Plant Physiol. (1986) 81, 55–59
0032-0889/86/81/0055/05/$01.00/0

Inhibitors of Proton Pumping

EFFECT ON PASSIVE PROTON TRANSPORT

Received for publication October 8, 1985 and in revised form January 17, 1986

MARY A. BISSON
Department of Biological Sciences, State University of New York at Buffalo, Buffalo New York 14260

ABSTRACT

Reported inhibitors of the Characean plasmalemma proton pump were tested for their ability to inhibit the passive H^+ conductance which develops in *Chara corallina* Klein ex Willd. at high pH. Diethylstilbestrol inhibits the proton pump and the passive H^+ conductance with about the same time course, at concentrations that have no effect on cytoplasmic streaming. *N*-Ethylmaleimide, a sulfhydryl reagent which is small and relatively nonpolar, also inhibits both pumping and passive conductance of H^+. However, it also inhibits cytoplasmic streaming with about the same time course, and therefore could not be considered a specific ATPase inhibitor. *p*-Chloromercuribenzene sulfonate (PCMBS), a sulfhydryl reagent which is large and charged and hence less able to penetrate the membrane, does not inhibit pumping or conductance at low concentration. At high concentration, PCMBS sometimes inhibits pumping without affecting H^+ conductance, but since streaming is also inhibited, the effect on the pump cannot be said to be specific. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide, a water soluble carbodiimide, weakly inhibits both pump and conductance, apparently specifically.

Under normal conditions at neutral pH, the membrane potential (E_m)[1] of charophytes is dominated by a proton pump at the plasmalemma which generates a highly negative potential (21). This negative potential serves as a driving force for the accumulation of cations such as ammonium (22). The pump generates a large electrochemical potential gradient for protons ($\Delta\bar{\mu}_H$), which serves as the driving force for the symport of such transported ions as Cl^- (4, 17).

At high pH, either imposed by high pH buffers or generated by the cell itself in the 'alkaline bands' (see below), the membrane potential is dominated by passive H^+ (or OH^-) conductance (G_H or G_{OH}), characterized by a dramatic increase in electrical conductance of the membrane (G_m) and a membrane potential near the equilibrium potential for protons (or hydroxyls: $E_H = E_{OH}$). In a banding internodal cell, regions of the membrane which pump protons alternate with proton conducting regions. This mosaic membrane results in two distinct domains along the cell: those which export acid ('acid band') and generate a high $\Delta\bar{\mu}_H$, powering cotransport systems; and those which import acid (alkaline band), have $\Delta\bar{\mu}_H$ close to zero, and precipitate $CaCO_3$. The functions of this banding system have not been well elucidated, but it is associated with photosynthetic uptake of CO_2 from relatively bicarbonate-rich (*e.g.* nonacid) aqueous medium (16, 23).

This situation, where proton-pumping and proton-conducting regions of membrane are contiguous, presents the questions of how these regions of membrane maintain their distinct transport properties. One possibility is that the membrane components responsible for the different functions are discretely distributed along the length of the membrane; the complementary possibility is that the components are uniformly distributed but the control of their functioning is different in the different bands.

Previous studies have looked at agents which affect the function of the proton pump or proton conductance, such as darkness (6), Ca^{2+} (5), and DCCD (8), to see whether these functions are linked, that is, whether inhibition of one function inhibits the other. This study examines several inhibitors. DES has been used as an ATPase inhibitor (13, 18), but it may also affect ATP levels (14). Sulfhydryl reagents were chosen because of indications that H^+ pumping was inhibited by the small, relatively nonpolar, and presumably permeant NEM but not by the large, charged, and presumably impermeant PCMBS (15). A water soluble carbodiimide, EDAC, was also chosen for study, because of its similarity to DCCD, and because of a report that the nature of its effect on E_m was unclear (13).

These four inhibitors, DES, NEM, PCMBS, and EDAC were tested on both proton pumping and proton conductance. In addition, the effect of all inhibitors on cytoplasmic streaming was noted. Cytoplasmic streaming is slowed by a decrease in ATP or an increase in Ca^{2+} concentration in the cytoplasm (19, 25). An effect of a given inhibitor on streaming indicates that the inhibitor cannot be assumed to be acting specifically on the pump, because there could be an effect on energy sources or a nonspecific effect on membrane function, involving an increase in permeability to Ca^{2+} or decreased ability to sequester or extrude Ca^{2+}.

