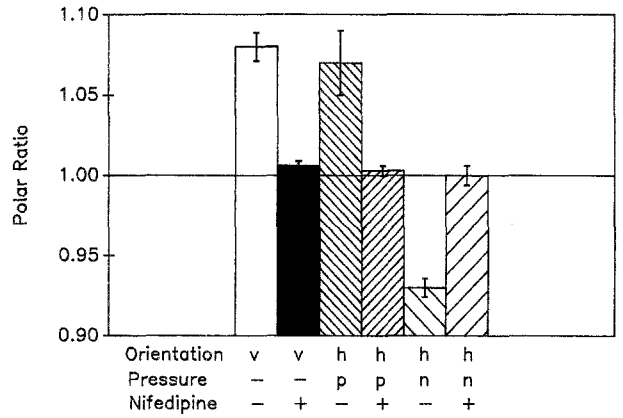

Fig. 9. The effect of 100 µM nifedipine on the ability of *Chara* cells to respond to gravitational and hydrostatic pressure. Internodal cells were placed in a vertical orientation (*v*) and the gravity-induced polar ratio was determined in the absence (−) and presence (+) of nifedipine. Horizontally oriented (*h*) cells were placed in a double chamber and a hydrostatic pressure of + 490 Pa (*p*, for positive pressure) or − 490 Pa (*n*, for negative pressure) was applied before the polar ratio was determined. Data represent the mean values ± S.E. (n = 3)

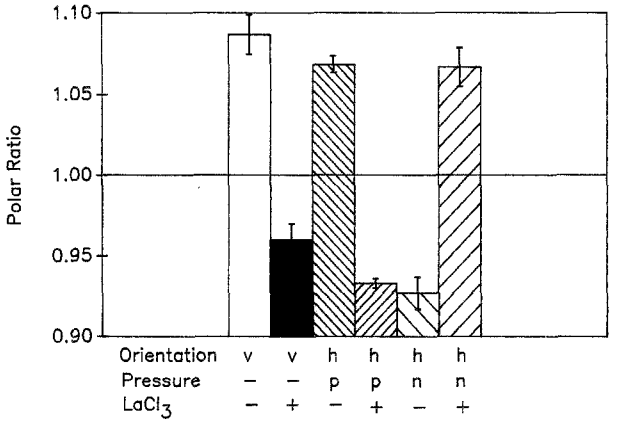

Fig. 10. The effect of 100 µM $LaCl_3$ on the ability of *Chara* cells to respond to gravitational and hydrostatic pressure. Internodal cells were placed in a vertical orientation (*v*) and the gravity-induced polar ratio was determined in the absence (−) and presence (+) of $LaCl_3$. Horizontally oriented cells (*h*) were placed in a double chamber and the hydrostatic pressure-induced polar ratio was determined while the cells experienced + 490 Pa (*p*) and − 490 Pa (*n*) applied pressure. Data represent mean values ± S.E. (n = 3)

Effect of neutral red and TEA Cl^-

Chara internodal cells were treated with neutral red to determine whether it caused a reversal of the normal gravity-induced and pressure-induced polarity of cytoplasmic streaming, as it does the gravity-induced polar ratio in *Nitellopsis* (Wayne et al. 1990). Both gravitational pressure and hydrostatic pressure induce a polarity of cytoplasmic streaming in *Chara*: the cytoplasm streams at a higher velocity down or away from the applied pressure, respectively (polar ratio > 1). However, when the same cells are placed in APW containing 0.001% neutral red, the cytoplasm streams up faster in gravity-stimulated cells, and faster toward the applied pressure in hydrostatic pressure-stimulated cells (polar ratio < 1; Fig. 11). Neutral red is known to open a K^+ channel in characean cells and consequently hyperpolarize the plasma membrane (Kawamura and Tazawa 1980). Therefore we tested the effect of the K^+ channel blocker, TEA Cl^-, on the ability of neutral red to reverse these responses. Subsequent incubation of neutral red-treated cells in 0.001% neutral red containing 10 mM TEA Cl^- for 1 h results in a reversal of the neutral red effect (Fig. 11). These results lead us to think about the possibility that the membrane potential may modulate both gravitational pressure and hydrostatic pressure sensing.

