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Nature of the Water Channels in the Internodal Cells of *Nitellopsis*

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Summary. The hydraulic resistance was measured on internodal cells of *Nitellopsis obtusa* using the method of transcellular osmosis. The hydraulic resistance was approximately 2.65 pm^{-1} sec Pa, which corresponds to an osmotic permeability of 101.75 $\mu m\ sec^{-1}$ (at 20°C). *p*-Chloromercuriphenyl sulfonic acid (*p*CMPS) (0.1–1 mM, 60 min) reversibly increases the hydraulic resistance in a concentration-dependent manner. *p*CMPS does not have any effect on the cellular osmotic pressure. *p*CMPS increases the activation energy of water movement from 16.84 to 32.64 kJ mol^{-1}, indicating that it inhibits water movement by modifying a low resistance pathway. *p*CMPS specifically increases the hydraulic resistance to exosmosis, but does not influence endosmosis. By contrast, nonyltriethylammonium (C_9), a blocking agent of K^+ channels, increases the hydraulic resistance to endosmosis, but does not affect that to exosmosis. These data support the hypothesis that water moves through membrane proteins in characean internodal cells and further that the polarity of water movement may be a consequence of the differential gating of membrane proteins on the endo- and exosmotic ends.

Key Words *Characeae* · *Nitellopsis* · K^+-channel · nonyltriethylammonium · *p*CMPS · transcellular osmosis · water channels

Introduction

Water moves across the plasma membrane of characean cells with an osmotic permeability (P_{os}) of around 100 $\mu m\ sec^{-1}$ when the cells are subjected to transcellular osmosis (Kamiya & Tazawa, 1956). The activation energies of water movement in *Chara corallina* (Kiyosawa, 1975), *Nitella flexilis* (Tazawa & Kamiya, 1966) and *Nitellopsis obtusa* (Wayne & Tazawa, 1988) are not significantly different from the activation energy of the self-diffusion of water (Kohn, 1965). Furthermore, Steudle and Tyerman (1983), following an analysis based upon irreversible thermodynamics, observed frictional interactions between the transport of water and acetone or ethanol in *Chara corallina*. These data taken together indicate that water moves through aqueous pores in the membranes of characean cells.

Using the method of transcellular osmosis, Kamiya and Tazawa (1956) observed that the rate of water movement into a *Nitella flexilis* cell is 2.6 times greater than the rate of water movement out of the cell. We found that the polarity of *Chara corallina* and *Nitellopsis obtusa* can be abolished or even reversed by treating the cells with cytochalasins A, B and E (Wayne & Tazawa, 1988) as well as cytochalasin D (*unpublished observations*). We suggested that the polarity results, in part, from an actin-mediated aggregation of membrane proteins on the endosmotic side. We also suggested that the various proteins that can serve as water channels can be gated differentially depending on the physiochemical milieu that exists on the endo- and exoosmotic cell ends. Explicit in this hypothesis is the assumption that water moves through aqueous pores created by proteins. Here we test this hypothesis by treating internodal cells of *Nitellopsis obtusa* with various inhibitors of protein function. Using the sulfhydryl inhibitor, chloromercuriphenyl sulfonic acid (*p*CMPS), which inhibits water movement in animal cells (Naccache & Sha'afi, 1974; Brown et al., 1975; Benga et al., 1983, 1987, 1989; Lukacovic, Toon & Solomon, 1984; Whittembury et al., 1984; Fischbarg, Liebovitch & Koniarek, 1987), we show that a sulfhydryl-containing protein serves as an aqueous channel for exosmosis. In addition, using nonyltriethylammonium (C_9), we show that a K^+ channel serves as an aqueous channel for endosmosis.

Materials and Methods

Nitellopsis obtusa (Desv. in Lois.) J. Gr. was grown in a soil-water mixture in large plastic containers at 25 ± 2°C with a 15 hr

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L, 9 hr D photoperiod. Cylindrical, young internodal cells (about 70 mm in length and about 0.7 mm in diameter) were isolated and placed in artificial pond water (APW: 0.1 mM NaCl, 0.1 mM $CaCl_2$ and 0.1 mM KCl) buffered by 10 mM Mes titrated with Tris to pH 5.5. The cells were then placed on a shaker for 3 hr to remove the $CaCO_3$ deposited on the wall. The cells were then transferred to APW buffered by 2 mM HEPES-NaOH (pH 7.3). The cells remained in this medium at least overnight before use. There is tendency for the total transcellular hydraulic resistance to increase as the isolated cells age.