MATERIALS AND METHODS

Algae were cultured and electrophysiological experiments done as described previously (5, 7), except that light intensity was 25 to 40 μmol m^{-2} s^{-1}, instead of the value incorrectly reported previously (7). Electrophysiological experiments were performed in the light in the presence of 2 mM K^+, a slightly elevated concentration which makes E_K (−104 mV) significantly more positive than E_H at pH 11 (−180 mV) and the electrogenic potential at pH 7.5 (more negative than −200 mV). In contrast to reports elsewhere (3), in my hands this never resulted in loss of hyperpolarization at neutral pH. Membrane conductance was measured by first clamping the membrane to a given potential (−150 mV unless otherwise noted); once a steady state current was obtained, a bipolar staircase of command voltages was imposed to generate a current voltage curve. I-V curves were run at one clamp potential, regardless of the spontaneous (unclamped

[1] Abbreviations: E_m, membrane potential; E_j, equilibrium potential for ion j; G_m, membrane conductance; G_j, specific conductance for ion j; $\Delta\bar{\mu}_H$, electrochemical potential for H^+; DCCD, dicyclohexylcarbodiimide; DES, diethylstilbestrol; NEM, *N*-ethyl maleimide; PCMBS, *p*-(chloromercuri)benzene sulfonate; EDAC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

or open-circuit) E_m, because of the well documented voltage-dependence of the membrane conductance (5, 12, 20). These I-V curves were generally linear around the steady state clamp potential; if not, the tangent at the steady state potential was selected as measure of G_m.

For DES and NEM, stock solutions were made up in ethanol and added to the medium just before use. Ethanol in the medium did not exceed 0.1%, at which concentration the ethanol alone has no effect on membrane properties. To avoid contamination by free Hg^{2+}, PCMBS was purified according to Will and Hopfer (24) just before use, with concentration determined using the extinction coefficient at 265 nm.

RESULTS

For each of the inhibitors, E_m and G_m were measured at pH 7.5 and pH 11. (Cells remained at pH 7.5 most of the time, and were in pH 11 only the minimum time necessary to make the measurements, about 2 min.) In each case, a depolarization of the membrane at pH 7.5 is taken to indicate an inhibition of the proton pump (see "Discussion"). A decrease in G_m at pH 11 indicates a decrease in G_H, although in most cases E_m does not change at pH 11, presumably because G_H is still greater than any other conductance (9). Nonspecific effects on ATP concentrations and/or membrane permeability to Ca^{2+} are indicated by a cessation in streaming. Effects on G_K are indicated by alterations in the G_m and K^+-dependence of E_m or clamp current. Effects on the voltage-dependence of G_K are indicated by clamping E_m to different values and measuring G_m at each value.

DES. Twenty μM DES causes E_m at pH 7.5 to become gradually less negative, eventually becoming more positive than E_K, as shown for a typical experiment in Figure 1. This suggests that the pump is being inhibited. G_m at pH 11 decreases with the same time course indicating simultaneous decrease in G_H. There is no change in the rate of cytoplasmic streaming even when inhibition is complete, indicating that the effect is relatively specific. Average values of streaming rate and of E_m and G_m at the two values of pH are shown in Table I.

DES does have an effect other than the effect on proton pumping and proton conductance. When E_m becomes less negative at pH 7.5, it alters with the external K^+ concentration, but only by 22 mV/10× concentration change. This is considerably less than what would be expected if $G_m = G_K$, that is, if $E_m = E_K$, and indicates an influence by other permeant ions. At pH 11, E_m does not depend on K^+ concentration (data not shown), which is typical of control cells (9). The voltage-dependence of G_m at pH 7.5 is also affected by DES, as shown by Figure 2. For cells depolarized by other means (10; see also Figs. 4 and 7), G_m is high at potentials positive to about −125 mV, and decreases dramatically at more negative values. In the presence of DES, the conductance remains low at all potentials (Fig. 2), except for a variable tendency for an increase at potentials more positive than the spontaneous potential. This increase may not be due to the K^+ channel opening, but may indicate an opening of other channels, such as a nonspecific leak or the Cl^- channel (although this has been reported to be inhibited by DES [1]). Thus, we can conclude that in the presence of DES, the voltage-sensitivity of

Table I. *Effect of 20 μM DES on Cytoplasmic Streaming and on Membrane Potential and Conductance at Neutral and Alkaline pH*

ΔG_m is the increase in conductance when cells are subjected to pH 11 (G_m at pH 11 minus G_m at pH 7.5). Cells were in solutions containing DES for 40 to 130 min; recovery in DES-free medium required 75 to 300 min. Data given as mean ± SE (n). Data for 8 cells. $n > 8$ indicates multiple measurements on individual cells.

