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Calcium Effects on Electrogenic Pump and Passive Permeability of the Plasma Membrane of *Chara corallina*

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Summary. Removal of Ca^{2+} from the medium results in depolarization of the *Chara* internodal cell and an increase in membrane conductance (G_m). The increase in conductance is associated with an increase in K^+ conductance, as judged by Ca^{2+} effects on the K^+ dependence of clamp current. The voltage dependence of G_m is also affected by Ca^{2+}, as is the time course of the response of clamp current to a step change in voltage. Mg^{2+} restores the low conductance and the fast response to a voltage change, but not hyperpolarization at neutral pH, suggesting that there is an additional, independent effect on the electrogenic pump. The membrane does not show the normal ability to increase proton conductance at high pH in the absence of Ca^{2+}; this is also restored by Mg^{2+} as well as by Ca^{2+}.

Key Words *Chara* · calcium · protons · potassium · electrogenic pump · conductance

Introduction

The plasma membrane of the large internodal cells of the freshwater alga *Chara corallina* is capable of sustaining a large negative electrical potential difference by the action of an electrogenic proton pump. Under some conditions (e.g., increased K^+ concentration, removal of Ca^{2+}, inhibition of the pump, removal of ATP) the cell depolarizes and the membrane potential takes on the characteristics of a potassium diffusion potential (Hope & Walker, 1961; Oda, 1962; Spanswick, 1974; Hope & Walker, 1975; Keifer & Spanswick, 1978, 1979). Recently, a new kind of membrane potential, a H^+ diffusion potential, was demonstrated in *Chara* internodes exposed to high pH (Bisson & Walker, 1980). This study was undertaken to elaborate the nature of the control of, and the relation between, the proton pumping and passive proton permeation. An earlier study (Bisson & Walker, 1981) showed that dicyclohexylcarbodiimide (DCCD), an inhibitor of transport H^+-ATPases (Keifer & Spanswick, 1979; Goffeau & Slayman, 1981) also prevented the cell from generating the H^+ diffusion potential in alkaline solutions. The time course of this inhibition was the same as that of the inhibition of proton pumping, and different from that of the effect on ATP concentrations, suggesting a relation between the H^+ pump and the H^+ leak. Ca^{2+} was chosen to be examined next. Removing Ca^{2+} from the medium collapses the electrogenic potential. Keifer and Lucas (1982) presented evidence that this is due to an increase in K^+ permeability, which shunts the electrogenic potential, suggesting that the H^+ pump is active but masked. The study reported here was initiated to compare the effect of Ca^{2+} removal on the proton pump and on the passive proton permeability; but in order to do this satisfactorily, the effect on K^+ permeability also had to be studied.

Materials and Methods

Plants were cultured as reported earlier (Bisson & Bartholomew, 1984). Internodes and the adjacent nodes were isolated and trimmed of their branchlets at least 24 hr before an experiment and incubated under the same light (14:10 light/dark cycle) and temperature (20 to 22°C) conditions in a solution containing (mM): K_2SO_4 0.1, NaCl 1, $CaSO_4$ 0.5, and N-2-hydroxyethylpiperazine-N′-2-ethane sulfonic acid (HEPES) 5, buffered to pH 7.5 with NaOH (which raised the sodium concentration by 2 to 3 mM).

For electrophysiological experiments, K_2SO_4 concentration was raised to 1 mM. A higher potassium concentration was used to make the equilibrium potential for K^+ (E_K) more positive, and distinctly different from both the electrogenic potential and from E_H. After impalement, this solution was replaced by a similar solution but without Ca^{2+}. In this medium cells depolarized, usually in 1 to 2 hr. Measurements were made in the zero calcium solution, then Ca^{2+} was added back and measurements made during recovery. In some cases, Mg^{2+} was added instead of Ca^{2+}. In these experiments, Ca^{2+} was later substituted for Mg^{2+}.

Measurements of membrane potential (ψ_m) and conductance (G_m) were made as described previously (Bisson & Walker, 1980). Unless otherwise specified, G_m was measured after clamping ψ_m to -150 mV. This is because of the potential dependence of G_m, reported to be due to the potential dependence of K^+ permeability (P_K) (Krawczyk, 1975; Smith & Walker, 1981; Sokolik & Yurin, 1981; Bisson & Walker, 1982). The activity of the electrogenic pump was shown when the membrane potential was more negative than the equilibrium potential for any ion. The ability to increase P_H and generate the H^+ diffusion potential was measured by subjecting the cells to more alkaline solutions, buffered by 2[N-cyclohexylamino]ethane sulfonic acid (CHES) (pK 9.3) or cyclohexylaminopropane sulfonic acid (CAPS) (pK 10.4). The experimental medium was brought to pH 12 by addition of NaOH (approximately 3 mM) without any other added buffer. Increase in P_H was indicated by a shift in membrane potential towards E_H (-180 mV at pH 11: Bisson & Walker, 1981) and by an increase in G_m.

