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Sodium efflux from perfused giant algal cells

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Abstract. Internodal cells of the giant alga *Chara corallina* were perfused internally to replace the native cytoplasm, tonoplast and vacuole with artificial cytoplasm. Sodium efflux from perfused cells, measured by including ^{22}Na in the perfusion media, was increased by increasing the internal sodium concentration and by decreasing the external pH, and was inhibited by external application of the renal diuretic amiloride. The sodium efflux was markedly ATP-dependent, with a 50-fold decrease in efflux observed after perfusion with media lacking ATP. Efflux in the presence of ATP was reduced by 33% by inclusion of 10 μM N,N'-dicyclohexylcarbodiimide in the perfusion medium. The membrane potential of the perfused cells approximated that of intact cells from the same culture. It is suggested that sodium efflux in perfused *Chara* cells proceeds via a secondary antiporter with protons, regulated by ATP in a catalytic role and with the proton motive force acting as the energy source.

Key words: *Chara* (Na^+ efflux) – Membrane transport – Sodium efflux.

Introduction

The study of ionic and osmotic relations in plants has, in the past, relied on the use of experimental systems in which physical parameters such as ion fluxes and membrane potential are measured in situations where the chemical and environmental conditions on one side of the membrane can be controlled – chosen, within limits – and held constant. This approach has allowed us to establish, in several systems, the nature of the transport processes which exist and their roles in the maintenance of ion balance or in osmoregulation, but has, as yet, contributed little towards an understanding of the molecular basis of membrane transport phenomena, or how such processes are regulated. Progress by plant biologists towards this end has been hampered by the dearth of suitable experimental systems. One system which has been developed, however, is the internally perfused giant algal cell, and this should allow the investigation of membrane transport to proceed one stage further: towards the analysis of the kinetics of individual transport proteins.

It is now more than twenty years since the first reports were received from Japan that these giant cells could be perfused internally and remain alive (Tazawa 1964). The technique requires the severing of the ends of the cell under conditions of reduced turgor followed by perfusion with new media to replace either the vacuolar sap alone, or the cytoplasm, tonoplast and vacuolar sap. The cell is then ligated and allowed to regain turgor. If care is taken during the physical manipulation of the cell and if perfusion media are prepared which perform adequately as artificial cytoplasm or vacuolar sap, then the perfused cells remain healthy for extended periods (between 10 h and several days, depending on the severity of the treatment).

So far, the perfusion technique has been used extensively to investigate the factors affecting cytoplasmic streaming (Tazawa et al. 1975, 1976), and for electrical work (Tazawa et al. 1976; Shimmen and Tazawa 1977; review by Tazawa and Shimmen 1982), but very little flux work has been done. Takeshige, Shimmen and Tazawa (1985, 1986) have shown that ATP-dependent H^+ efflux in *Nitellopsis obtusa* can account, quantitatively, for the electrogenic pump current identified by electrical work, thus providing firm evidence for the postulated role of a proton-translocating ATPase in the electrophysiological characteristics of the plasma-

Abbreviations: DCCD = N,N'-dicyclohexylcarbodiimide; EGTA = ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; Mes = 2-(N-morpholino)ethanesulphonic acid; Mops = 3(N-morpholino)propanesulphonic acid; Taps = tris(hydroxymethyl)methylaminopropanesulphonic acid

lemma. The technique has also been used in the study of active chloride uptake by *Chara corallina*, by measurement of $^{36}Cl^-$ fluxes (Sanders 1980b; Reid and Walker 1984), and the detailed analysis of such flux measurements led to the proposal of a $2H^+:1Cl^-$ cotransport as the mechanism of the active chloride influx (Sanders and Hansen 1981).

The value of this experimental system, and related techniques, is that it is now possible to gain access to, and control conditions at, both sides of both the plasmalemma and the tonoplast, and investigate transport phenomena at each of these membranes separately.