	E_m	G_m	Streaming Velocity
	mV	$S\ m^{-2}$	$\mu m\ s^{-1}$
pH 7.5			
Control	−209 ± 4.8 (12)	1.29 ± 0.24 (12)	59 ± 6.8 (6)
+DES	−82 ± 4.7 (8)	0.33 ± 0.31 (8)	64 ± 3.5 (7)
Recovery	−198 ± 13 (7)	0.82 ± 0.26 (7)	59 ± 5.7 (7)
			$\frac{\Delta G_m}{S\ m^{-2}}$
pH 11			
Control	−167 ± 1.6 (8)	5.02 ± 1.53 (8)	3.83 ± 1.51 (8)
+DES	−167 ± 2.1 (8)	1.16 ± 0.30 (8)	0.82 ± 0.29 (8)
Recovery	−165 ± 2.8 (8)	3.17 ± 0.90 (8)	2.35 ± 0.81 (8)

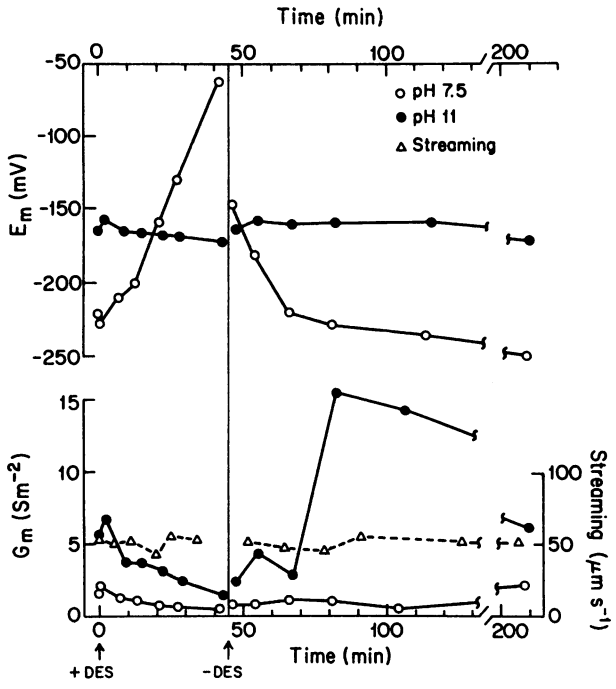

FIG. 1. Time course of effect of 20 μM DES on membrane potential (E_m) and on conductance (G_m) at pH 7.5 (○) and pH 11 (●). Also shown is the effect on cytoplasmic streaming (△). Typical time course from single cell.

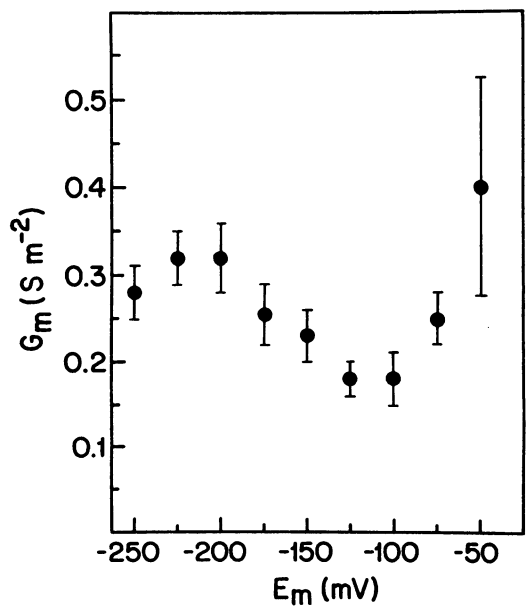

FIG. 2. Membrane conductance as a function of membrane potential in the presence of 20 μM DES at pH 7.5. Average ± SE; $n = 5$.

the K^+ channels is impaired, so that they do not open at normal potentials. This is similar to the conclusions of Beilby (2). She shows no increase in conductance at less negative potentials, as shown here, but her plots of conductance as a function of potential are generated from rapid I-V curves, while those presented here represent steady state conductance at each potential. Beilby (2) does say that conductance increases at less negative potentials with time.

