Biochimica et Biophysica Acta, 983 (1989) 186–192
Elsevier

BBAMEM 74509

The membrane potential of the cellular slime mold *Dictyostelium discoideum* is mainly generated by an electrogenic proton pump

Bert Van Duijn and Sake A. Vogelzang

Department of Physiology and Physiological Physics, and Cell Biology and Genetics Unit, Zoological Laboratory, Leiden University, Leiden (The Netherlands)

(Received 27 December 1988)
(Revised manuscript received 12 April 1989)

Key words: Membrane potential; Proton efflux; Electrogenic proton pump; Potassium ion/proton cotransport; Microelectrode measurement; (*D. discoideum*)

Trans membrane potential or ionic current changes may play a role in signal transduction and differentiation in the cellular slime mold *Dictyostelium discoideum*. Therefore, the contribution of electrogenic ion pumps to the membrane potential of *D. discoideum* cells was investigated. The (negative) peak-value of the rapid potential transient, seen upon microelectrode impalement, was used to detect membrane potential changes upon changes in the external pH in the range of 5.5 to 8.0. The membrane potential was close to the Nernstian potential for protons over the pH range 5.5 to 7.5. The acid-induced changes in membrane potential were consistent with outward-proton pumping. The maximal membrane potential was at pH 7.5. Furthermore, the proton pump inhibitors diethylstilbestrol, miconazole and zearalenone directly depolarize the membrane. Cyanide and temperature decrease cause membrane depolarization as well. During recovery from cyanide poisoning a H^+ efflux is present. From these measurements we conclude that the membrane potential of *D. discoideum* cells is mainly generated by an electrogenic proton pump. Measurements in cells with different extracellular potassium and H^+ concentrations suggest a role for potassium in the function of the electrogenic proton pump. These results provide a framework for future research towards a possible role for the proton pump in signal transduction and differentiation.

Introduction

The cellular slime mold *Dictyostelium discoideum* lives in the soil as amoebae, feeding on bacteria. Exhaustion of the food supply induces periodic secretion of cyclic AMP (cAMP). cAMP acts as a chemoattractant for neighboring cells which relay the signal. This process leads to aggregation and development to a multicellular organism [1,2]. *D. discoideum* is regarded as a good model to study hormone signal transduction, morphogenesis and differentiation processes [3–5]. Ions and ion fluxes may play a role in signal transduction and differentiation in *Dictyostelium* [6–14]. However, little is known about the electrophysiological properties of *Dictyostelium* and the role of these properties in signal transduction and differentiation.

Amoeboid cells of *D. discoideum* live under various environmental ionic conditions. To prevent large fluctuations in membrane potential and intracellular ion concentrations due to changes in the extracellular ionic conditions, electrogenic ion pumps may play an important role in the origin of the membrane potential. The intracellular potassium concentration in *D. discoideum* is about 50 mM [6,9,11]. A previous study showed that at an extracellular potassium concentration of 50 mM still a large negative membrane potential is present [15]. Hence, despite a dependency of the membrane potential on extracellular potassium was found, these measurements suggest that a large part of the membrane potential might be generated by an electrogenic ion pump [15]. In the non-cellular slime mold *Physarum polycephalum* the membrane potential is mainly determined by an electrogenic proton pump [16], as is the case in plant cells (see, for example, Refs. 17 and 18), other fungi [19–21] and yeast cells (see, for

Abbreviations: ADA, *N*-[2-acetamido]-2-iminodiacetic acid; DES, diethylstilbestrol; $[H^+]_e$, external H^+ concentration; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid.

Correspondence: B. Van Duijn, Department of Physiology and Physiological Physics, Leiden University, Wassenaarseweg 62, NL-2300 RC Leiden, The Netherlands.

example, Ref. 22). Biochemical investigations indicate the presence of an electrogenic proton pump in *D. discoideum* [23,24] which probably plays a role in stalk cell differentiation [12,13] and cAMP signal transduction [25]. Other ion dependent plasma membrane ATPases have also been demonstrated in *Dictyostelium* [26–28].