Determination of Endosmotic and Exosmotic Hydraulic Resistances with Transcellular Osmosis Combined with Cell Ligation

The rate of transcellular osmosis was determined in an apparatus designed by Tazawa and Kamiya (1966) and illustrated in Wayne and Tazawa (1988). Internodal cells were treated in APW with or without inhibitors in a covered chamber. The cells were then placed in the apparatus such that the length of the cell part in chamber B was twice the length of the cell part in chamber A. Chambers A and B were physically separated by a 7-mm silicone seal (HVG, Toray Silicone, Tokyo, Japan), and the cell parts in both chambers A (endosmotic side) and B (exosmotic side) were bathed in APW with or without the inhibitors. Forward transcellular osmosis was initiated by replacing the solution in chamber B with 100 mM sorbitol (0.244 MPa at 20°C), and the quantity of water movement was recorded every 10 sec for 40 sec. The magnitude of water movement was measured by following the rate of movement of a column of APW in a glass capillary with an Olympus CH microscope equipped with a 10× objective lens. The solution in the capillary was continuous with the solution in chamber A. The solution in chamber B was replaced with APW with or without the inhibitors to initiate backward transcellular osmosis, and the cells were allowed to reach their original equilibrium condition during the next 10 min. Subsequently, the cell part in chamber B was ligated (Kamiya & Kuroda, 1956) at its middle so that the length of the cell in chamber B that could participate in transcellular osmosis was equal to the length of the cell part in chamber A. Again transcellular osmosis was initiated with 100 mM sorbitol and was recorded every 10 sec for 40 sec. At the end of the experiment, the cell end distal to the ligation was cut off to ensure that the ligation was complete. Some cells were placed in the transcellular osmometer symmetrically (*see* Wayne & Tazawa, 1988, for details).

In order to eliminate any interference from the small volume change that occurs during the onset of transcellular osmosis (0–5 sec) (Tazawa & Kamiya, 1966) or the build-up of unstirred layers (more than 40 sec after the onset of transcellular osmosis) the rate of water transport between 10 and 30 sec was used to calculate the rate of transcellular osmosis. Experiments were performed at room temperature (20–23°C) unless stated otherwise.

Analysis of Results

The hydraulic conductivities for endosmosis (L_{pen}) and exosmosis (L_{pex}) were calculated using the following equations. The general equations can be found in Kamiya and Tazawa (1956) and they are based on the following assumptions: (*i*) The membranes form the main resistance to water permeability; and (*ii*) The resistance to endosmosis and the resistance to exosmosis are independent, both work in series and can be summed. Details of the method for the determination of L_{pen}^{-1} and L_{pex}^{-1} are described in Wayne and Tazawa (1988).

The initial flow of water (J_v, in $pm^3\ sec^{-1}$) is proportional to the osmotic gradient ($\Delta\pi$, in Pa) used to initiate transcellular osmosis.

$$J_v = K\Delta\pi. \tag{1}$$

The transcellular osmosis constant (K), which relates the observed flow to the driving force (Tazawa & Kamiya, 1965), was calculated by dividing the initial flux of transcellular water movement by the magnitude of the osmotic gradient. K is a constant that depends on the surface area of the endosmotic and the exosmotic sides of the cell and the ratio between these two areas such that K is given as $A_{en}A_{ex}L_{pen}L_{pex}/(A_{en}L_{pen} + A_{ex}L_{pex})$. K is expressed in $pm^3\ sec^{-1}\ Pa^{-1}$.

In the transcellular osmosis before ligation $A_{en} = A_{ex}/2 = A$ and the rate of transcellular osmosis (K_1) equals:

$$K_1 = \frac{2A\ L_{pen}\ L_{pex}}{(L_{pen} + 2L_{pex})}. \tag{2}$$

After ligation, $A_{en} = A_{ex} = A$ and the rate of transcellular osmosis (K_2) equals:

$$K_2 = \frac{A\ L_{pen}\ L_{pex}}{(L_{pen} + L_{pex})}. \tag{3}$$

The polarity of water movement (α) is defined as the ratio of L_{pen} to L_{pex}. That is:

$$\alpha = L_{pen}/L_{pex}. \tag{4}$$

We can substitute (α) for L_{pen}/L_{pex} and replace L_{pen} in Eq. (4) and (5) with $[(\alpha)(L_{pex})]$ to get the following equations:

$$K_1 = \frac{2\ (\alpha)\ L_{pex}}{(\alpha + 2)} \tag{5}$$

$$K_2 = \frac{A\ (\alpha)\ L_{pex}}{(\alpha + 1)}. \tag{6}$$

From these equations, we get α and L_{pex}, and then L_{pen} can be derived from Eq. (4) as $[(\alpha)\ L_{pex}]$.