Current relaxation experiments were done by clamping the membrane at -100 to -125 mV, waiting for a steady-state current to be achieved, then suddenly altering the command potential to -150 mV. Current at each time I_t was measured either manually off the recorder trace or entered by a digitizer into a Datax computer. The current relaxation $I_t - I_\infty$, where I_∞ is the current at the steady state, was usually well fit by a single exponential term; occasionally two exponentials were needed to describe the relaxation. Fitting was done by using a linear regression on $\ln(I_t - I_\infty)$, and the time constant k obtained from the slope.

Results

When Ca^{2+} was removed from solution, cells usually depolarized to a potential near E_K (-104 mV; Bisson & Walker, 1981) after 1 to 2 hr. Occasionally, cells would take as long as 7 hr to depolarize. If action potentials occurred soon after the removal of Ca^{2+}, the potential often did not recover completely to the electrogenic potential, but only to E_K. However, when such cells were clamped to -150 mV for measurement of G_m, they invariably hyperpolarized to the electrogenic potential on release from the clamp. If cells showing such action potentials were allowed to stand for 2 hr, however, clamping to the more negative value would not result in hyperpolarization. Values given for electrical parameters of cells in Ca^{2+}-free solutions are from cells which had depolarized without an action potential, or which had been in Ca^{2+}-free solution at least 2 hr after an action potential.

Table 1. Electrical characteristics in the presence or absence of Ca^{2+} and Mg^{2+} at elevated K^+ concentrations

		0 Ca^{2+}	+ Ca^{2+}	+ Mg^{2+}
$\psi_m^{7.5\,a}$	mV	$-109 \pm 2(23)^b$	$-130 \pm 5(52)$	$-114 \pm 12(12)$
Final $\psi_m^{7.5}$			$-167 \pm 15(11)$	$-119 \pm 20(5)$
ψ_m^{11}		$-129 \pm 4(11)$	$-169 \pm 3(23)$	$-161 \pm 9(9)$
$G_m^{7.5}$	S m^{-2}	$4.9 \pm 0.8(19)$	$1.6 \pm 0.2(29)$	$1.9 \pm 0.5(14)$
G_m^{11}		$8.3 \pm 1.3(10)$	$12.9 \pm 6.4(22)$	$15.1 \pm 4.7(9)$
ΔG_m		$3.9 \pm 1.4(10)$	$11.1 \pm 6.3(22)$	$13.0 \pm 4.1(9)$

[a] Explanation of symbols: $\psi_m^{7.5}$—membrane potential at pH 7.5; ψ_m^{11}—at pH 11; $G_m^{7.5}$—membrane conductance at pH 7.5; G_m^{11}—at pH 11.0; ΔG_m—change in membrane conductance with increase in pH; *see* text for details.
[b] Data presented as mean ± standard error (number).

ψ_m is near E_K in the absence of calcium, but is more negative in its presence. The average value for the membrane potential after the addition of Ca^{2+} is -130 mV (Table 1) but this includes measurements very soon after the addition of Ca^{2+} to previously Ca^{2+}-free solutions. Since the cells tend to hyperpolarize slowly over a period of hours (as shown in Fig. 1), the average potential measured immediately before the termination of the experiment, -167 mV, is also given in Table 1.

The membrane potential at pH 11 is also more negative in the presence than in the absence of Ca^{2+} ($P < 0.01$). In contrast to the slow hyperpolarization of $\psi_m^{7.5}$ after the addition of Ca^{2+}, ψ_m^{11} reaches a value near its maximum negative value as soon as measured (usually about 2 min) after the addition of Ca^{2+} and changes only little with further incubation in Ca^{2+} (Fig. 1).

Membrane conductance at pH 7.5 is higher in 0 Ca^{2+} than in normal Ca^{2+} ($P < 0.01$) (Table 1). At pH 11, although the mean conductance is higher in the presence of Ca^{2+}, the difference is not significant ($P > 0.1$). $\Delta G_m (= G_m^{11} - G_m^{7.5})$ is greater than in the absence of Ca^{2+}, but this is not statistically significant. G_m^{11} is greater than $G_m^{7.5}$ in both the absence ($P < 0.02$) and in the presence ($P < 0.01$) of Ca^{2+}.