Knowledge of the origin of the membrane potential of *Dictyostelium* may help to resolve the role of ions and ion fluxes in signal transduction and differentiation. The method used in the present study to measure membrane potentials [15] has revealed part of the origin of the membrane potential in *Dictyostelium* and provides possibilities for future investigations of the role of membrane electrophysiological properties in signal transduction and differentiation. In this paper we report that the electrogenic ion pump, suggested by previously reported experiments [1], contributes largely (> 60%) to the membrane potential and is a DES-sensitive electrogenic proton pump. The experiments suggest a role for potassium in the membrane potential of *Dictyostelium*.

Materials and Methods

Cell culture

Cells of *D. discoideum* NC-4 (H) were grown in association with *Escherichia coli* 281 as described [15]. Cells were harvested before clearing of the bacterial lawn occurred and washed free of bacteria three times by centrifugation at $150 \times g$ during 2 min in cold 10 mM sodium/potassium phosphate buffer (pH 6.5). Cells in suspension were deposited on glass coverslips (with a thickness of 0.17 mm) at a density of about $5 \cdot 10^4$ cells/cm^2, and used within three hours.

Membrane potential measurements

For membrane potential measurements the glass cover slips, with adhered cells on it, were attached to a teflon culture dish which permitted measurements under 100× objective magnification [29]. Membrane potentials were measured with fine-tipped open-ended glass microelectrodes filled with 3 M potassium chloride. Microelectrodes had resistances ranging from 14 Mohm to 71 Mohm and were capacitively compensated. Rise time (i.e., the time to reach two-thirds of the potential response upon a current pulse) of compensated microelectrodes was 37 μs (S.E. = 3.6 μs, n = 43). For microelectrode impalement of cells a piezo-stepper device was used [15]. Furthermore, potentials were recorded as described [15], except for the storage of signals, which was directly on a digital storage oscilloscope (Nicolet 3091), and thereafter displayed on paper with a *X-Y* recorder (Hewlett-Packard 7035B).

D. discoideum cells are relatively small (diameter < 10 μm). Due to this small size, impalement of cells causes a rapid depolarization of the membrane potential. This depolarization is caused by the introduction of a microelectrode-induced shunt resistance [15,30]. Therefore, no reliable stationary membrane potential measurements can be made in *D. discoideum* cells [15]. However, analysis of the negative-going potential transient seen in the first millisecond after microelectrode impalement has shown that the peak value, E_p, of this transient (Fig. 1) can be used as a reliable indicator of the true resting membrane potential before impalement [15,30]. Consequently, the value of E_p was used as an indicator of the membrane potential of *D. discoideum*, rather than the semi-stationary depolarized potential E_n (Fig. 1).

Measurements were carried out at room temperature unless specified otherwise. For measurements at 3–4°C the bath was continuously perfused with cold saline solution. Potential values are expressed as means ± S.E. with n = number of cells. Differences between measured values were tested with the Student's t-test (95% level).

H^+-efflux measurements

For measurements of H^+ efflux during recovery from cyanide poisoning cells were incubated for 10 min in 10 mM sodium/potassium phosphate buffer (pH 6.5) supplemented with 1 mM KCN. Thereafter, cyanide was removed by centrifugation for 1 min at $150 \times g$ and resuspension of the cell pellet in non-buffered Na^+/K^+-saline solution ($27 \cdot 10^7$ cells/ml). Directly after resuspension the extracellular pH in the continuously stirred suspension was measured. H^+ efflux per cell was calculated from ΔpH. Correction for passive H^+ efflux was made by measurement of the extracellular pH after resuspension of the cell pellet in non-buffered Na^+/K^+-saline solution with 1 mM KCN, and subtraction of this H^+ efflux from the H^+ efflux measured without CN^- in the suspension.