The reciprocal of L_{pen} and L_{pex} are the transcellular hydraulic resistances for endosmosis and exosmosis, respectively. When the cell is partitioned into equal halves ($A_{en} = A_{ex} = A$), we can calculate the transcellular hydraulic resistance, R

$$R = K^{-1} = (L_{pen}^{-1} + L_{pex}^{-1})/A. \tag{7}$$

The total transcellular hydraulic resistance (r_{tot}) is given by R/A; therefore,

$$r_{tot} = K^{-1}A^{-1} = L_{pen}^{-1} + L_{pex}^{-1}. \tag{8}$$

Measurement of Membrane Potential (E_m) and Membrane Resistance (R_m)

Measurements were made using conventional microcapillary electrodes connected to an electrometer as described earlier (Wayne & Tazawa, 1988).

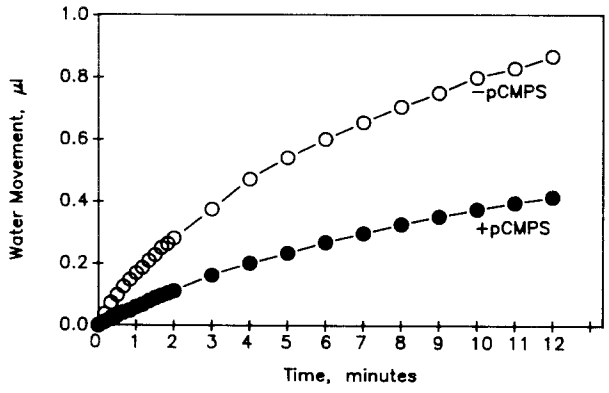

Fig. 1. Time course of transcellular water movement through an internodal cell of *Nitellopsis obtusa* in the presence or absence of 1 mM *p*CMPS

Measurement of Cellular Osmotic Pressure

Cellular osmotic pressure was measured by expressing the cell sap, collecting 8 µl from each cell and placing it in a Wescor 5100C vapor pressure osmometer.

Chemicals

Nifedipine and *p*CMPS were purchased from Sigma. Dithiothreitol and N-ethylmaleimide were purchased from Nakarai Chemicals, Tokyo. 9-Anthracenecarboxylic acid was purchased from Aldrich Chemical, Milwalkee, WI. Tetrodotoxin was a gift from Prof. S. Miyachi of Tokyo University, and nonyltrimethylammonium Br was a gift from Prof. K. Koga and Dr. N. Ikoda of Tokyo University. D-600 was a gift from Knoll Pharmaceuticals.

Results

The Effect of *p*CMPS on the Time Course of Transcellular Water Movement

Water moves through characean internodal cells at an approximately linear rate for 30 sec. Thereafter the rate of water movement slows down due to the build-up of a concentration gradient of intracellular solutes between the endosmotic and the exosmotic parts of the cell that consequently reduces the driving force for transcellular osmosis (Kamiya & Tazawa, 1956). The initial rate of water movement is substantially reduced after treating the cell with 1 mM *p*CMPS for 60 min (Fig. 1). Since the hydraulic resistance is influenced by the cellular osmotic pressure (Kiyosawa & Tazawa, 1972) and since *p*CMPS is known to increase the intracellular cation concentration in a variety of cells (Will & Hopfer, 1979), we tested whether or not *p*CMPS inhibits transcellular osmosis by increasing the cellular osmotic pressure. Table 1 shows that *p*CMPS has no effect on the cellular osmotic pressure.

Table 1. The effect of *p*CMPS, A-9-C, C_9, D-600 and TTX on the cellular osmotic pressure of internodal cells of *Nitellopsis*

Treatment	(*n*)	Cellular osmotic pressure	
		(mOsm)	MPa (at 20°C)
APW (7.0)	10	284.70 ± 0.50	0.675
1 mM *p*CMPS	10	285.75 ± 0.91	0.678
APW (7.2)	5	285.80 ± 1.42	0.678
1 mM A-9-C	5	284.80 ± 1.30	0.676
APW (5.6)	10	288.94 ± 0.78	0.686
1 mM C_9	10	285.40 ± 0.80	0.677
APW (7.3)	5	290.20 ± 0.65	0.689
100 µM D-600	5	288.80 ± 0.70	0.685
APW (7.3)	5	281.40 ± 2.52	0.668
31.4 µM TTX	5	284.80 ± 4.29	0.676

The osmotic pressure of the cell sap was measured with a vapor pressure osmometer. The cells used in the experiments ± *p*CMPS were analyzed after a 60-min treatment. The rest of the cells were analyzed after a 30-min treatment. Values for each treatment are grouped with controls run simultaneously.