Sulfhydryl Reagents. Ten μM NEM also results in depolarization of the cell at pH 7.5 and decreased G_m at pH 11 (Fig. 3; Table II). However, there are two significant differences between this effect and that of DES. First, cytoplasmic streaming is also inhibited, and with about the same time course (Fig. 3). This indicates that the effect may not be specific, but could be exerted on aspects of cell activity other than the pump itself; 100 μM NEM depolarizes the cell within seconds, but also stops streaming simultaneously (data not shown), so that the two effects are not separable. The second difference between NEM and DES is that the effect is not readily reversible (Fig. 3), even if 1 mM DTE is added when NEM is washed out. However, when cells are treated with NEM for 2 h, then placed in DTE for 24 to 48 h, membrane function recovers (Table II).

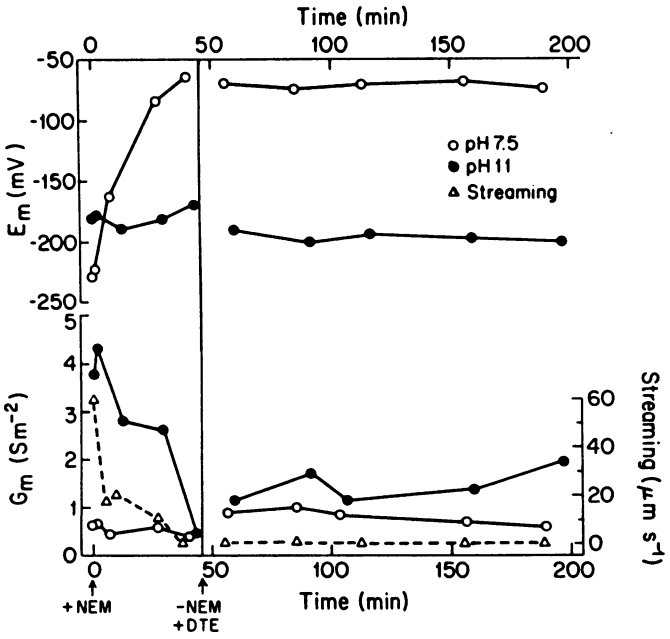

FIG. 3. Time course of the effect of 10 μM NEM on membrane potential and conductance at pH 7.5 (O) and pH 11 (●). At 47 min NEM is replaced by 1 mM DTE. Also shown is the effect on cytoplasmic streaming (Δ). Typical time course from single cell.

Table II. *Effect of 10 μM NEM on Streaming and on Membrane Potential and Conductance at Neutral and Alkaline pH*

ΔG_m is the increase in conductance when cells are subjected to pH 11 (G_m at pH 11 minus G_m at pH 7.5). Cells were in solutions containing NEM for 10 to 70 min. Recovery in the presence of 1 mM DTE required 24 to 48 h. Control and +NEM represent the same cells. Recovery represents different cells from the same batch (see text). Mean ± SE (n).

	E_m	G_m	Streaming Velocity
	mV	$S\,m^{-2}$	$\mu m\,s^{-1}$
pH 7.5			
Control	−235 ± 3.5 (5)	1.01 ± 0.14 (5)	62 ± 3 (5)
+NEM	−65 ± 3.7 (5)	0.59 ± 0.082 (5)	0 ± 0 (5)
Recovery	−209 ± 10 (7)	0.58 ± 0.062 (6)	58 ± 5 (6)
			$\frac{\Delta G_m}{S\,m^{-2}}$
pH 11			
Control	−174 ± 2.6 (5)	12.6 ± 3.1 (5)	11.6 ± 3.1 (5)
+NEM	−181 ± 4.9 (5)	1.40 ± 0.39 (5)	0.84 ± 0.34 (5)
Recovery	−173 ± 3.9 (7)	10.8 ± 2.3 (6)	10.2 ± 2.3 (6)

In the presence of NEM, E_m shows a strong K^+-dependence (49 mV/decade). The dependence of clamp current on K^+ concentration (68 mamp m^{-2}/decade), a more sensitive indication of G_m, is similar to that seen when the cell is depolarized by other methods (5). The dependence of G_m on E_m is more typical (Fig. 4), although some decrease in G_m at less negative potentials is seen after treatment with DTE.

Ten μM PCMBS does not inhibit either E_m or G_m at any pH (data not shown). At 100 μM, PCMBS occasionally has some effect after long exposure. This effect is quite variable: one cell showed no effect, one cell showed an inhibition of hyperpolarization at pH 7.5 and a decrease in G_m at pH 11, and two cells showed an inhibition of hyperpolarization at pH 7.5 without any effect on G_m at pH 11. All cells which depolarized at pH 7.5 also showed a slowed or stopped streaming rate, so the effect cannot be said to be specific to the proton pump. Moreover, at long incubation times in PCMBS, some Hg^{2+} could be released and could be the cause of these effects.