The work described in this present paper, involves the use of tonoplast-free perfused cells of *Chara corallina* in an investigation of the transport of sodium across the plasmalemma, using tracer flux studies with $^{22}Na^+$. It has been argued for other plant cells that a Na^+/H^+ exchange system is involved in the active efflux of Na^+ at the plasmalemma (Ratner and Jacoby 1976; Jeschke 1979). In this case "uphill" Na^+ efflux is coupled to the "downhill" movement of protons, and the necessary proton gradient is set up and maintained by the primary active transport at the plasmalemma, the proton-translocating ATPase. There is direct evidence of Na^+/H^+ antiport in tonoplast vesicles (Blumwald and Poole 1985), powered by the proton graident set up by the proton-translocating ATPase in the tonoplast membrane (Bennett et al. 1984). Direct evidence for the proposal of Na^+/H^+ antiport in the plasmalemma is much needed. In the present work the sodium efflux is measured as a function of imposed gradients of pH and of sodium concentration across the plasmalemma. The effects on sodium efflux of treatment with the renal diuretic amiloride [a specific inhibitor of Na^+/H^+ antiport and of Na^+-channel activity in some animal systems (Cuthbert and Shum 1976; Benos 1982)], of internal ATP concentration, and of internal treatment with N,N′-dicyclohexylcarbodiimide (DCCD) are also presented. The hypothesis that sodium efflux proceeds via a Na^+/H^+ antiport is reviewed in the light of the flux measurements made.

Material and methods

Plant material. Internodal cells of *Chara corallina* were taken from cultures grown on the roof of the Botany School, Cambridge in large tanks which were provided with additional heating to ensure a minimum water temperature of 15° C. Cells having diameters of between 0.6 and 1.1 mm and lengths of between 5 and 12 cm were used. Care was taken to avoid those cells which had pronounced calcification of the cell wall, since in these cases ligation was often unsuccessful.

Table 1. Calculated free EGTA, ATP, Mg and Mg-ATP concentrations in perfusion media

	Total (mM)	Free (mM) pH 7.2	pH 7.7
EGTA	5	3.47	1.89
ATP	2	0.019	0.017
Mg^{2+}	12	8.5	6.92
Mg-ATP		1.97	1.97

Perfusion media. Perfusion media for tonoplast-free perfusion contained the following: 5 mM ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA); 15 mM KCl; 5 mM K_2SO_4; 10 mM $MgSO_4$; 2 mM Mg-ATP; 5% Ficoll (dialysed); 50 mM 2-(N-morpholino)ethanesulphonic acid (Mes); 65 mM KOH, pH 7.7. In addition, media contained between 1 and 20 mM Na^+ added as Na_2SO_4, and between 120 and 148 mM sorbitol (depending on the concentration of Na^+ employed) so that the osmotic pressure approximated that measured for intact cells and could be held constant whilst the concentration of Na^+ was varied. Perfusion media at pH 7.2 were obtained by back-titrating media at pH 7.7 using Mes. This required the addition of a further 4.5 mM Mes but in other respects the composition of perfusion media at pH 7.2 was identical to those at pH 7.7. Calculations were made of the free EGTA, ATP, Mg and Mg-ATP concentrations in perfusion media using the dissociation constants and equations given by Fabiato and Fabiato (1979). These are listed in Table 1.

External media. Prior to an experiment, cells were incubated overnight in artificial pond water (0.1 mM KCl, 0.1 mM NaCl, 0.5 mM $CaCl_2$, 2 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (Hepes) adjusted to pH 7.5 with NaOH). After perfusion, cells were incubated in Na-free artificial pond waters with sorbitol (APW-S) at three different values of pH, having the following composition: 0.1 mM KCl; 1.0 mM $Ca(OH)_2$; 4.0 mM buffer [Mes: pH 6.2, 3-(N-morpholino)propanesulphonic acid (Mops): pH 7.2 or 3-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]-amino}-1-propanesulphonic acid (Taps): pH 8.4]; 150 mM sorbitol.