The Effect of *p*CMPS on the Total Hydraulic Resistance

Inhibiting the sulfhydryl groups of proteins with 1 mM *p*CMPS (60 min) results in a 36% increase in the total transcellular hydraulic resistance (r_{tot}) from 2.72 to 3.69 pm^{-1} sec Pa (Table 2). Preliminary experiments indicated that *p*CMPS takes a long time (>40 min) to influence the hydraulic resistance of the cell, even though it causes a depolarization of the membrane potential within 15 min. Treatment with *p*CMPS results in a decrease in the hydraulic conductivity ($L_p = 2(r_{tot})$) from 0.74 to 0.54 pm sec^{-1} Pa^{-1}. If we express the difference in the osmotic pressure across the membrane as a difference in the water concentration, then the units of L_p can be converted into the units of the osmotic permeability coefficient (P_{os}) by multiplying L_p by $RT/\overline{V}_w$ (Hansson Mild & Løvtrup, 1985). Therefore *p*CMPS decreases the P_{os} from 101.85 to 72.42 µm sec^{-1}. If we assume that the plasma membrane is 7 nm wide, we see that *p*CMPS decreases the diffusion coefficient of water (D_w) from 7.06×10^{-13} m^2 sec^{-1} to 5.07×10^{-13} m^2 sec^{-1}, which is about 0.03% of the self-diffusion coefficient of water (1.85×10^{-9} m^2 sec^{-1} at 20°C, Kohn, 1965).

*p*CMPS has no effect on cytoplasmic streaming

Table 2. The effect of *p*CMPS on the total transcellular hydraulic resistance, the hydraulic conductivity, the osmotic permeability and the diffusion of water through the membrane

Treatment	*n*	r_{tot} pm^{-1} sec Pa	L_p pm sec^{-1} Pa^{-1}	P_{os} μm sec^{-1} (at 20°C)	D_w m^2 sec^{-1} (at 20°C)
APW (7.0)	6	2.65 ± 0.29	0.75	101.85	7.06×10^{-13}
1 mM *p*CMPS	6	3.69 ± 0.34	0.54	72.42	5.07×10^{-13}

The total transcellular hydraulic resistances (r_{tot}) were determined for cells treated in APW (7.0) with or without 1 mM *p*CMPS for 60 min. After temperature equilibration (10 min), symmetrically placed cells were subjected to transcellular osmosis using a driving force of 100 mM sorbitol. The hydraulic conductivities (L_p), the osmotic permeabilities (P_{os}) and the diffusion coefficients for water (D_w) were calculated from the following formulas:

$$L_p = 2(1/r_{tot})$$

$$P_{os} = L_p RT/\bar{V}_w$$

and

$$D_w = (P_{os})(d)$$

where R is the gas constant, T is the absolute temperature in K (set to 293°K), $\bar{V}_w$ is the partial molar volume of water and d is the thickness of the plasma membrane (set to 70 nm). Please note that the total transcellular hydraulic resistance represents the total resistance to both endosmosis and exosmosis; therefore, L_p, P_{os} and D_w represent average values. The values for the total transcellular hydraulic resistance represent the mean ±1 SEM ($P < 0.05$).

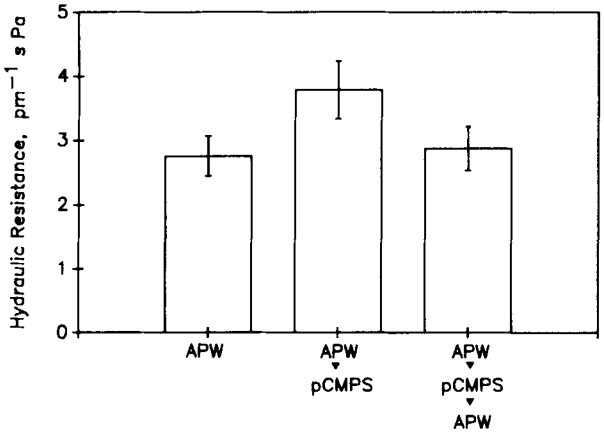

Fig. 2. The reversibility of the *p*CMPS-induced increase in the total transcellular resistance (r_{tot}) in *Nitellopsis*. Symmetrically placed cells were sequentially treated with APW (7.0), 1 mM *p*CMPS in APW (7.0) and APW (7.0) for 60 min each and after each treatment subjected to transcellular osmosis. Each bar represents the mean ±SEM for five cells. Using a *t* test, the level of significance between the controls and the *p*CMPS treated cells is $P < 0.05$. The difference between the control and the reversed cells is not significant