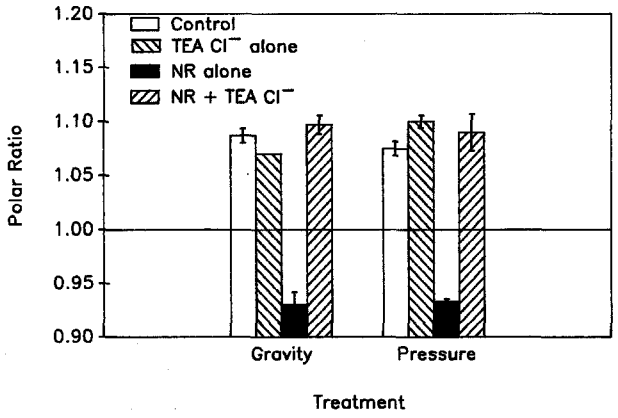

Fig. 11. The effect of neutral red on the ability of *Chara* cells to respond to gravitational and hydrostatic pressure. Internodal cells were gravistimulated in a vertical position or were placed in a double chamber and experienced an applied pressure of + 490 Pa on one end. The polar ratios were first determined for untreated cells, then for cells treated with 0.001% neutral red. Neutral red-treated cells were subsequently treated with 10 mM TEA Cl^- and the polar ratios determined. Data represent mean values ± S.E. (n = 3)

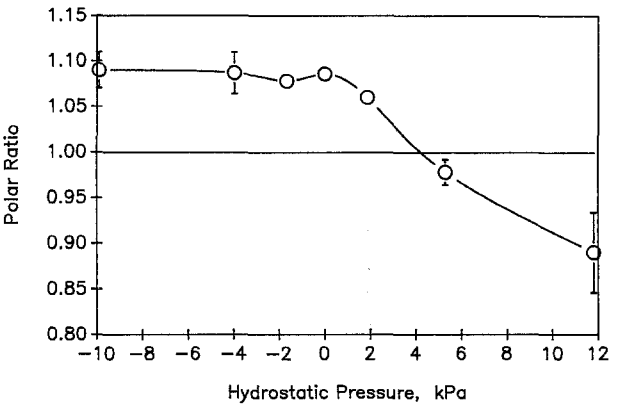

Fig. 12. The effect of hydrostatic pressure on gravisensing in vertical *Chara* cells. The lower nodes of gravistimulated cells were exposed to the indicated hydrostatic pressures and the polar ratios were determined. Data represent mean values ± S.E. (n = 3–5)

Effect of hydrostatic pressure on gravity-induced polarity of cytoplasmic streaming

A hydrostatic pressure of ca. 4,000 Pa applied to the bottom of a vertical cell abolishes the gravity-induced polarity of cytoplasmic streaming (Fig. 12). Higher pressures cause the polar ratio to fall below 1.0. However, an applied negative pressure on the bottom end does not augment the gravity-induced polar ratio, indicating that the response is saturated at 1 **g**. Since the two stimuli are not additive, these data indicate that both gravity and hydrostatic pressure may act on the same point.

Discussion

When a column of water in air is in a horizontal position, there is no pressure differential between the ends of the column. When this column is rotated to the vertical position, a pressure gradient is formed with pressure increasing with depth. This is the gravitational pressure. The magnitude of this pressure is proportional to the difference in density between the water and air. If the column of water in air is surrounded by an elastic membrane and rests on a solid surface, it will bulge at the bottom and become more narrow at the top. It can be argued that the protoplast of a *Chara* internodal cell is analogous to such a column of water. Thus it would be expected to be subject to physical deformation dependent on the differences between its density and that of the surrounding medium.