In a voltage-clamped cell, a step change in membrane potential to a final value of -150 mV from an initial value of -100 to -125 mV produces a transient current (*see* Fig. 2). The transient measured here is much too slow to be a capacitative transient, using the values for capacitance obtained by Findlay and Hope (1964) for a step change in cur-

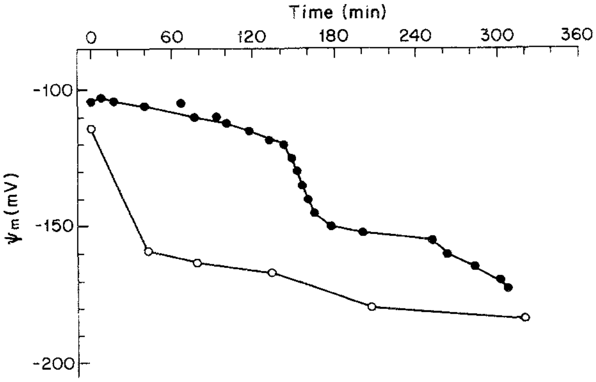

Fig. 1. Time course of recovery of ψ_m after addition of Ca^{2+}. Closed circles: membrane potential at pH 7.5; open circles, at pH 11. Cell 7/22/82

rent. The transients discussed here show time constants of the order of seconds or tens of seconds. This transient, or relaxation, is markedly affected by Ca^{2+}, as shown in Fig. 2. The relaxation is quantified in Table 2. The plot of $\ln(I_t - I_\infty)$ (where I_∞ is the steady-state value of current at −150 mV) against time usually gives a linear plot, with slope = k. An example is given in Fig. 3. Sometimes, the plot could be decomposed into two linear plots, as shown in Fig. 4. Sometimes, there was a lag of 1 to 16 sec before the current relaxation began. This lag was seen four times; three of those times it occurred in Ca^{2+}-free solutions. The constant points during the lag were not used in the computation of k. Table 2 divides the data according to whether one or two time constants were measured. In the presence of Ca^{2+}, the time constant is more negative, indicating a faster rate of relaxation ($P < 0.01$). In the cells which show two phases of response, the fast response is not different in the absence of Ca^{2+} ($P > 0.1$), but the slow response is 4.5 times faster in the presence of Ca^{2+} ($P < 0.01$).

The total change in current, $\Delta I = I_0 - I_\infty$, normalized to membrane area, was also calculated for each cell, including some cells which showed no relaxation ($\Delta I = 0$), and which were not included in the current relaxation computations. This measures the extent of relaxation, as opposed to the speed of relaxation. There is no significant difference in the extent of relaxation ($P > 0.1$). This is still true even if only those cells which experience the same change in membrane potential (−50 mV, from −100 to −150 mV) are used in the computation.

Finally, the total time to steady state is also recorded in Table 2. As expected, it takes significantly longer to reach steady state in the absence of Ca^{2+} ($P < 0.01$).

In some experiments, after measurements were taken in 0 Ca^{2+}, 0.5 mM magnesium sulfate was added instead of calcium sulfate. These results are also shown in Tables 1 and 2. Mg^{2+} is generally as effective as Ca^{2+} in restoring membrane functions. The one notable exception was membrane potential at neutral pH. Most cells do not hyperpolarize in

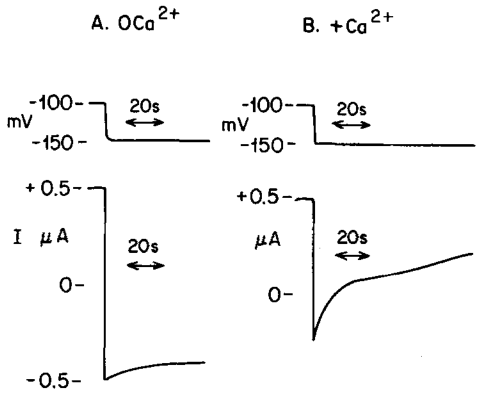

Fig. 2. Current relaxation in the absence (*A*) and presence (*B*) of Ca^{2+}. In both cases, a step change in membrane potential, from −100 to −150 mV, is shown in the upper trace, the current response is shown in the lower trace. Membrane area 3.1×10^{-5} m^2. Cell 8/3/82

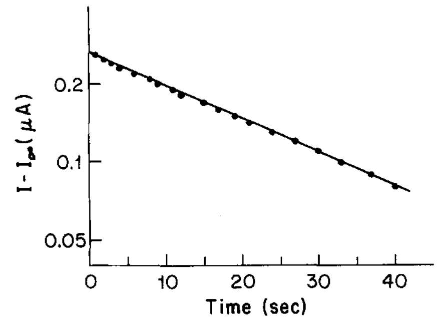

Fig. 3. Current relaxation plot on decimal logarithmic scale. k computed for natural logarithmic transform = -0.029 sec^{-1}, $r = -0.993$. In the presence of Ca^{2+}. Membrane potential = −132 mV. Membrane area = 3.3×10^{-5} m^2. Cell 7/22/82

Table 2. Time course of the voltage-dependent changes in G_m

		0 Ca^{2+}	Ca^{2+}	Mg^{2+}
k^a	s^{-1}	−0.041 ± 0.014(9)	−0.16 ± 0.041(19)	−0.30 ± 0.046(9)
$k_1{}^b$		−0.23 ± 0.19(5)	−0.26 ± 0.080(3)	
k_2		−0.021 ± 0.014(6)	−0.098 ± 0.072(3)	
ΔI	mA m^{-2}	16.9 ± 5.4(17)	8.5 ± 1.4	10.6 ± 5.7(11)
Δt	s	142 ± 45(12)	38 ± 6(15)	12 ± 2(9)

[a] k is computed time constant when relaxation shows single logarithm time course.