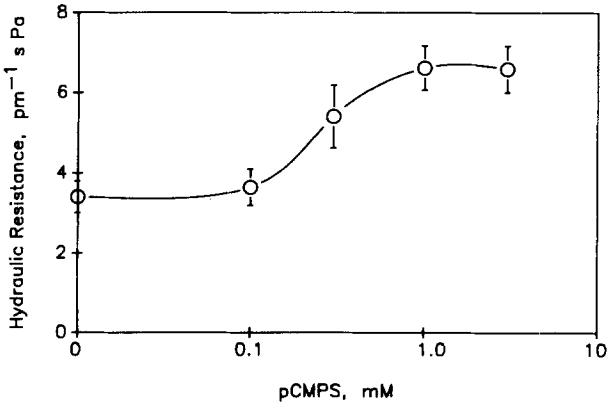

Fig. 3. The effect of various concentrations of *p*CMPS on the total transcellular hydraulic resistance (r_{tot}) in *Nitellopsis*. Each bar represents the mean ±SEM for three cells

(streaming rate = 56 ± 1 (12) and 59 ± 1 (12) μm sec^{-1} at 20–23°C for the control and *p*CMPS cells, respectively), indicating that it exerts its effect on the outside of the plasma membrane (Lucas & Alexander, 1980). The effect of *p*CMPS is completely and always 100% reversible by just washing the drug away (Fig. 2). *p*CMPS increases the hydraulic resistance in a concentration-dependent manner from 0.1 to 1 mM. The response is saturated by 1 mM *p*CMPS and approximately 0.2 mM supports a half-maximal response (Fig. 3). Figure 4 shows that the increase in the hydraulic resistance induced by *p*CMPS can be prevented by simultaneously treating the cells with 5 mM DTT. DTT alone has no effect on the total specific hydraulic resistance.

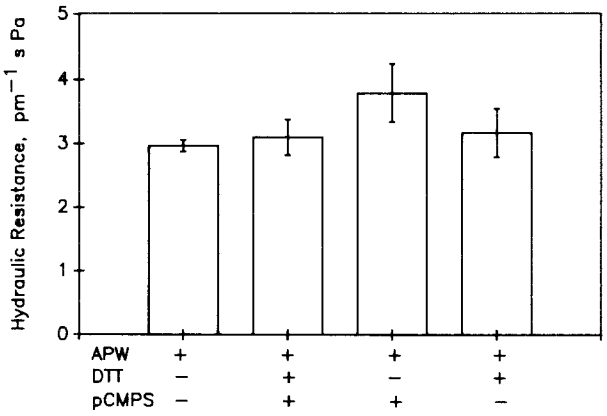

Fig. 4. The effect of DTT in rendering *p*CMPS incapable of increasing the total hydraulic resistance (r_{tot}) in *Nitellopsis*. The bars on APW, *p*CMPS + DTT, pCMPS alone and DTT alone represent the mean ±SEM for 8,5,5 and 3 cells, respectively

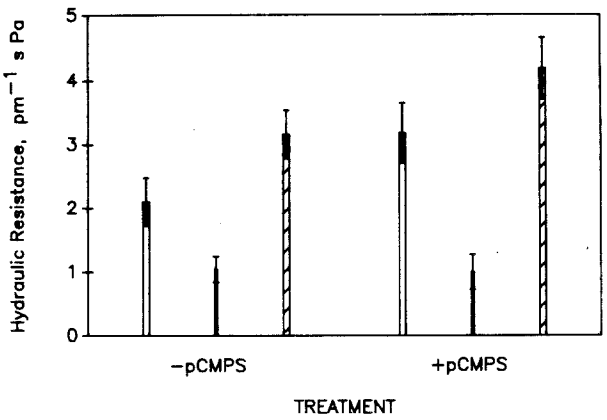

Fig. 5. The effect of *p*CMPS on the endosmotic (L_{pen}^{-1}), exosmotic (L_{pex}^{-1}) and total transcellular (r_{tot}) hydraulic resistance in *Nitellopsis*. Cells were treated in APW (7.0) with or without 1 mM *p*CMPS for 60 min and then subjected to the ligation method of transcellular osmosis. Each bar represents the mean ±SEM for six cells. L_{pen}^{-1}: ■; L_{pex}^{-1}: □; and r_{tot}: ▨

Table 3. The effect of *p*CMPS on the activation energy of transcellular osmosis in *Nitellopsis*

Cell number	APW (7.0)		1 mM *p*CMPS	
	kJ mol^{-1}	kcal mol^{-1}	kJ mol^{-1}	kcal mol^{-1}
1	19.8	4.7	—	—
2	—	—	34.3	8.2
3	—	—	30.9	7.4
4	13.8	3.3	—	—
$\bar{X}$	16.8	4.0	32.6	7.8
SE	2.9	0.7	1.6	0.4

Experiments were performed at temperatures ranging from 5–25°C. Symmetrically placed cells were treated in APW (7.0) with or without 1 mM *p*CMPS for 60 min prior to the first transcellular osmosis. One and the same cell was subjected to all temperatures. Using a *t* test, the level of significance is $P < 0.01$.