Since the densities of the endoplasm and vacuolar sap of *Chara* are 1013.9 and 1005.0 kg/m^3, respectively, and the relative volumes of the endoplasm and vacuole are 0.064 and 0.936, respectively (Takeshige and Tazawa 1989, Wayne and Staves 1991), the calculated density of the protoplast is 1005.6 kg/m^3. The density of the culture medium is ca. 1000.5 kg/m^3. Thus the density of the protoplast is greater than that of the surrounding medium, and a vertically-oriented *Chara* protoplast will be subject to gravitational pressure. The rigid cell wall of characean cells prevents the protoplast from becoming pear-shaped. However gravitational pressure will tend to push the protoplast against the bottom of the cell while causing it to pull away from the cell wall at the top of the cell. Thus, tensile and compressive stresses are induced between the plasma membrane and the cell wall at the top and bottom of the cell, respectively. According to the gravitational pressure model for plant graviperception, these differential tensile and compressive stresses at opposite ends of a cell enable the cells to perceive gravity (Wayne et al. 1990).

A hydrostatic pressure applied to one end of a horizontal *Chara* cell will induce a polarity of cytoplasmic streaming, mimicking the gravitational pressure-induced polarity seen in vertically oriented cells. A positive hydrostatic pressure will cause the plasma membrane to pull away from the cell wall at the end where the pressure is applied, and move towards the opposite end. This results in the induction of tensile and compressive stresses between the plasma membrane and the cell wall at the ends proximal and distal to the applied hydrostatic pressure, respectively. Both gravitational pressure and hydrostatic pressure induce a polarity of cytoplasmic streaming such that the velocity away from

the site of a "tension" is greater than that moving away from the site of "compression".

We use the terms "tension" and "compression" to emphasize the effects at the plasma membrane/cell wall interface resulting from the vectorial forces induced by gravity and hydrostatic pressure. Since the pressure generated by turgor is much larger than that generated by hydrostatic pressure, a positive hydrostatic component will subtract from the turgor-induced pressure between the plasma membrane and the cell wall at the end where pressure is applied. There will be a concomitant increase in pressure at the other end of the cell. Hydrostatic and gravitational pressure will induce compressive and tensile stresses on the cell ends even if the cell has been ligated or irradiated with a UV microbeam. The observations that cellular ligation and UV microbeam irradiation of the cell end eliminates the ability of the internodal cell to respond to gravity and hydrostatic pressure, while having no effect on the motile machinery, underscore the importance of the intact cell ends for sensing, or at least responding to, compressive and tensile stresses. We have presented evidence elsewhere that leads us to think that the gravity- and pressure-receptors may be integrin-like proteins (Wayne et al. 1992).

The realization of a response requires the conversion of the energy of the stimulus (e.g., hydrostatic pressure) into energy directly usable by the cell to induce a polarity of cytoplasmic streaming. The potential energy (in J) available to the internodal cell as a consequence of the applied hydrostatic pressure is given by the following equation:

$$\text{potential energy} = \alpha P_a A d \qquad (6)$$

where P_a is the applied hydrostatic pressure (490 Pa), A is the cross sectional area of the cell ($1.96 \times 10^{-7}\,m^2$), d is the assumed distance the protoplast moves within the cell wall (1×10^{-9} m) and α is a dimensionless number that relates the calculated pressure to the actual pressure at the plasma membrane. The potential energy available is thus α (9.60×10^{-14}) J. If α is assumed to be 1, this is considerably greater than the potential energy available as a consequence of gravity (1.9×10^{-16} J).

The quantification of the energies required to induce a polarity of streaming by hydrostatic pressure and that generated by gravitational pressure raises several questions. For example, why do we need to apply 9.62×10^{-14} J to get a polar ratio of 1.1 when gravity only supplies 1.9×10^{-16} J? In our experimental system, hydrostatic pressure is applied to the outside of a node. Pressure on the internodal cell plasma membrane is induced by water passing through the apoplastic compartments between plasma membrane and the exposed nodal cells. Perhaps these compartments act as baffles, providing a resistance to the applied pressure thus reducing α and consequently lowering the actual energy at the plasma membrane by 500-fold.