Using 'Good' buffers at or near their pK values it was thus possible to prepare buffered external media having a range of pH values but with constant Ca^{2+}, K^+ and Cl^- concentrations. The addition of 150 mM sorbitol limited the degree of hydrostatic pressure developed by the cell after perfusion and reduced the stress placed upon the ligatures, whilst permitting the cell to regain considerable turgor.

Other chemicals. The renal diuretic amiloride was obtained as the hydrochloride (Sigma Chemical Co., Poole, Dorset, UK) and used at a final concentration of 25 μM in aqueous solution. The inhibitor DCCD (Koch-Light Laboratories, Colnbrook UK) was used at a final concentration of 10 μM from a stock solution of 1 mM in ethanol.

The perfusion procedure. The apparatus used was based on a design by Sanders (1980a) but with modifications, and is illustrated in Fig. 1. The intact internodal cell was carefully blotted and placed on the raised portion of the perspex plinth. Looselytied loops of polyester thread or lightly-waxed dental floss were passed over the ends of the cell (three at each end) before the end champers were placed in position over the ends of the cell. The larger central chamber was then placed in position.

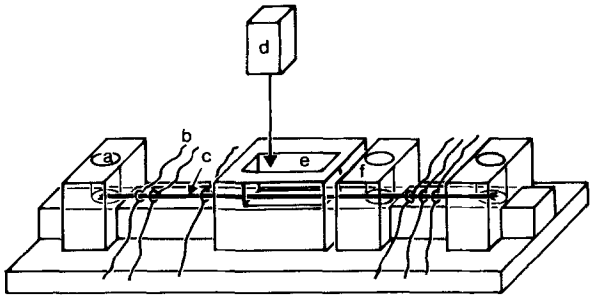

Fig. 1. Diagram of perfusion apparatus. *a*, end chamber containing perfusion medium; *b*, ligation; *c*, cell; *d*, stirrer; *e*, central chamber containing pond water with sorbitol

All chambers were lightly greased with high-vacuum grease (Apiezon L; Apiezon Products, London, UK) before use to ensure a watertight seal. A few drops of perfusion medium were placed in each end chamber, and the plinth was levelled using a small spirit level. The cell was allowed to lose turgor and then the ends of the cell within the small end chambers were removed using small scissors. Perfusion medium was made to flow through the cell either by providing a pressure head at one end by the addition of more perfusion medium, or else by tilting the plinth slightly. Perfusion of the cell was monitored using a binoccular microscope by watching the movement of intracellular particles along the cell. The rate of perfusion measured by these means was about 1 $mm \cdot s^{-1}$ [compared with rates of 4–8 $mm \cdot s^{-1}$ used by Sanders (Sanders 1980a, b) and 0.17–0.33 $mm \cdot s^{-1}$ used by Tazawa and co-workers (Tazawa 1964; Tazawa et al. 1976)]. Perfusion was continued for 2 min, with additions of new perfusion medium to and withdrawal of old medium from the end chambers being made as appropriate. The perspex plinth was then levelled again and the outermost pair of ligatures was pulled tight using flat-ended forceps, with the "downstream" ligature being tied first. External medium (APW-S) was placed immediately in the central chamber, and then the other two pairs of ligatures were tied, working from the outside inwards and with the innermost pair being tied close to the outside walls of the central chamber.

Where perfusion had been successful, turgor was regained by the cell within about 2 min from replacement of the external bathing medium, and cytoplasmic streaming recommenced within 4 or 5 min. Disintegration of the tonoplast has been shown to occur either during the perfusion procedure itself, or within the next 20 min (Tazawa et al. 1976). The success rate of perfusion was found to be increased significantly if the procedure was performed at reduced temperature. In this work a temperature of 15° C was employed, by cooling the apparatus using an aluminium block through which cooled water was circulated.