Table 4. The effect of *p*CMPS on the membrane potential (E_m), the membrane resistance (R_m) and the ability to generate an action potential in response to transcellular osmosis in *Nitellopsis*

Treatment	*n*	E_m (mV)	R_m (Ω m^2)	Excitability %
APW (7.0)	10	−168.30 ± 1.98	1.38 ± 0.24	90
1 mM *p*CMPS	10	−120.70 ± 1.59	1.37 ± 0.22	0

Cells were treated with or without *p*CMPS for 60 min. E_m was measured with conventional microcapillary electrodes. R_m was measured by applying 0.1 μA square pulses at a frequency of 1.11 sec^{-1}. The transcellular osmosis-induced action potential on the endosmotic side was induced by replacing the solution in one chamber with 200 mM sorbitol.

Cells were subjected to transcellular osmosis at various temperatures ranging from 5 to 25°C, in order to calculate the activation energy for transcellular water movement from Arrhenius plots of the data. We found that 1 mM *p*CMPS increases the activation energy by 95%, from 16.8 to 32.6 kJ mol^{-1} (Table 3).

In order to determine whether *p*CMPS increases the total specific transcellular hydraulic resistance by increasing the endosmotic or exosmotic specific hydraulic resistance or both we subjected the cells to the ligation method of transcellular osmosis (Wayne & Tazawa, 1988). We find that *p*CMPS specifically increases the exosmotic specific hydraulic resistance and consequently the polarity of water movement increases (Fig. 5). By contrast, 1 mM N-ethylmaleimide, another sulfhydryl blocker, has little effect on hydraulic resistance (*data not shown*).

In order to further characterize the action of *p*CMPS on the membrane we measured the membrane potential and resistance of cells treated with and without 1 mM *p*CMPS. Table 4 shows that treatment of *Nitellopsis* internodal cells with 1 mM *p*CMPS induces a membrane depolarization amounting to about 47 mV but no consistent change in the membrane resistance. *p*CMPS also inhibits the generation of the action potential on the endosmotic side that is induced by transcellular osmosis (Table 4).

Table 5. The effect of A-9-C, D-600 and TTX on the total hydraulic resistance in *Nitellopsis*

Cell number	r_{tot} (pm^{-1} sec Pa)		% change
	APW (7.2)	1 mM A-9-C	
1	2.00	2.00	0.000
2	1.95	1.95	0.000
3	2.70	2.68	0.007
$\bar{X}$	2.22	2.21	0.002
SE	0.24	0.24	0.002
	APW (7.3)	100 μM D-600	
4	2.32	2.32	0.000
5	2.08	2.08	0.000
6	3.27	3.27	0.000
$\bar{X}$	2.56	2.56	0.000
SE	0.36	0.36	0.000
	APW (7.3)	31.4 μM TTX	
7	3.73	3.75	0.005
8	2.51	2.51	0.000
9	3.29	3.29	0.000
$\bar{X}$	3.18	3.18	0.002
SE	0.36	0.36	0.002

The hydraulic resistances were measured in symmetrically placed cells treated with APW with or without inhibitors for 15 min.

Table 6. The effect of A-9-C, D-600 or TTX on membrane potential (E_m) and membrane resistance (R_m) in *Nitellopsis*

Treatment	n	E_m (mV)	R_m (Ω m^2)
APW (7.2)	4	190.25 ± 1.42	1.65 ± 0.08
1 mM A-9-C	4	194.00 ± 1.31	1.75 ± 0.06
APW (7.3)	4	216.00 ± 1.81	1.87 ± 0.21
100 μM D-600	4	211.75 ± 1.93	1.95 ± 0.22
APW (7.3)	4	233.50 ± 1.20	1.87 ± 0.07
31.4 μM TTX	4	233.50 ± 1.20	1.87 ± 0.07

Cells were treated with APW with or without inhibitors for 15 min. E_m was measured with conventional microcapillary electrodes. R_m was measured by applying 0.1 μA square pulses at a frequency of 1.11 sec^{-1}.

The Effect of Specific Ion Channel Blockers on Hydraulic Resistance

In order to determine whether or not specific ion channels are involved in transcellular water movement, we tested the effect of various ion channel blockers on r_{tot}. Although the chloride channel blocker A-9-C (1 mM, 15 min) increases only slightly the membrane electrical resistance from 1.65 to 1.75 Ω m^2 it has no effect on hydraulic resistance. Likewise, D-600 (100 μM, 15 min), an inhibitor of voltage-dependent Ca^{2+} channels has no effect on the hydraulic resistance (Table 5) even though it causes a slight depolarization of the membrane (Table 6). Tetrodotoxin affected neither the hydraulic resistance nor the electrical properties of the membrane, indicating that either TTX-binding proteins may be absent in *Nitellopsis* or these proteins have no effect on the electrical properties of the membrane of the resting cell.