It is unclear to us why ca. 10 times as much energy is required to *negate* the gravity-induced polarity of streaming in vertical cells with hydrostatic pressure compared with that which is required to *induce* polarity in a horizontal cell by hydrostatic pressure. We did experiments in two ways: beginning with a gravistimulated vertical cell, then applying a hydrostatic pressure; and beginning with a hydrostatic pressure on a horizontal cell and subsequently providing a gravity stimulus. The results were the same in each case, suggesting that the first stimulus did not become amplified in such a way as to require more energy from the second stimulus to reverse the response.

Our data indicate that a turgid cell is required for both the gravity and hydrostatic pressure-induced polarity. However, it is problematic to understand how a small pressure induced by gravity can be detected over the relatively large turgor pressure (Audus 1979, Björkman 1988). Let us consider an internodal cell that is 2×10^{-2} m long (l) and 0.5×10^{-3} m wide (2 r). The volume of the cell ($v = \pi r^2 l$) is thus $3.9 \times 10^{-9}\,m^3$. The density of the vacuolar sap, which takes up 93.4% of the protoplast is $1005.0\,kg/m^3$ and the density of the endoplasm, which takes up 6.4% of the protoplast is $1013.9\,kg/m^3$ (Takeshige and Tazawa 1989, Wayne and Staves 1991). The density of the protoplast is thus $1005.6\,kg/m^3$. We can calculate the force that the protoplast will exert in a gravitational field using the equation for static buoyancy:

$$B = \mathbf{g}\,\text{Vol}\,(\rho_m - \rho_p) \qquad (7)$$

where B is the static buoyancy (in N); **g** is the acceleration due to gravity ($9.8\,m\,s^{-2}$), Vol is the volume of the protoplast (in m^3); and ρ_m and ρ_p are the densities (in kg/m^3) of the medium and the protoplast, respectively. Thus, gravity will cause the protoplast to exert a force against the lower wall that is equal to -2.1×10^{-7} N. We can determine the gravity-induced pressure on a surface using the formula:

$$P = |B|/A \qquad (8)$$

where P is the pressure (in Pa); and A is the area (in m^2) being acted upon. Thus, if the force calculated above is exerted against the entire end wall, which has

an area (πr^2) of 1.96×10^{-7} m^2, the pressure that the membrane would experience due to gravity would be 1.1 Pa. The membrane at the top of the cell would be relieved of a pressure of the same magnitude. It is also possible that some force focusing mechanisms exist, so that the force is exerted on a smaller area and the pressure at the focus due to gravity is consequently larger.

Now, let's compare the magnitude of this pressure to those experienced by the cell. In our culture conditions, the osmotic pressure of the external medium ranges from 2,500–29,700 Pa (1–12 mOsm). In nature a freshwater charophyte may be growing in a 12,400 Pa (5 mOsm) medium (Bisson and Bartholomew 1984). We measured the osmotic pressure of our cells to be 637,000 Pa (257 mOsm). Thus assuming an external osmotic pressure of 12,400 Pa, the turgor pressure of the cells will be 624,000 Pa. A 10% fluctuation in the external osmotic pressure would result in a change of turgor pressure of 0.2% or 1,250 Pa. How can the plasma membrane respond to the small pressures induced by gravity (1.1 Pa) when it already experiences 637,000 Pa of pressure as a consequence of turgor? The gravity-induced pressure is 1.73×10^6 times smaller than the turgor pressure, and 8×10^4 times smaller than typical fluctuations. Since turgor pressure, by definition, is equal on all parts of the plasma membrane, no differential pressure is produced by turgor alone. The vectorial nature of gravitational pressure allows for the establishment of a small but constant pressure differential between the top and bottom of the cell. If there were only one gravireceptor on the plasma membrane it might not be able to sense the small pressure due to gravity since the normal fluctuations in turgor pressure (1,250 Pa) that occur in response to changes in the water status on the plant exceed the small pressure induced by gravity. However, we find that both cell ends are necessary for the realization of the graviresponse and thus we proposed that there are at least two gravireceptors in the membrane that sense the differential pressure on the membrane at the top and bottom. Two receptors, unlike one, can sense a difference in the pressure on the membrane, the sign of which represents the vector of gravity. Any mechanism that may be used for communication by the two receptors remains unknown. Turgor pressure does not preclude this method of sensing but actually is necessary to poise the plasma membrane in a strained condition where it can respond to small differences in pressure induced by gravity. An analogy between a signaling mechanism dependent on both the cell exerting a force on the extracellular matrix and the extracellular matrix resisting these forces, and the operation of stringed instruments has been made (Ingber 1991). In both cases, tension is required to transmit mechanical information; whether in the form of biological signals, or musical tones.