Measurement of the membrane potential. Membrane-potential measurements were made on perfused cells using external electrodes and the K^+-anaesthesia method similar to that used by Moriyasu et al. (1984). In this case an additional small chamber was placed over the cell before perfusion and the innermost ligation was made close to the outside wall of this chamber. This chamber was filled with 100 mM KCl so that the portion of the cell enclosed within it was depolarised, and the electrical potential recorded between this chamber and the central chamber containing APW-S gave a measure of the membrane potential of the cell. Salt-bridges (100 mM KCl in 2% agar in thin polythene tubing) connected the chambers to calomel electrode half-cells, and potential difference was registered on a high-impedence amplifier (model FD223, electrometer WPI Inc. Newhaven, Conn., USA) and recorded on a chart recorder.

Flux measurements. Efflux of Nat^+ from perfused cells was measured by including ^{22}Na (Amersham International, Amersham, Bucks., UK) in the perfusion medium (185 kBq/ml, 11–22 kBq/μmol), and counting the radioactivity that appeared in the outside medium in a given time. Changes of the external medium were made every 20 min, with a double wash-out procedure to ensure complete replacement of the solution each time. Stirring of the external medium throughout the experiment was effected using a sealed hypodermic needle attached to the shaft of 1.5-V direct-current motor. Each experiment involved measurements on between three and six replicate perfused cells per treatment.

Efflux into Na-free external media was measured to avoid changes in internal specific activity as a result of Na^+ influx. In these conditions the efflux is therefore downhill, although in the intact cell the transport is uphill, requiring an energy input. The efflux measurements started after a recovery time of at least 45 min after perfusion, with several changes of solution in this period. There was therefore no free-space or exchange component in the measured flux. The fraction of cell activity lost during the course of an experiment was small (2–7%), and therefore changes in efflux reflect changes in the activity of the transport system and are not the result of changing Na_i during the period of measurement.

Results

In the first experiments the variability between cells was very great. This was greatly improved by working at 15° C instead of 25° C, and by stirring the outside solution. Although both pH 7.7 and pH 7.2 were used in the internal perfusion medium, most of the experiments in the improved conditions were done using pH_i of 7.2.

The effects of external pH on Na efflux. Figure 2 shows a plot of the rate of tracer sodium efflux against time, for perfused cells having an internal pH of 7.2 and an internal Na concentration of 1 mM, exposed to changes in the pH of their bathing medium. On transfer from pH 7.2 to pH 6.2 (solid lines) there is a rise in the value of the rate constant (and thus in the efflux) which remains constant for subsequent efflux periods at this pH. On transfer back to pH 7.2 the rate constant falls to its original value. On transfer to pH 8.4 the value of the flux falls, but is again restored on transfer of the cells back to pH 7.2. Similar behaviour is exhibited by cells taken through the pH regime in the opposite order, i.e. subjected first to a rise in pH (dotted lines). It should be noted that the absolute values of the rate constants exhibited by replicate cells in the same pH is variable, although the behaviour is not.

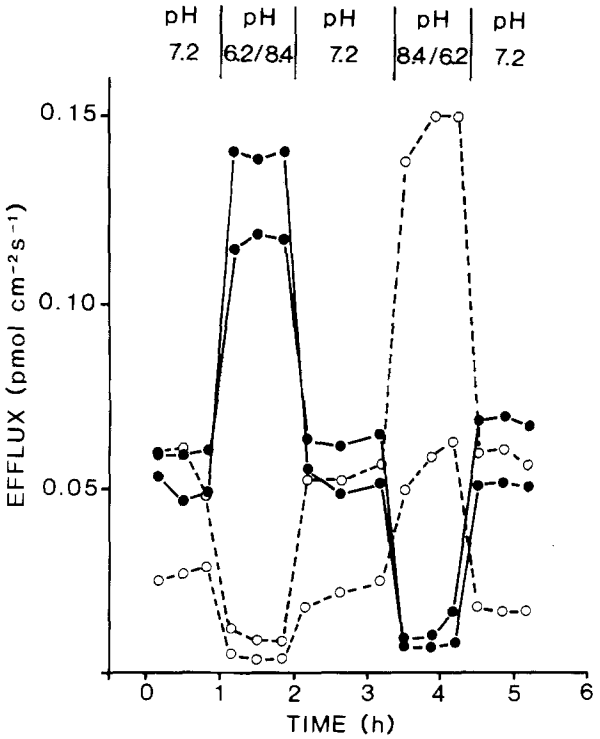

Fig. 2. Plot of sodium efflux against time, from perfused *Chara* cells with internal sodium concentration 1 mM and internal pH 7.2. ●—●, external pH changed in the order 7.2→6.2→7.2→8.4→7.2, at times shown on the graph. o---o, pH changed in the order 7.2→8.4→7.2→6.2→7.2. Each line represents the behaviour of one cell