By contrast, the potassium channel blocker C_9 (1 mM, 15 min) increases the hydraulic resistance by approximately 35%. Specifically, it increases the hydraulic resistance of the endosmotic side (Fig. 6). C_9 induces a membrane depolarization of approximately 20 mV and increases the membrane resistance from 1.11 to 1.49 Ω m^2 (Table 7). None of the above ion channel blockers affected the cellular osmotic pressure (Table 1). (For references on the effect of channel blockers on the electrophysiology of characean cells, *see* Tazawa & Shimmen, 1980; Tyerman, Findlay & Paterson, 1986; Shiina & Tazawa, 1987*a*,*b*; Tsutsui et al., 1987).

Discussion

The high osmotic permeability (Kamiya & Tazawa, 1956; Dainty & Ginzburg, 1964*b*; Kiyosawa & Tazawa, 1973; Steudle & Zimmermann, 1974), the low activation energy (Tazawa & Kamiya, 1966; Kiyosawa, 1975; Wayne & Tazawa, 1988) and the observation of frictional interactions (Dainty & Ginzburg, 1964*a*; Steudle & Tyerman, 1983) argue strongly for the presence of aqueous channels in the membranes of characean cells. This interpretation is strongly supported by the observations that water movement is inhibited by cytochalasins (Wayne & Tazawa, 1988) and by the sulfhydryl reagent *p*CMPS and the K^+ channel blocker C_9.

*p*CMPS increases the total transcellular hydraulic resistance of cells of *Nitellopsis obtusa* by approximately 36%. This corresponds to a 27% decrease in the hydraulic conductivity (L_p), indicating that 27% of the water moving across the plasma membrane, moves through a SH-containing protein(s). This protein acts as a low resistance pathway to water movement since *p*CMPS increases the activation energy of transcellular osmosis. This SH-containing protein specifically acts as a water channel on the exosmotic side. *p*CMPS induces a depolarization of the membrane potential, but the depolarization is not the cause of the increase in hydraulic resistance since 1 mM NEM, which also depolarizes the membrane, does not change the hydraulic resistance (Wayne & Tazawa, 1988). The H^+-ATPase of characean cells is inhibited by

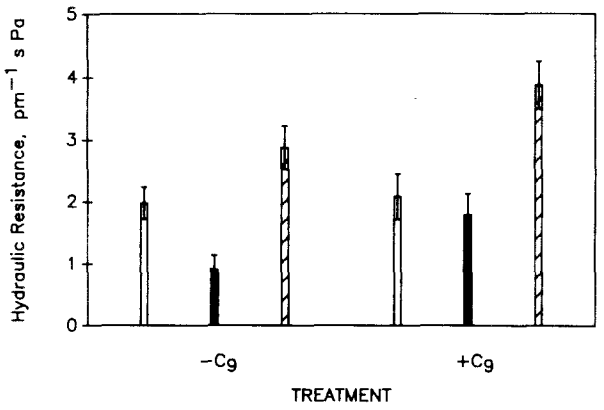

Fig. 6. The effect of C_9 on the endosmotic (L_{pen}^{-1}), exosmotic (L_{pex}^{-1}) and total transcellular (r_{tot}) hydraulic resistance in *Nitellopsis*. Cells were treated in APW (5.6) with or without 1 mM C_9 for 15 min and then subjected to the ligation method of transcellular osmosis. Each bar represents the mean ±SEM for eight cells. L_{pen}^{-1}: ■; L_{pex}^{-1}: □; and r_{tot}: ▨

Table 7. The effect of C_9 on membrane potential (E_m) and membrane resistance (R_m) in *Nitellopsis*

Treatment	n	E_m (mV)	R_m (Ω m^2)
APW (5.6)	10	167.30 ± 1.59	1.11 ± 0.19
1 mM C_9	10	148.80 ± 1.59	1.49 ± 0.28

Cells were treated with APW (5.6) with or without C_9 for 15 min. E_m was measured with conventional microcapillary electrodes. R_m was measured by applying 0.1 μA square pulses at a frequency of 1.11 sec^{-1}.