[b] k_1 the faster time constant when relaxation shows double logarithmic time course; k_2 is the slower time constant.

the presence of Mg^{2+}. In one cell, Mg^{2+} promoted a hyperpolarization to −185 mV, but the cell did not maintain this potential. In every case, when Ca^{2+} was substituted for Mg^{2+} the membrane potential at pH 7.5 became more negative ($P < 0.1$). However, membrane potential at pH 11, G_m at both pH 7.5 and pH 11, ΔI and k (only single exponential relaxations were observed) are all the same whether Ca^{2+} or Mg^{2+} is added back. The only other parameter which the two divalent cations affect differently is the time required to come to the new steady-state value after a negative shift in clamp potential, 27 sec faster with Mg^{2+} ($P < 0.01$).

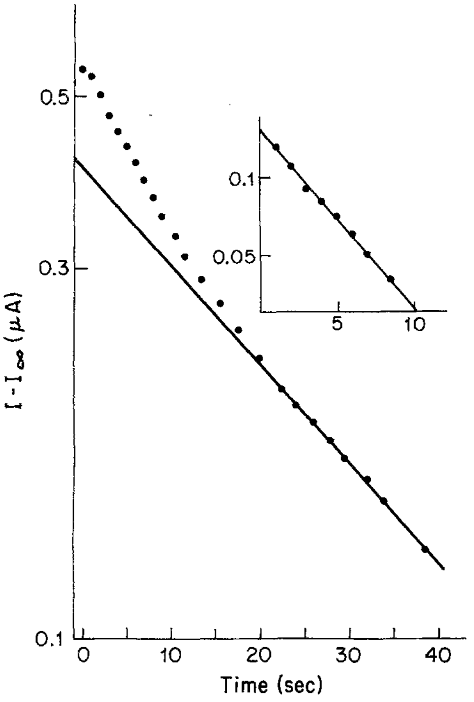

Fig. 4. Current relaxation plot on decimal logarithmic scale showing 2 logarithmic constants. k_2 computed for natural logarithmic transform = $-0.030\ sec^{-1}$, $r = -0.992$, using the last 9 points. *Inset:* Subtraction of computed line from points. $k_1 = -0.16\ sec^{-1}$, $r = 0.997$. In the absence of Ca^{2+}. Membrane potential = −118 mV. Cell 8/1/82

Table 3. Changes in membrane potential and conductance at elevated Ca^{2+} concentration[a]

		Ca^{2+}	$10 \times Ca^{2+}$
$\psi^{7.5}$	mV	$-193 \pm 16(6)$	$-170 \pm 11(7)$
ψ^{11}		$-170 \pm 20(4)$	$-168 \pm 11(5)$
$G_m^{7.5}$	$S\ m^{-2}$	$2.7 \pm 0.8(5)$	$1.8 \pm 0.4(7)$
G_m^{11}		$18.1 \pm 11.8(3)$	$15.4 \pm 4.5(4)$
ΔG_m		$15.7 \pm 10.9(3)$	$13.7 \pm 4.8(4)$
ΔI	$mA\ m^{-2}$	$5.2 \pm 0.9(5)$	$11.3 \pm 1.0(3)$
Δt	s	$21.3 \pm 3.6(5)$	$3.0 \pm 2.0(3)$

[a] Symbols as in Table 1.

To test whether the normal Ca^{2+} concentration (0.5 mM) saturates the Ca^{2+}-dependent responses, cells at high Ca^{2+} concentration (5 mM) were compared with controls. The data are shown in Table 3. There is no significant difference in any steady-state membrane potential or conductance. Because the recorder tracings were not suitable, no time constants for current relaxation were measured. However, the time to come to steady-state current after a −50 mV shift in clamp potential is significantly less ($P < 0.01$) in the presence of high Ca^{2+}, and the total change in current is significantly greater ($P < 0.01$). Although not measured, the relaxation must therefore be faster.