The effect of internal sodium concentration on sodium efflux. Table 2 summarises the values of the efflux measured as a function of both internal sodium concentration and of external pH. For each pH_0 value considered there is an increase in the Na efflux with increasing internal Na concentration up to about 10 mM $[Na^+]_i$. A double-reciprocal plot of flux against concentration was drawn (data not shown). Since the plot involved the comparison of data from different experiments, and natural variation in the tissue was high, it was not possible to demonstrate unambiguously that a Michaelis-Menten relationship existed. If such a relationship is valid then the present data indicate, for internal pH 7.2, that the K_m for Na efflux is between 0.5 and 5 mM for an external pH of 6.2, with K_m values in the range 2.5–7.0 mM for pH 7.2 and of about 20 mM for pH 8.4.

Table 2 also shows, for comparison, one experiment in which an internal pH of 7.7 was used, with an internal Na^+ of 15 mM. At pH_0 6.2 the Na^+ efflux is somewhat higher at pH_i 7.7 than at pH_i 7.2 but the variability in the group at pH 7.2 is such that the difference is not significant ($0.10 > P > 0.05$); there is no difference at higher external pH.

Table 2. Sodium efflux from perfused *Chara* cells as a function of internal sodium concentration (Na_i) and of external pH (pH_o). Effluxes shown as mean ± SE ($n = 6$)

Na_i (mM)	Na^+ efflux (pmol·cm^{-2}·s^{-1}) at pH_oX		
	6.2	7.2	8.4
Internal pH 7.2			
1	0.09 ±0.02	0.03 ±0.001	0.01 ±0.001
5	0.12 ±0.03	0.07 ±0.01	0.06 ±0.02
10	0.19 ±0.06	0.10 ±0.01	0.07 ±0.02
15	0.18 ±0.05	0.10 ±0.01	0.10 ±0.002
20	0.14 ±0.02	0.10 ±0.01	0.12 ±0.01
Internal pH 7.7			
15	0.29 ±0.03	0.10 ±0.01	0.07 ±0.01

The effect of amiloride on sodium efflux. Figure 3 shows the effects (at pH_i 7.2) of adding 25 μM amiloride to the external solution during the course of an efflux experiment. It can be seen that amiloride induces a marked reduction in the rate constant for efflux, and that this inhibition of efflux is fully reversible. Absolute values for the flux before, during and after treatment with amiloride are given in Table 3 where it can be seen that amiloride approximately halves the efflux.

The ATP requirement of the sodium efflux. The dependence of the flux on internal ATP supply was investigated by comparing control cells (2 mM ATP) with those perfused with ATP-free perfusion medium. From Table 4 it can be seen that Na efflux is drastically reduced in cells perfused without ATP, at internal pH 7.2.

The effect of DCCD on sodium efflux. Table 5 shows the effects of incorporating 10 μM DCCD in the perfusion medium, at pH 7.2, on the value of the sodium efflux. The time of DCCD treatment before the start of the flux measurements was at least 85 min, a time long enough for external DCCD to produce depolarisation in intact cells (Keifer and Spanswick 1978) or in perfused cells (Takeuchi and Kishimoto 1983). Treatment with DCCD in the presence of ATP resulted in a reduction in efflux of about 35% compared with the control value. Cells perfused without ATP did not show a further significant depression of the efflux when treated with DCCD. As in Table 4 it should be noted that conclusions drawn from this section