*p*CMPS and NEM (Lichtner, Lucas & Spanswick, 1981). We originally thought that a *p*CMPS-sensitive, NEM-insensitive region of this protein serves as a water channel and is responsible for exosmotic water movement. However, the affect of NEM appears to be more complicated. While 1 mM NEM has no effect on r_{tot} we find that 30 μM NEM causes a 15% increase in r_{tot}. It is therefore possible that low concentrations on NEM also inhibited the *p*CMPS-sensitive protein, but then at higher concentrations, NEM decreases the resistance by opening K^+ channels (*see* Lichtner et al., 1981).

The hydraulic resistance of erythrocytes also increases upon treatment with *p*CMPS. This sulfhydryl agent specifically inhibits the membrane water permeability by binding bands 3 and 4.5 (Benga, 1989). The water-conducting capability of band 3 has been confirmed by placing the purified protein in black lipid membranes (Benz, Tosteson & Schubert, 1984). At present, it is completely unknown whether or not any homology exists between the characean water-conducting protein and the erythrocyte proteins that bind *p*CMPS. However, the identification and purification of characean water-conducting proteins is possible using radiolabeld *p*CMPS.

In order to characterize further the proteins that contribute to water flow in characean cells, we tested the effects of ion channel blockers on the hydraulic resistance. Ion channels are pores and may be able to pass water as well as ions (Kukita & Yamagishi, 1983). In characean cells, it is known that pores exist that pass water, but not ions (Kiyosawa & Ogata, 1987). Since the plasma membrane of characean cells are equipped with ion channels for K^+, Cl^- and Ca^{2+}, we began our investigation using inhibitors of the channels that transport these ions. Of all the ion channel blockers tested only C_9 specifically increased the hydraulic resistance on the endosmotic side. These data indicate that C_9-sensitive K^+ channels (Tazawa & Shimmen, 1980) may serve as water channels in characean cells. The observations that C_9 induces a depolarization of the membrane potential, and an increase in membrane resistance supports the contention that it is acting on a K^+ channel.

In interpreting the effect of C_9 we should be cautious, because C_9 is a very hydrophobic molecule with a long hydrocarbon chain. Aliphatic monohydric alcohols also increase r_{tot} (Tazawa & Kamiya, 1965) and this inhibition increases with the increase in carbon chain length (Kiyosawa, 1975). Increases in viscosity upon the addition of alcohol are not enough to account for the decrease in r_{tot} (Kiyosawa, 1975). The incorporation of alcohol molecules into the membrane may cause packing of the membrane structure. If C_9 increases r_{tot} unspecifically due to its hydrophobic nature, not only L_{pen}^{-1} but also L_{pex}^{-1} should be affected to the same extent. The polar effect of C_9, therefore, may suggest that a channel, probably a K^+ channel is involved specifically in endosmosis.

While there is strong evidence that water moves through channels in characean cells, erythrocytes, epithelial and endothelial cells, this may not be a widespread phenomenon. The osmotic permeability coefficient of these cells and tissues are considerably higher than the P_{os} of most other cells (Dick, 1966; Finkelstein, 1987). It is clear in *Valonia*, for example where the P_{os} is only 2.4 μm sec^{-1}, that water does not move through aqueous pores (Gutknecht, 1967, 1968). In fact, the low P_{os} of *Valonia* may be a consequence of the lack of aqueous pores. It then is interesting to see whether or not cytochalasins, *p*CMPS or C_9 affects the P_{os} of *Valonia*.

We assume that the proteins that are inhibited by *p*CMPS and C_9 are homogenously distributed along the membrane of the cell. However, the SH-containing protein and the K^+ channel are only involved in water flows on the exosmotic or endosmotic side, respectively. We have previously suggested that the flow of water though the plasma membrane sweeps away ions from the protoplasmic surface of the plasma membrane on the endosmotic side and leads to an accumulation of ions at the protoplasmic surface of the plasma membrane on the exosmotic side. The differential ion concentrations on the endosmotic and exosmotic sides may lead to a differential gating of channels on the two sides. We suggest that the ionic conditions on the endosmotic side lead to the opening of the K^+ channel and the ionic conditions on the exosmotic side lead to the opening of the channel formed by the SH-containing protein. This differential gating could lead to the observed polarity of transcellular water movement in characean cells. The putative actin-mediated aggregation of proteins on the endosmotic side also contributes to this polarity. We look forward to the discovery of the physiological and environmental factors that regulate the hydraulic resistance of the membrane so that the cell can maintain a homeostasis in the face of adversity (*see*, for example, Kang & Burg, 1971; Carceller & Sanchez, 1972; Weisenseel & Smeibidl, 1973; Dowler et al., 1974; Pike, 1976; Boyer & Wu, 1978; Loros & Taiz, 1982). From the point of view of water stress biology, it is quite reasonable that L_{pex}^{-1} is greater than L_{pen}^{-1}.

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