EDAC. One mM EDAC slowly inhibits E_m, but does not reduce it completely to the passive diffusion potential (Fig. 5). This is similar to the findings of Keifer and Spanswick (13). It also decreases G_m at pH 11, with about the same time course, and has no effect on streaming. The effects are summarized in Table III. The summary of all pH 11 G_m data indicates that the decrease with EDAC is not significant. However, of the 7 cells studied, 6 showed a marked decrease in G_m at pH 11, while one showed a marked increase, to 15 S m^{-2}. Since such an increase in measured conductance can be due to microelectrode plugging or exclusion from the cytoplasm, this value was eliminated from the analysis. When this is done, EDAC is seen to have a significant ($P < 0.05$) effect on G_m at pH 11. The decrease in streaming rate is not significant ($P > 0.1$).

The smooth, monotonic change in E_m at pH 11 shown in Figure 5 is somewhat misleading. Generally, cells respond to a change from pH 11 to pH 7.5 by an action potential (8). In most cells, recovery from the action potential to the hyperpolarized potential is rapid and spontaneous. With longer exposures to EDAC, this recovery is slower; what is plotted in Figure 5 is the most negative value achieved after the action potential. Figure 6

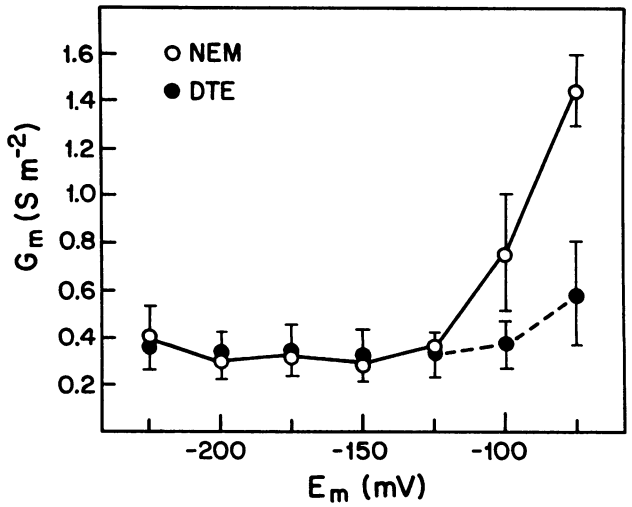

FIG. 4. Potential dependence of G_m in 10 μM NEM (O) and immediately following NEM treatment in the presence of 1 mM DTE (●). Average ± SE; n = 4 to 6.

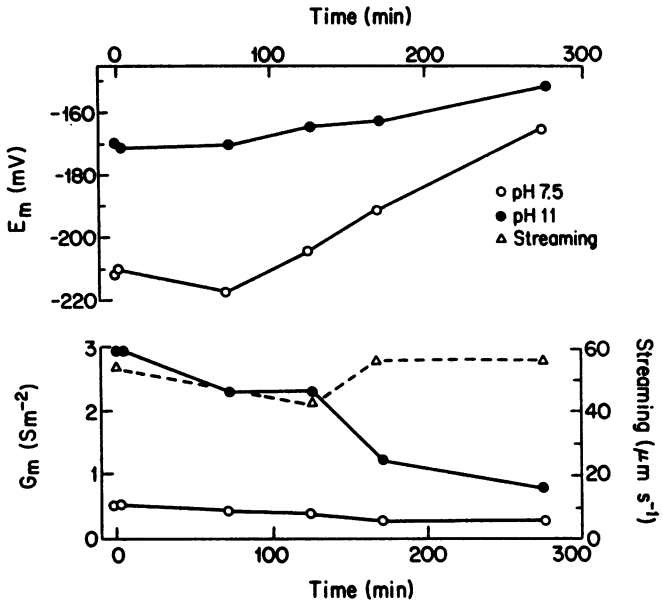

FIG. 5. Time course of the effect of 1 mM EDAC on membrane potential and conductance at pH 7.5 (●) and pH 11 (○). Typical time course from single cell.

shows the recovery from successive action potentials in a single, representative cell. Eventually E_m recovers only to the passive diffusion potential. However, when such a cell is clamped to −150 mV for measurement of G_m, the current needed to maintain the clamp declines slowly to zero. When the clamp is released, such a cell remains at −150 mV, and will slowly hyperpolarize further to a plateau value. With longer exposures to EDAC, the time required for the clamp current to diminish to zero increases from a few seconds to about 60 min, and the plateau becomes less negative. It may be that with time the cell would not hyperpolarize past the diffusion potential even with a long hyperpolarizing clamp, but the experiments were never taken out that long. However, after a long time in EDAC, cells would remain depolarized long enough to measure G_m as a function of E_m and to study E_m and clamp current as a function of K^+ concentration while the cell remained dominated by K^+ conductance. The depolarized values of E_m are given in Table III. E_m changed by 41 mV/10× K^+ concentration change, while clamp current changed by 65 mamp m^{-2}/decade. Dependence of G_m on E_m was typical (Fig. 7).