The pH dependence of the membrane potential at high pH in the presence and absence of Ca^{2+} is shown in Fig. 5. Note that at pH 7.5, the membrane potential is the same for the two treatments. This is because the experiments performed in the presence of Ca^{2+} were done in the first hour after addition of Ca^{2+} to previously Ca^{2+}-free cells, so that the cells had not yet hyperpolarized at neutral pH. In the presence of Ca^{2+} we see that ψ_m becomes more negative with increasing pH. (As in all experiments, each measurement at high pH is bracketed by measurements at pH 7.5. For these experiments, membrane potential returns quickly to the control value after the measurement at high pH.) The maximum slope of the potential *vs.* pH occurs between pH 11 and 12, −51 mV/pH unit. The dependence of potential on pH is not so marked in the absence of Ca^{2+}. In fact, the cell only hyperpolarizes significantly at pH 12, and the slope between pH 11 and 12 is only

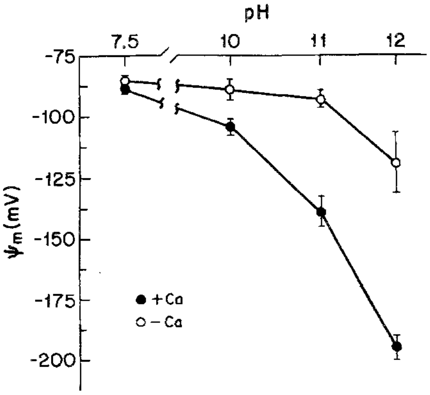

Fig. 5. Membrane potential as a function of pH in the presence (closed circles) and absence (open circles) of Ca^{2+}. For 0 Ca^{2+}, 9 cells, $n = 9$ for each high pH, 31 for 7.5; for + Ca^{2+}, 8 cells, $n = 8$ for each high pH, 26 for 7.5

−29 mV/pH unit. This suggests that the membrane potential is not dominated by H^+ permeability at high pH to the same extent in the absence of Ca^{2+} as it is in its presence. Is this due solely to an increase in G_K, or does G_H also fail to increase? The data in Table 1 are equivocal, because although ΔG is greater in the presence than in the absence of Ca^{2+}, the variability is high enough that the difference is not significant. In this set of experiments, however, ΔG is significantly lower at pH 11 ($P < 0.001$) and at pH 12 ($P < 0.1$) in the absence of Ca^{2+} (Fig. 6).

The effect of membrane potential on G_m in the presence and absence of Ca^{2+} is shown in Fig. 7. In these experiments ψ_m is varied by clamping the cell to different potentials and waiting for steady-state current before measuring G_m by running a bipolar staircase of command voltages. The effect of Ca^{2+} on G_m is seen to be minimal at potentials more negative than −175 mV. It increases with decreasing ψ_m, reaching a maximum at −100 mV, near the unclamped (open-circuit) membrane potential of the cells. (Again, the $+Ca^{2+}$ measurements were done shortly after the addition of Ca^{2+}, so that the cell had not yet hyperpolarized.)

The effects of Ca^{2+} and of potential on G_m have been assumed to be due to effects of G_K. This seems reasonable because in the depolarized state, the K^+ dependence of the potential indicates that G_K dominates G_m (Hope & Walker, 1961; Oda, 1962), and if G_m decreases, it can only be due to a decrease in G_K. I tested this by measuring the K^+ dependence of ψ_m in open-circuit (unclamped) cells and of clamp current in a voltage-clamped cell. Figure 8 shows the effect on membrane potential. The dashed line shows the result anticipated for a purely Nernstian response, that is, $G_K \gg G_j$. The closed circles show the response at neutral pH in the absence of Ca^{2+}. The slope is close to that predicted. The fact that it is slightly less, and that the values are in general positive to those predicted, suggests that conductance of some other ion, e.g., Na^+ or Cl^-, affects the membrane potential, consistent with earlier reports (Hope & Walker, 1961). The same holds true in the presence of Ca^{2+} (again, measured before the cells hyperpolarized at neutral pH). At high pH in the absence of Ca^{2+} (open circles), the membrane potential is also close to that predicted, although the slope is even less, and the value may be more nega-

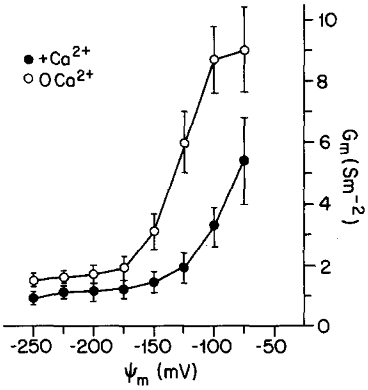

Fig. 7. Membrane conductance as a function of potential in the presence (closed circles) and absence (open circles) of Ca^{2+}. $n = 13$

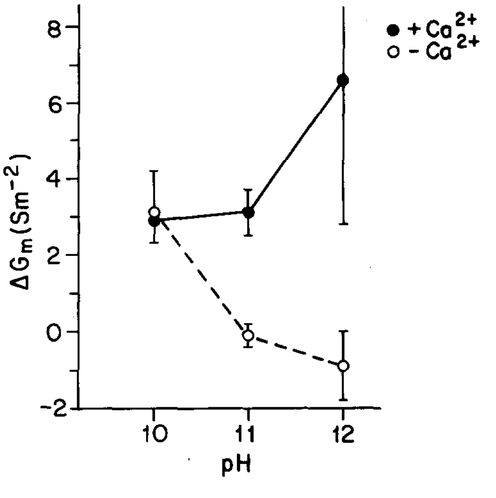

Fig. 6. Change in membrane conductance (the difference between conductance at high pH and bracketing values at pH 7.5) in the presence (closed circles) and absence (open circles) of Ca^{2+}. Same cells as Fig. 5