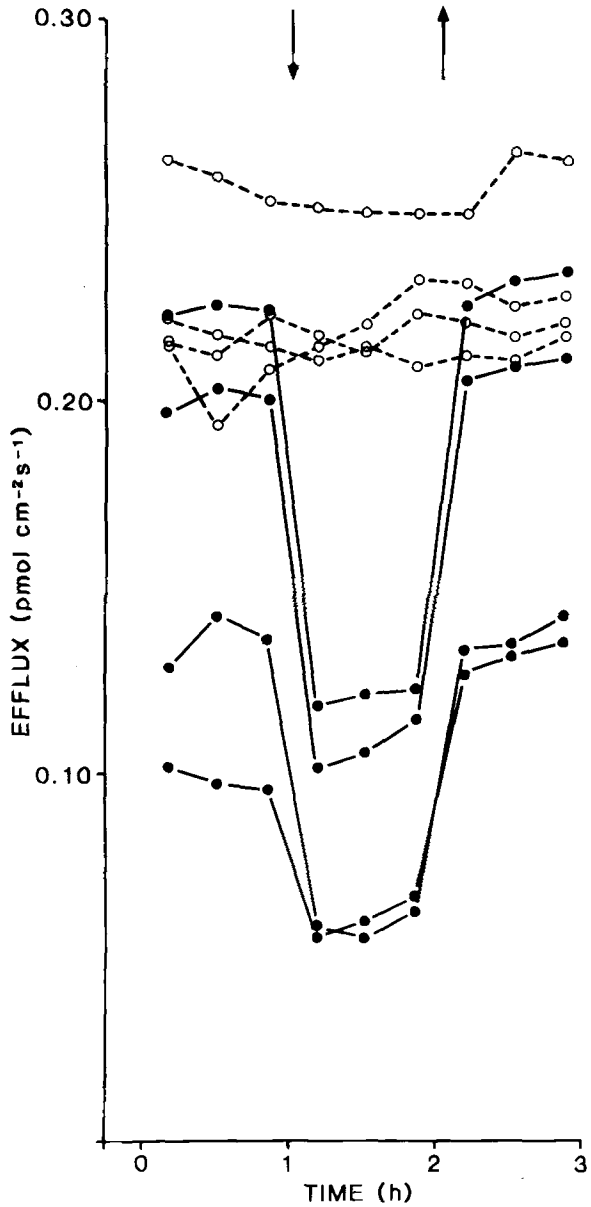

Fig. 3. Plot of sodium efflux against time, from perfused *Chara* cells with internal sodium concentration 10 mM, external pH 6.2, and internal pH 7.2. Amiloride added externally at 25 μM and withdrawn again at the times indicated by the *arrows* on the graph. ●—●, treatment cells; o---o, controls (no amiloride). Each line represents the behaviour of one cell

Table 3. The effect of amiloride on sodium efflux from perfused cells. Internal sodium concentration = 10 mM; external pH = 6.2; internal pH = 7.2; amiloride applied externally at 25 μM. Effluxes shown as mean ± SE (n = 6)

Treatment	Na^+ efflux (pmol·cm^{-2}·s^{-1})
Control	0.16 ± 0.02
1 h in amiloride	0.09 ± 0.01
1 h in amiloride, 1 h washout	0.17 ± 0.01

Table 4. Effect of ATP on sodium efflux from perfused *Chara* cells. Internal sodium concentration = 10 mM; external pH = 6.2; internal pH = 7.2. Effluxes shown as mean ± SE ($n = 6$)

Treatment	Na^+ efflux (pmol·cm^{-2}·s^{-1})
Control (2 mM added ATP)	0.19 ± 0.06
No added ATP	$0.0038 \pm 2.3 \times 10^{-5}$

Table 5. Effect of DCCD on sodium efflux from perfused *Chara* cells. Internal sodium concentration = 10 mM; external pH = 6.2; internal pH = 7.2. The DCCD was used internally at 10 μM; ATP, when present, at 2 mM internally. Efflux shown as mean ± SE (n = 3)