When EDAC was removed from solution, E_m at neutral pH began to go immediately but slowly more negative. Because of the length of the experiments, however, they were not carried out to full recovery.

DISCUSSION

The use of inhibitors in intact cells is fraught with hazards. Depolarization of an electrogenic potential may be due not only to an inhibition of an electrogenic pump, but also to an increase in conductance of a passive transport process. In the past, G_m has been used as a measure of the two effects: inhibiting the pump should decrease G_m, while increasing passive conductance should increase it. However, it is now known that in *Chara* the K^+ conductance is voltage-dependent (5, 12, 20). Thus, a depolarization of the cell will *ipso facto* result in an increase in G_K, even though the initial effect may be on the pump itself. To control for this, I measured all values of G_m at one clamped potential, −150 mV. If the initial effect of the inhibitor is to increase G_K, this should be evident in an increased G_m even when the cell is hyperpolarized by the voltage clamp. This difference in measuring conductance at different membrane potentials can explain some differences with previous authors (*e.g.* Ref. 13) who found increases in G_m as the cell depolarized when this study did not. However, it does not explain why the increase in G_m which Keifer and Spanswick (13) found with EDAC was irreversible; if it was due to the voltage-dependence of the K^+ channels, it should have been reversed as the cell repolarized. There is no obvious explanation for the differences in our results here. In my hands, all the inhibitors which depolarize the cell at neutral pH also decrease G_m at that pH. Recovery to the more negative potential is accompanied by a partial recovery in G_m with DES, but not with NEM.

Even if an inhibitor effect is known to be on the electrogenic pump rather than G_K, the effect may be indirect, *e.g.* on the energy source or on some controlling factor. These kinds of nonspecific effects were monitored by measuring the cytoplasmic streaming rate, which decreases with decreased ATP. A high streaming rate indicates that ATP is not decreased, although a decreased streaming rate could indicate decreased ATP, an increase in cytoplasmic Ca^{2+}, or an effect on the streaming mechanism itself. Using this criterion, DES and EDAC appear to have their effects while ATP remains high. Keifer and Spanswick (14) found that DES inhibited ATP, but they were using higher concentrations than are employed here; they also found EDAC to have no effect on ATP. Inhibition by NEM or PCMBS is always accompanied by an inhibition of streaming. Since Keifer and Spanswick (14) found no effect on ATP, it may be that the effect on streaming is due to a direct effect on the streaming mechanism itself, which is known to be sensitive to NEM (11). It is also possible that the effect is via an alteration in cytoplasmic

Table III. *Effect of 1 mM EDAC on Cytoplasmic Streaming and on Membrane Potential and Conductance at Neutral and Alkaline pH*

ΔG_m is the increase in conductance at pH 11 (G_m at pH 11 minus G_m at pH 7.5). Cells were in solutions containing EDAC for 140 to 400 min. Data given as mean ± SE (*n*). Data for 5 to 7 cells.

	E_m	G_m	Streaming Velocity
	mV	$S\ m^{-2}$	$\mu m\ s^{-1}$
pH 7.5			
Control	−224 ± 5.3 (7)	1.2 ± 0.18 (7)	53 ± 2.6 (7)
+ EDAC hyperpolarized	−168 ± 4.0 (7)	0.37 ± 0.10 (7)	47 ± 5.9 (6)
+EDAC depolarized	−78 ± 6.6 (5)	0.38 ± 0.06 (5)	
			$\frac{\Delta G_m}{S\ m^{-2}}$
pH 11			
Control	−168 ± 3.2 (7)	5.5 ± 0.87 (7)	4.3 ± 0.90 (7)
+ EDAC	−156 ± 7.3 (7)	3.5 ± 2.0 (7)	3.1 ± 1.97 (7)
+ EDAC[a]		1.6 ± 0.46 (6)	1.2 ± 0.48 (6)

[a] Represents same data in previous row, but with suspect outlying point removed (see text).