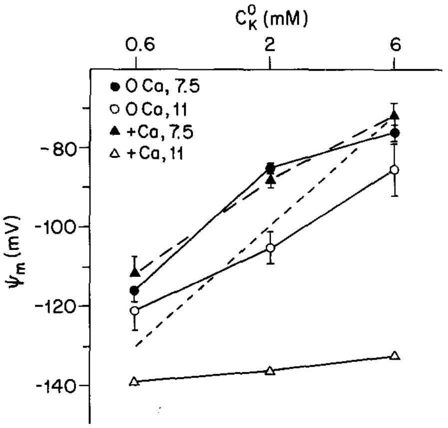

Fig. 8. Membrane potential as a function of K^+ concentration. Closed circles: 0 Ca^{2+}, pH 7.5 (n = 13 for 0.6 and 6 mM, 37 for 2 mM); open circles: 0 Ca^{2+}, pH 11 (n = 14, 28); closed triangles: + Ca^{2+}, pH 7.5 (n = 13, 27); open triangles: + Ca^{2+}, pH 11 (n = 11, 22). Note logarithmic scale for K^+ concentration

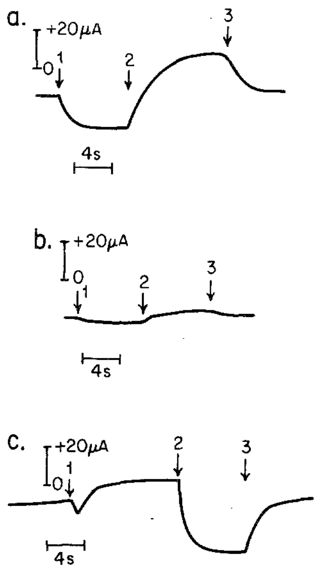

Fig. 9. Dependence of clamp current on K^+ concentration at pH 7.5. Open circuit potential = −79 to −82 mV. Cell area = 3.5 × 10^{-5} m^2. Cell 9/14/83. *a.* 0 Ca^{2+}. Command voltage = −100 mV. Initially, K^+ concentration is 2 mM. At the arrow labeled 1,the solution is changed to one with 0.6 mM K^+. At arrow 2, K^+ concentration = 6 mM. At arrow 3, K^+ concentration = 2 mM. *b.* 0 Ca^{2+}. Command voltage = −200 mV. Order of presentation of solutions is the same as in part *a*. *c.* + Ca^{2+}. Command voltage = −100 mV. The order of presentation of the different K^+ concentrations is reversed, that is, 6 mM at arrow one and 0.6 mM at arrow 2. (Presentation order was randomized.) The transient following arrow 1 is due to some 0.6 mM K^+ solution in the line when 6 mM K^+ was introduced

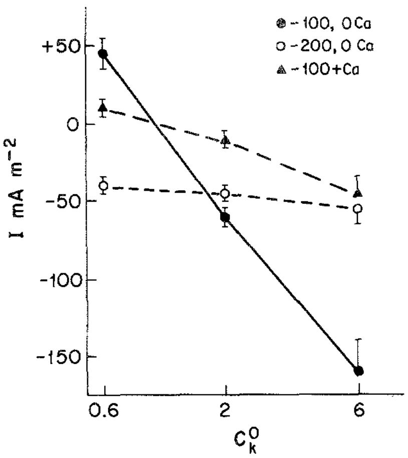

Fig. 10. Dependence of clamp current on K^+ concentration at pH 7.5. Closed circles: 0 Ca^{2+}, command potential = −100 mV (n = 7 for 0.6 and 6 mM, 16 for 2 mM); open circles, 0 Ca^{2+}, command potential = −200 mV (n = 7, 14); triangles, + Ca^{2+}, clamp potential = −100 mV (n = 5, 9). Note logarithmic scale for K^+ concentration

tive than E_K. The only equilibrium potential which is more negative than E_K is E_H (−180 mV at pH 11). This suggests that there is some contribution of H^+ diffusion to the membrane potential, although it remains dominated by K^+ diffusion. In the presence of Ca^{2+}, however, ψ_m shows very little dependence on K^+ concentration at pH 11, suggesting G_H, not G_K, now dominates the membrane potential.