Treatment	Na^+ efflux (pmol·cm^{-2}·s^{-1})
+ATP − DCCD	0.21 ± 0.04
+ATP + DCCD	0.14 ± 0.01
−ATP − DCCD	$0.0052 \pm 2.2 \times 10^{-5}$
−ATP + DCCD	$0.0047 \pm 2.0 \times 10^{-5}$

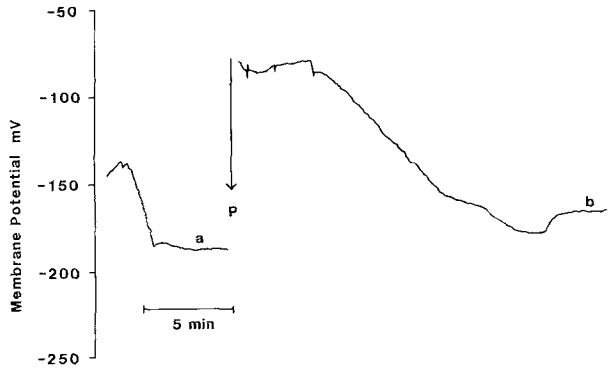

Fig. 4. Typical chart-recorder trace illustrating the behaviour of the membrane potential of the *Chara* cell during the perfusion procedure, as measured using external electrodes and the K^+-anaesthesia method. *Trace a*, steady potential after mounting cell in apparatus but before perfusion; *P*, point at which perfusion takes place; *trace b*, steady membrane potential after disruption of the tonoplast

require the comparison of results obtained from different sets of cells.

Electrical measurements. Figure 4 shows a typical trace of membrane potential of a cell in the first 30 min following perfusion and ligation. After recovery of turgor the value of the membrane potential recovers to a value typical of the membrane potential of intact cells from the same culture. Frequently, a further gradual increase in membrane potential is seen, occurring at about the same time that the tonoplast disintegrates. Thereafter the potential declines slightly, levelling off and becoming steady after about 25 min.

Figure 5 illustrates the dependence of the membrane potential of a perfused cell on the external

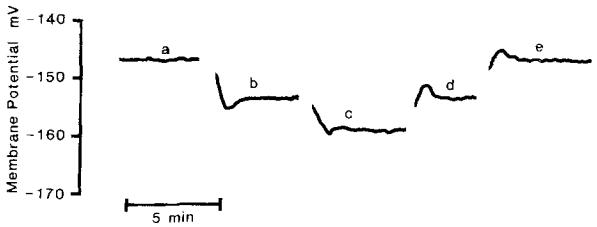

Fig. 5. Typical chart-recorder trace illustrating the behaviour of the membrane potential of perfused *Chara* cells subjected to changes in pH of the outside medium (buffered pondwater plus 150 mM sorbitol). pH changes were as follows: *trace a*, 6.2; *trace b*, 7.2; *trace c*, 8.4; *trace d*, 7.2; *trace e*, 6.2. Internal pH 7.2

pH, for a typical cell 2 h after perfusion and with APW-S externally. A change of potential of some 5 mV per pH unit is common.

The electrical behaviour of the cell during and after perfusion indicates that the plasmalemma has not been damaged by the perfusion procedure.

Discussion

In plant cells, lower eukaryotes and bacteria, sodium is extruded by active transport at the plasmalemma. In these organisms the electrical driving force on protons is in an inward direction as a consequence of the cytoplasm being alkaline and electrically negative with respect to the outside, so that a secondary Na^+/H^+ antiporter could lead to extrusion of Na^+ against its electrical gradient without direct coupling to an energy source, in accordance with Mitchell's hypothesis (Mitchell 1966). Evidence supporting the operation of such a Na^+/H^+ antiporter has been found in barley root tips (Ratner and Jacoby 1976) and in many bacteria, fungi and blue-green algae.

If sodium efflux across the plasmalemma of *Chara* proceeds via a secondary Na^+/H^+ antiport then the following predictions about the response of the sodium flux to experimental treatments can be made:

1) The sodium efflux should be sensitive, at least within some limits, not only to internal and external sodium concentration, but also to external and internal pH.