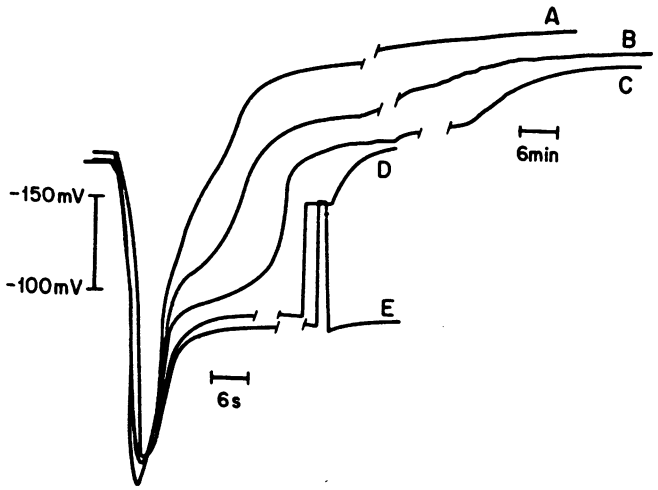

FIG. 6. Recovery from action potentials at different times after the addition of 1 mM EDAC. Trace A, 4 min (essentially identical to control before EDAC); trace B, 103 min; trace C, 259 min; trace D, 355 min (note clamp which results in a hyperpolarization); trace E, 383 min (note clamp which does not result in a hyperpolarization). Traces from a single, typical cell.

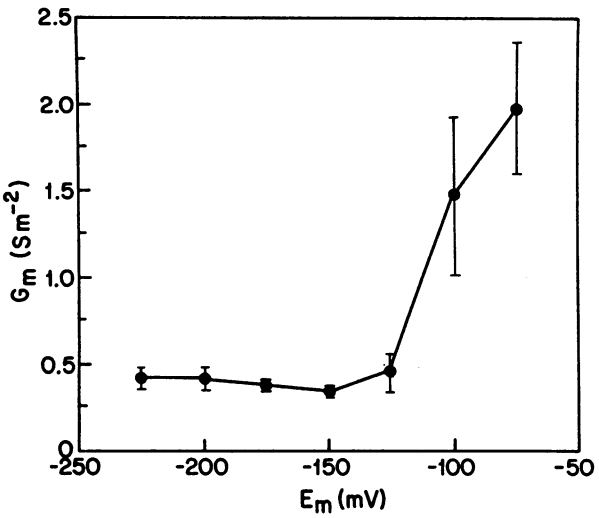

FIG. 7. Potential dependence of G_m in 1 mM EDAC. Average ± SE; n = 6.

Ca^{2+}, but this is less likely because the effect of 100 μM NEM is so rapid. Although one cannot conclude that NEM does not have a direct effect on the pump, nonetheless the cessation of streaming indicates that this highly reactive molecule has entered the cytoplasm, and may be having many different effects which would affect the pump directly or indirectly.

DES would seem to be the most specific and most effective of the inhibitors studied here. However, it apparently also inhibits K^+ conductance. This is indicated by the decreased dependence of E_m on K^+ concentration (22 mV/10× decade). This could also be due to an increase in conductance to other ions, but since G_m at pH 7.5 is not significantly different in DES as compared to NEM or EDAC (Tables I–III; $P > 0.1$) these two possibilities cannot be distinguished. However, the voltage- dependence of G_m (Fig. 2) indicates that K^+ channels do not open as the cell depolarizes beyond −125 mV, indicating that at least part of the effect is on G_K.

The main goal of this study is to compare the effect of inhibition of active H^+ pumping at neutral pH and H^+ conductance at high pH. This is done by comparing the effect of the inhibitors on E_m at pH 7.5 and on the conductance at pH 11. If we consider only those inhibitors that do not affect streaming, eliminating possible indirect effects, we see that DES and EDAC inhibit E_m at pH 7.5 and G_m at pH 11 with about the same time course. This is similar to the effects of DCCD (9). This suggests that inhibition of the two H^+ transport functions occurs simultaneously. Previous studies showed that darkness and the photosynthesis inhibitor DCMU inhibit G_m at pH 11 without effect on E_m at pH 7.5 (6). Also, removal of Ca^{2+} inhibits both functions, but high G_m at pH 11 is restored rapidly upon addition of Ca^{2+} or Mg^{2+}, while recovery of hyperpolarization at neutral pH occurs only slowly with Ca^{2+}, and not at all with Mg^{2+} (5). These results can be summarized by saying that chemical inhibitors have a similar effect on the two functions, while alteration of possible physiological controls may affect the two independently.