Are there other biological receptors that are capable of sensing a small difference in stimulation in the presence of a large background? Many cells, including pollen tubes, myoblasts and neurites are capable of aligning themselves in an electric field (Nucitelli 1988). The membrane potential of *Xenopus* neurites is approximately -0.06 V but the cell can recognize and respond to an electric field by turning toward the cathode when there is a voltage drop as small as 0.000025 V across each membrane (Patel and Poo 1984). Such a small change (4×10^{-4}) in the membrane potential is thought to be incapable of activating or inactivating membrane channels. Could the high sensitivity be due to the fact that there are two membranes that experience the electric field? As a consequence of the vectorial nature of the field, one membrane becomes depolarized and the other becomes hyperpolarized. Perhaps these two membranes are capable of producing a stimulus that is proportional to the differences in the membrane potential between the two membranes, and this stimulus is actually what is recognized by the cell.

The phototactic response of single celled algae, including *Dunaliella salina*, is another case where cells detect small differences in a stimulus in the presence of a large background (Wayne et al. 1991). The actual difference depends on the mechanism of perception of the light direction. Let's assume that the cell has two photoreceptors, one in the front and one in the back of the cell. Let's further assume that the molar extinction coefficient (ϵ) of the photoreceptor pigment is 400 m^2/mol, the cell is 10^{-5} m long (d) and the concentration of the photoreceptor pigment (c) is approximately 10^{-3} mol/m^3. Using the Beer-Lambert law, where

$$\log I_o/I = \epsilon d c \qquad (9)$$

we find that the ratio of light that hits the front photoreceptor (I_o) to the amount of light that hits the rear photorecptor (I) is 1.00000921. That is, the two photoreceptors would detect the directional signal when the difference in the energy fluence rate between the photoreceptor in the front and back is 9×10^{-6}.

Now let's assume that a swimming cell uses a single photoreceptor that measures the difference in the photon fluence rate at two instants in time. Let's say that the cell measures a photon fluence rate difference every 1 s and it swims 50×10^{-6} m/s. Let's assume that the

cells are swimming toward a light source that has an photon fluence rate of 10^{21} photons/m^2/s at the initial cell position. After swimming toward the light for 1 s, the cell would be irradiated by light with a photon fluence rate of 1.0001×10^{21} photons/m^2/s according to the inverse square law:

$$I_1/I_2 = d_2^2/d_1^2 \quad (10)$$

where I_i (in photons/m^2/s) is the photon fluence rate at a given distance (d in m) from the light source.

Thus if cell used the "two instant mechanism", it would be able to detect a difference in the photon fluence rate of 10^{17} photons/m^2/s when there is a background of 10^{21} photons/m^2/s. The ratio of the differences to the background photon fluence rate is 10^{-4}. If the cell measures a photon fluence rate difference every 0.1 s, then the ratio of the difference to the background photon fluence rate is 10^{-5}, which is similar to the ratio determined for the "one instant mechanism".