The dependence of ψ_m on K^+ is identical in the presence and absence of Ca^{2+}, even though G_m is very different. This kind of experiment gives information only about the ratios of conductances, not the absolute values. To investigate this further, the effect of K^+ on clamp current at various potentials in the presence or absence of Ca^{2+} was measured. Illustrative individual results of this are shown in Fig. 9 and average results from all cells in Fig. 10. Cells in the absence of Ca^{2+}, clamped at −100 mV (Fig. 9*a*; Fig. 10, closed circles) show the greatest sensitivity to K^+ concentration. When clamped at −200 mV (Fig. 9*b*; Fig. 10, open circles), the sensitivity is much less. Immediately after Ca^{2+} is added back, before the hyperpolarization at neutral pH takes place, clamp current at −100 mV is much less sensitive to K^+ (Fig. 9*c*; Fig. 10, triangles). This occurs despite the fact that the open-circuit voltage dependence is the same in similar circumstances (*cf.* Fig. 8, closed circles and closed triangles). Clamp current at −200 mV in the presence of Ca^{2+} shows no K^+ sensitivity (*data not shown*).

Discussion

This study began with one question: is the effect of Ca^{2+} the same on proton pumping at neutral pH and proton permeability at high pH? In order to answer this question, however, it had to deal with the question of the nature of the effect of Ca^{2+} on the proton pump.

Depolarization of the cell in the absence of Ca^{2+} is known to be accompanied by an increase in G_m (Keifer & Lucas, 1982; Table 1). One would predict that inhibiting the proton pump would cause a decrease in G_m (Spanswick, 1974; Keifer & Spanswick, 1978). However, when cells are in a state where the pump is inactive and membrane potential is manipulated with a voltage clamp, hyperpolarization itself is seen to lead to a decrease in G_m (Krawczyk, 1975; Smith & Walker, 1981; Sokolik & Yurin, 1981; Bisson & Walker, 1982). This has been suggested to be due to an effect on K^+ permeability, primarily because in these cells G_K dominates G_m. This is confirmed by this study, which shows that clamping to a more negative potential results in less

K^+ sensitivity of the clamp current (Figs. 9 and 10). The green alga *Hydrodictyon africanum* shows similar voltage-dependent K^+ conductance, although the time course of response to a step change in potential is more complicated than that seen here (Findlay & Coleman, 1983).

It has been suggested (Keifer & Lucas, 1982) that removal of Ca^{2+} results in an increase in K^+ permeability. This also is confirmed by this study (Figs. 9 and 10). I also show two new related effects. The effect of Ca^{2+} on conductance is voltage sensitive (Fig. 7). Ca^{2+} also affects the rate of current change after a step-change in voltage (Fig. 2; Table 2), suggesting that it affects the time dependence of the voltage dependence of G_K. These facts are accommodated by a model for G_K similar to that used for K^+ conductance in animal systems (Latorre & Alvarez, 1981), to wit, a channel which can be in an open or closed state, with the probability of being in a given state affected by the membrane potential, presumably because the act of closing or opening depends on the movement of a charged group through the electric field. G_K in a given state would result from the average number of channels open at any given time. A more negative membrane potential promotes the closing of K^+ channels, resulting in fewer being open at any given time. Keifer and Lucas (1982) suggest that Ca^{2+} affects the closing, but not the opening of channels. Their evidence for this is that removal of Ca^{2+} does not itself open the K^+ channels, but that the K^+ channels must first be opened by increasing K^+ concentration or by an action potential (both of which depolarize the membrane and which increases G_K). While I found it to be true that with low (0.2 mM) K^+ concentrations cells could remain hyperpolarized for weeks without Ca^{2+} in the medium, removal of Ca^{2+} with EGTA could result in a depolarization to a value somewhat positive to E_K without an action potential (with Ca^{2+}, $\psi_m = -198 \pm 5$ mV ($n = 27$); without Ca^{2+}, $\psi_m = -133 \pm 6$ mV ($n = 16$)). It is true that depolarization occurs more readily in the presence of higher K^+. I initially hypothesized that high K^+ resulted in the exchange for Ca^{2+} out of the cell wall. However, measurements of residual Ca^{2+} in isolated cell walls showed no difference at the two K^+ levels. K^+ may have some direct effect on the tendency of the channels to open, but this is not clear. However, let us assume that Ca^{2+} promotes the closing of channels, without ruling out the possibility that it may also affect the opening. Then, at a given ψ_m, more channels should be closed in the presence than in the absence of Ca^{2+}. There is an interaction between the effects of Ca^{2+} and of ψ_m, however, as shown in Fig. 7. If ψ_m is sufficiently negative, Ca^{2+} has less influence on G_m, that is, on the average number of open channels, suggesting that requirement of Ca^{2+} for closing is not absolute. In animal systems, the opening of some channels is affected by the presence or absence of Ca^{2+} on the inside (cytoplasmic side) (Meech, 1978; Lew & Ferreira, 1978), but I know of no evidence for an effect of external Ca^{2+} in animal systems. The effect seen here is not specific for Ca^{2+}, but is mimicked quite well by Mg^{2+} (Table 2), which suggests that the effect may be due to a general screening of charge rather than a specific interaction with the putative channel or its putative gate.