LITERATURE CITED

1. BEILBY MJ 1982 Cl^- channels in *Chara*. Philos Trans R Soc Lond B Biol Sci 299: 435–445
2. BEILBY MJ 1984 Current-voltage characteristics of the proton pump at *Chara* plasmalemma. I. pH dependence. J Membr Biol 81: 113–125
3. BEILBY MJ 1985 Potassium channels at *Chara* plasmalemma. J Exp Bot 36: 228–239
4. BEILBY MJ, NA WALKER 1981 Chloride transport in *Chara*. I. Kinetics and current-voltage curves for a probable proton symport. J Exp Bot 32: 43–54
5. BISSON MA 1984 Calcium effects on electrogenic pump and passive permeability of the plasma membrane of *Chara corallina*. J Membr Biol 81: 59–67
6. BISSON MA 1985 The effect of darkness on active and passive transport in *Chara corallina*. J Exp Bot 37: 8–21
7. BISSON MA, D BARTHOLOMEW 1984 Osmoregulation or turgor regulation in *Chara*? Plant Physiol 74: 252–255
8. BISSON MA, NA WALKER 1980 The *Chara* plasmalemma at high pH. Electrical measurements show rapid specific passive uniport of H^+ or OH^-. J Membr Biol 56: 1–7
9. BISSON MA, NA WALKER 1981 The hyperpolarization of the *Chara* membrane at high pH. Effects of external potassium, internal pH, and DCCD. J Exp Bot 32: 951–971
10. BISSON MA, NA WALKER 1982 Control of passive permeability in the *Chara* plasmalemma. J Exp Bot 33: 520–532
11. CHEN JCW, N KAMIYA 1976 Localization of myosin in the internodal cell of *Nitella* as suggested by differential treatment with *N*-ethylmaleimide. Cell Struct Funct 1: 1–9
12. FINDLAY GP, HA COLEMAN 1983 Potassium channels in the membrane of *Hydrodictyon africanum*. J Membr Biol 75: 241–251
13. KEIFER DW, RM SPANSWICK 1978 Activity of the electrogenic pump in *Chara corallina* as inferred from measurements of the membrane potential, conductance, and potassium permeability. Plant Physiol 62: 653–661
14. KEIFER DW, RM SPANSWICK 1979 Correlation of adenosine triphosphate levels in *Chara corallina* with the activity of the electrogenic pump. Plant Physiol 64: 165–168
15. LICHTNER FT, WJ LUCAS, RM SPANSWICK 1981 Effect of sulfhydryl reagents on the biophysical properties of the plasmalemma of *Chara corallina*. Plant Physiol 68: 899–904
16. LUCAS WJ 1983 Photosynthetic assimilation of exogenous HCO_3^- by aquatic plants. Annu Rev Plant Physiol 34: 71–104
17. SANDERS D 1980 The mechanism of Cl^- transport at the plasma membrane of *Chara corallina* I. Cotransport with H^+. J Membr Biol 53: 129–141
18. SCHERER GFE 1984 Subcellular localization of H^+-ATPase from pumpkin hypocotyls (*Cucurbita maxima* L.) by membrane fractionation. Planta 160: 348–356
19. SHIMMEN T, M TAZAWA 1982 Cytoplasmic streaming in the cell model of *Nitella*. Protoplasma 112: 101–106
20. SMITH PT, NA WALKER 1981 Studies on the perfused plasmalemma of *Chara corallina*. I. Current voltage curves: ATP and potassium dependence. J Membr Biol 60: 223–236
21. TAZAWA M, T SHIMMEN 1982 Control of electrogenesis by ATP, Mg^{2+}, H^+ and light in perfused cells of *Chara*. Chap 5. *In* Current Topics in Membranes and Transport, Academic Press, New York, pp 49–67
22. WALKER NA, MJ BEILBY, FA SMITH 1979 Amine uniport at the plasmalemma of charophyte cells. I. Current-voltage curves, saturation kinetics, and effects of unstirred layers. J Membr Biol 49: 21–55
23. WALKER NA, FA SMITH, IR CATHERS 1980 Bicarbonate assimilation by freshwater Charophytes and higher plants. I. Membrane transport of bicarbonate ions not proven. J Membr Biol 57: 51–58
24. WILL PC, U HOPFER 1979 Apparent inhibition of active non-electrolyte transport by an increased sodium permeability of the plasma membrane. Mechanism of action of *p*-chloromercuribenzene sulfonate. J Biol Chem 254: 3806–3811
25. WILLIAMSON RE, CC ASHLEY 1982 Free Ca^{2+} and cytoplasmic streaming in the alga *Chara*. Nature 296: 647–651