2) The sodium efflux should be inhibited by a specific inhibitor of the Na^+/H^+ antiporter.

3) No direct coupling to an ATPase should be required.

The perfused cell has allowed us to test these predictions experimentally, and, with respect the first two points listed above, this paper has provided evidence in support of a secondary Na^+/H^+ antiporter (Tables 2, 3). Based on these results alone we cannot, however, conclude firmly that this mechanism operates since we have not demonstrated that there is an uptake of protons by the cell linked to the efflux of Na^+; nor do the results summarised in Tables 2 and 3 rule out the involvement of other efflux mechanisms. Indeed, the operation of other efflux processes may be indicated by the large dependence of the flux upon internal ATP, a fact which conflicts with the third prediction listed above, and whose interpretation needs further discussion.

The drastic reduction in sodium efflux which was observed in perfusion without ATP has several possible explanations. Firstly, the Na^+ flux, rather than mediated by a secondary antiporter, could be dependent on the operation of a Na^+-extruding ATPase. In the bacterium *Streptococcus faecalis*, Harold and Papineau (1972) produced evidence for active Na^+ extrusion by Na^+/H^+ antiport, and for electrogenic ATP-driven proton extrusion. However Heefner and Harold (1982) later argued that the Na^+ efflux was ATP-driven, by a Na^+-ATPase distinct from the F_0F_1-type proton-translocating ATPase. They suggested that cleavage of the ATPase, either in genetically defective mutants or by proteolysis in the preparation of membrane vesicles, degrades it to a form in which it operates as an Na^+/H^+ antiporter. Thus the present inhibition of Na^+ efflux in the absence of ATP in the perfusion medium might indicate a Na^+-ATPase.

Another explanation for the ATP-dependence of Na extrusion is that ATP is required catalytically in some way which does not involve a membrane ATPase, for example in a phosphorylation reaction. In this case the secondary porter would be activated by ATP, but energetically coupled to proton circulation and therefore dependent on the electrochemical driving force for protons. Blaustein (1976), for example, found that extrusion of calcium from squid axons was effected by a Na^+/Ca^{2+} antiporter which was driven by the electrochemical potential of Na but was regulated by ATP. In *Chara*, Shimmen and Tazawa (1977) found that Mg-ATP was required in the perfusion medium for excitability in tonoplast-less cells, and thus there may be regulatory effects of ATP on the opening of Ca^{2+} and Cl^- channels involved in the action potential; Shiina and Tazawa (1986) suggest that the Ca^{2+} channel is controlled by protein phosphorylation. Further work is needed to establish whether a similar mechanism is responsible for the ATP-dependence of the Na^+ efflux.

A third possibility is a voltage-sensitive antiport with a stoichiometry of $2H^+:1Na^+$, but the DCCD effects (Table 5) indicate that this is unlikely. The effect of DCCD was to give a relatively small reduction in Na^+ efflux. If the transporter

is electrogenic and voltage-sensitive then the effects of DCCD and of ATP-free conditions should be comparable, since both depolarise the tonoplast-less cell (Shimmen and Tazawa 1977; Takeuchi and Kishimoto 1983); in fact absence of ATP reduces the flux to 2–2.5% of the control value (Tables 4, 5), whereas the flux in the presence of DCCD is still about 67% of the control.

Clearly further work is required to establish the nature of the sodium-efflux processes in *Chara*, and the results presented here are preliminary. Other putative inhibitors of ATPase-linked transport processes such as diethylstilbestrol and orthovanadate (Bowman et al. 1978; Bowman and Slayman 1979) must be tested.

A modification of the existing perfusion apparatus has been developed recently which will allow several perfusions of different media to be made through the same cell. This new system will permit fluxes to be measured on a single cell for a range of internal conditions, such as $[Na^+]$, pH, +/− ATP, +/− inhibitor, and will greatly reduce the variation in results obtained by comparing replicate batches of cells from different treatments.

This paper serves also to confirm the validity and the usefulness of the perfused giant algal cell as an experimental system for the investigation of membrane transport phenomena.

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