An increase in G_K could alone generate the depolarization of membrane potential seen in the absence of Ca^{2+}. This was suggested by Keifer and Lucas (1982) because they showed that after depolarization in the absence of Ca^{2+}, cells would rapidly hyperpolarize if K^+ concentrations were lowered. They therefore suggested that the pump was active, but its potential was masked by the high G_K. When G_K was lowered by lowering K^+ concentration, the pump potential was expressed, due to the increase of the pump conductance relative to the K^+ conductance (G_p/G_K). An alternative explanation for their results is that the pump rapidly recovers when G_K is lowered. I also observed that cells which generated an action potential soon after the removal of Ca^{2+} and which failed to repolarize past the level of E_K would quickly repolarize when G_K was reduced, although in this case it was reduced by clamping the potential to a more negative value (*see* Results). However, this was not true of cells which were incubated in Ca^{2+}-free medium for several hours; these did not repolarize even after repeated clamping to -150 mV. There is therefore a difference in the activity of the pump after long times in Ca^{2+}-free medium. It may be that the pump is on but masked soon after the removal of Ca^{2+}, but after several hours the pump turns off. An alternative explanation is that the pump is off in the absence of Ca^{2+} when G_K is high, but turns on again when G_K is lowered and that the ability to recover is progressively slowed or weakened with time in Ca^{2+}-free medium. A possible explanation for the slow time course of this Ca^{2+} effect is that the effect is due to depletion of Ca^{2+} from the cytoplasm, which could be moderated by drawing from stores in mitochondria and the central vacuole. However, spontaneous hyperpolarization at neutral pH after the addition of Ca^{2+} to Ca^{2+}-free cells also requires several hours, and it does not seem likely that it would take this long to replenish Ca^{2+} stores in the cytoplasm. Hyperpolarization under these conditions can also be facilitated by reducing G_K, either by decreasing K^+ concentration, clamping the membrane to a more negative value, or both. The relationship between G_K and the control of the electrogenic pump is not simple, and needs to be further elucidated.

The suggestion that the pump may actually be nonfunctional after long incubation in Ca^{2+}-free medium is open to the criticism that a functional pump is required to control cytoplasmic pH, and without it, pH^i should decrease. With ψ_m at −90 mV, pH^i should decrease to 1.5 units less than the outside, or pH 6, which seems uncomfortably low for a cytoplasmic value (Smith & Raven, 1979). Since the proton permeability is lowered (*see* Results and *below*), the influx of protons should be mitigated, but it is not likely that it would be reduced to zero. To my knowledge, the value of pH^i has not been measured in the presence and absence of Ca^{2+}.

The *Chara* membrane may exhibit high H^+ permeability. [No electrical experiments have been able to distinguish between H^+ permeability and OH^- permeability, although arguments can be made for either ion permeating the membrane (Lucas, 1979; Bisson & Walker, 1980, 1981). The reader is admonished to keep in mind that wherever P_H and H^+ fluxes are invoked, P_{OH} and OH^- fluxes in the opposite direction could equally be employed, and would give equivalent results.] The permeability may be induced by subjecting the cell to high external pH, but it appears also to exist in the alkaline bands which cells are capable of generating in the light in unbuffered solution (Chilcott et al., 1983). This high passive permeability to H^+ is also inhibited in the absence of Ca^{2+}. The increase in P_H at high pH is not entirely abolished in the absence of Ca^{2+}, as can be seen from the increase in G_m ($\Delta G > 0$, Table 1, Fig. 6) and from the fact that the cell does hyperpolarize somewhat at pH 12 even in the absence of Ca^{2+} (Fig. 5). Although passive proton permeation and active proton pumping respond similarly to DCCD (Bisson & Walker, 1981), they respond differently to the removal of Ca^{2+}. The pump is slow to respond to removal and to restoration of Ca^{2+}, and Mg^{2+} will not substitute for Ca^{2+}. P_H, like P_K, is quickly restored when either Ca^{2+} or Mg^{2+} is added back. Two hypotheses could explain the similarity of the two effects of Ca^{2+} on the two passive transport processes. One is that both result from charge-screening by divalent cations. The other is that one effect depends on the other, e.g., that high P_H cannot be induced when P_K is high. Ordinarily, a hyperpolarization due to a slight increase in P_H would result in a decrease in P_K, due to the voltage sensitivity of the latter. The decrease in P_K would result in further hyperpolarization, perhaps resulting in a positive feedback effect, resulting in the coordination of H^+-channel opening. Since the response of P_K to hyperpolarization is diminished and slowed in the absence of Ca^{2+}, such facilitation of channel opening would not be present.

In summary, I find two distinct effects of Ca^{2+} on membrane properties. One effect is rapid, nonspecific, and acts on passive H^+ and K^+ permeabilities. The other is slow, specific, and may be acting directly on the H^+ pump.

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