Now let's consider the ability of cells to detect a thermal gradient and migrate toward the higher temperature. The pseudoplasmodia of *Dictyostelium discoideum* can detect a temperature gradient of less than 0.0004 °C across the 100 µm in diameter cell when the midpoint temperature is 23.5 °C (296.5 K) (Poff and Skokut 1977). To convert temperature into an absolute quantity we use K. In this cell the ratio of the difference in temperature to the background (midpoint) temperature is given by: $0.0004\,K/296.5\,K = 1.35 \times 10^{-6}$.

Such sensitive responses also occur in higher plants. The roots of corn are positively thermotropic; that is, at temperatures below 26 °C they bend toward the direction of higher temperature when placed in a temperature gradient. The roots respond to a gradient as small as 0.5 °C/cm or approximately 0.075 °C per root or 0.0025 °C per 50 µm cell (Fortin and Poff 1990, 1991). Thus at 26 °C (299 K), the ratio of the effective temperature differences across the root and a single cell to the background is 2.5×10^{-4} and 8.4×10^{-6} respectively.

Other examples of cells capable of detecting very small signals over a large background include: the altered swimming behavior of *E. coli* and *Salmonella typhimurium* grown at 35 °C when the temperature changes only 0.02 °C/min (Imae 1985); chemotropism of *Achlya bisexualis*, which takes place in a gradient of amino acids such that the concentration difference across the hypae is only 5 µM (Schreurs et al. 1989); and the hydrotropism of corn and pea roots which grow toward saturated filter paper in a chamber maintained at 93% relative humidity (Takahashi and Scott 1991).

In conclusion, a number of responses are known to take place in response to a small difference in stimulus relative to a large background signal, just as we propose occurs in the sensing of both hydrostatic pressure and gravitational pressure. Moreover, the hydrostatic pressure-induced polarity of cytoplasmic streaming of *Chara* has similar requirements and shows similar responses to the gravity-induced polarity of cytoplasmic streaming of *Chara* and *Nitellopsis*. These data indicate that both gravity and hydrostatic pressure may act at the same point of the signal transduction chain leading to the induction of a polarity of cytoplasmic streaming. Collectively, these results support the hypothesis that plants respond to gravity by sensing gravitational pressure.

Acknowledgements

We thank Dr. Michael Frohlich of the Department of Biological Sciences, Union College for suggesting the idea of subjecting horizontal cells to a hydrostatic pressure, Raj Ramen for helping us with the fluid mechanics, Dr. Robert Turgeon for emphasizing the importance of using degrees Kelvin and Dr. Tom Owens for the loan of the radiometer. We thank Dr. K. Niklas, Dr. M. Tazawa, and two anonymous reviewers for their helpful and insightful comments on the manuscript. This work was supported in part, by Hatch Funds to RW and grants from NASA to RW and ACL.

References

Audus LJ (1979) Plant geosensors. J Exp Bot 30: 1051–1073

Björkman T (1988) Perception of gravity by plants. Adv Bot Res 15: 1–41

Bisson MA, Bartholomew D (1984) Osmoregulation or turgor regulation in *Chara*? Plant Physiol 74: 252–255

Caspar T, Pickard BG (1989) Gravitropism in a starchless mutant of *Arabidopsis*. Planta 177: 185–197

Dainty J, Ginzburg BZ (1964) The reflection coefficient of plant cell membranes for certain solutes. Biochim Biophys Acta 79: 129–137

Ewart AJ (1903) On the physics and physiology of protoplasmic streaming in plants. Clarendon Press, Oxford

Fortin M-C, Poff K (1990) Temperature sensing of primary roots of maize. Plant Physiol 94: 367–369

– – (1991) Characterization of thermotropism in primary roots of maize: dependence on temperature and temperature gradient, and interaction with gravitropism. Planta 184: 410–414

Gersani M, Sachs T (1990) Perception of gravity expressed by vascular differentiation. Plant Cell Environ 13: 495–498

Hayashi T (1957) Some dynamic properties of the protoplasmic streaming in *Chara*. Bot Mag (Tokyo) 70: 168–174

Imae Y (1985) Molecular mechanism of thermosensing in bacteria. In: Eisenbach M, Balaban M (eds) Sensing and response in microorganisms. Elsevier, New York, pp 73–81

Ingber D (1991) Integrins as mechanochemical transducers. Curr Opin Cell Biol 3: 841–848

Kamiya N (1940) Control of protoplasmic streaming. Science 92: 462–463

Kamiya N, Tazawa M (1956) Studies on water permeability of a single plant cell by means of transcellular osmosis. Protoplasma 46: 394–422

Kawamura G, Tazawa M (1980) Rapid light-induced potential change in *Chara* cells stained with neutral red in the absence of internal Mg-ATP. Plant Cell Physiol. 21: 547–559

Kiss JZ, Sack FD (1989) Reduced gravitropic sensitivity in roots of a starch-deficient mutant of *Nicotiana sylvestris*. Planta 180: 123–130

– Hertel R, Sack FD (1989) Amyloplasts are necessary for full gravitropic sensitivity in roots of *Arabidopsis thaliana*. Planta 177: 198–206

Moore D (1991) Perception and response to gravity in higher fungi – a critical appraisal. New Phytol 117: 3–23

Moore R (1989) Root gravitropism and cellular differentiation in wild-type and a starchless mutant of *Arabidopsis thaliana*. Ann Bot 64: 271–277

Nobel P (1983) Biophysical plant physiology and ecology. WH Freeman, New York

Nuccitelli R (1988) Physiological electric fields can influence cell motility, growth, and polarity. Adv Cell Biol 2: 213–233

Patel N, Poo M-M (1984) Pertubation of the direction of neurite growth by pulsed and focal electric fields. J Neurosci 4: 2939–2947

Poff K, Skokut M (1977) Thermotaxis by pseudoplasmodia of *Dictyostelium discoideum*. Proc Natl Acad Sci USA 74: 2007–2010

Sack F (1991) Plant gravity sensing. Int Rev Cytol 127: 193–252

Schreurs WJA, Harold RL, Harold FM (1989) Chemotropism and branching as alternative responses of *Achlya bisexualis* to amino acids. J Gen Microbiol 135: 2519–2528

Takahashi H, Scott T (1991) Hydrotropism and its interaction with gravitropism in maize roots. Plant Physiol 96: 558–564

Takeshige K, Tazawa M (1989) Determination in the inorganic pyrophosphate level and its subcelluar localization in *Chara corallina*. J Biol Chem 264: 3262–3266

Tazawa M (1968) Motive force of the cytoplasmic streaming in *Nitella*. Protoplasma 65: 207–222

Wareing PF, Nasr TA (1958) Effect of gravity on growth, apical dominance and flowering in fruit trees. Nature 182: 379–380

Wayne R (1985) The contribution of calcium ions and hydrogen ions to the signal transduction chain in phytochrome-mediated fern spore germination. PhD Thesis, University of Massachusetts, Amherst, MA, USA

– Staves MP (1991) The density of the cell sap and endoplasm of *Nitellopsis* and *Chara*. Plant Cell Physiol: **32**: 1137–1144

– Tazawa M (1988) The actin cytoskeleton and polar water permeability in characean cells. Protoplasma [Suppl 2]: 116–130

– – (1990) Nature of the water channels in the internodal cells of *Nitellopsis*. J Membrane Biol 116: 31–39

– Kadota A, Watanabe M, Furuya M (1991) Photomovement in *Dunaliella*: fluence rate-response curves and action spectra. Planta 184: 515–524

– Staves MP, Leopold AC (1990) Gravity-dependent polarity of cytoplasmic streaming in *Nitellopsis*. Protoplasma 155: 43–57

– – – (1992) The contribution of the extracellular matrix to gravisensing in characean cells. J Cell Sci (in press)

Westing AH (1971) A case against the statoliths. In: Gorden SA, Cohen MJ (eds) Gravity and the organism. University of Chicago Press, Chicago, pp 97–101