Composition of solutions

All chemicals were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A., unless specified otherwise. For control experiments cells were bathed in a Na^+/K^+-saline solution composed of 40 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$ and 5 mM Hepes (pH 7.0). Experiments at different extracellular pH values were performed in Na^+/K^+-saline solution with various organic buffers (ADA, Hepes and Mes) dependent on the pH value desired, as well as with Na/K-phosphate buffered Na^+/K^+-saline solution.

The K^+-free saline solution was the Na^+/K^+-saline solution without addition of KCl. Solutions containing DES (Merck, Darmstadt, F.R.G.), zearalenone and miconazole were prepared just before use from 40 mM DES, 50 mM zearalenone and 10 mM miconazole stock-solutions in ethanol respectively. E_p and E_n values measured in cells bathed in Na^+/K^+-saline solution

with 0.1% (v/v) ethanol (DES solvent) were not different from control measurements (data not shown). Potassium cyanide containing solutions were made just before use. Na^+/K^+-saline solutions with 1 mM sodium orthovanadate or with 1 mM thimerosal (Serva Feinbiochemica, Heidelberg/New York, F.R.G./U.S.A.) were freshly made before use. Na^+/K^+-saline solutions with ouabain, also, were freshly made before use.

Results

The membrane potential of *D. discoideum* cells probably is largely generated by electrogenic ion pumps [15], e.g., proton pumps, calcium pumps and sodium/potassium pumps. The experiments to be reported here have been directed towards establishing of a possible role for proton pumps in generating the membrane potential of *D. discoideum*.

Measurement of the resting membrane potential

Upon impalement of a *D. discoideum* cell a rapid negative going potential transient is seen (Fig. 1) [15]. In Na^+/K^+-saline solution the mean of the peak value, E_p, was -27.2 mV, while the mean value of the semi-stationary potential E_n was -5.4 mV (Tables I and II). Using the analysis reported previously [15] it can be concluded that E_p is about 30% of the true resting membrane potential, E_m, of *D. discoideum*. Although E_p is an underestimation of the true membrane potential, it can be used as the best estimation for the detection of membrane potential changes. Mathematical analysis of the peak-potential transient [15,30] has shown that the ratio between the measured E_p and the true E_m does not depend on the value of E_m (i.e., E_p is a proportional underestimation of E_m). Therefore, E_p was used to study the effect of extracellular factors on the membrane potential of *D. discoideum*. It should be noted that E_n, the semi-stationary potential, is not always a good estimate of E_m. For example, Fig. 1 demonstrates that membrane potential changes are visible in E_p but not in E_n.

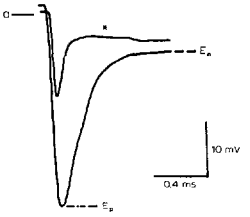

Fig. 1. Two potential transients measured upon microelectrode impalement of *D. discoideum* cells bathed in Na^+/K^+-saline solution with pH 7.0 and 6.0 (buffered with Hepes and ADA, respectively; transient at pH 6.0 indicated by *). The peak-value of the potential transient, E_p, is regarded as a more reliable measure for the resting membrane potential, E_m, prior to impalement than the semi-stationary potential E_n.

TABLE I

E_p and E_n values for D. discoideum cells bathed in Na^+/K^+-saline solutions with various buffers at room temperature, unless stated otherwise

Buffer type	E_p (mV)	S.E. (mV)	E_n (mV)	S.E. (mV)	n
Hepes, pH 7.0 [a]	−25.3	1.5	−5.5	0.3	124
Phosphate, pH 7.0 [a]	−31.4	3.8	−6.4	0.9	17
ADA, pH 6.0 [b]	−10.6	0.7	−7.4	0.6	61
Phosphate, pH 6.0 [b]	−9.7	0.9	−5.4	0.5	32
Hepes, pH 7.0 at 3–4 °C	−16.9	1.3	−7.8	0.6	34

[a] Differences in E_p and E_n between Hepes and phosphate-buffered solution (pH 7.0) are not significant.

[b] Differences in E_p and E_n between ADA and phosphate-buffered solution (pH 6.0) are not significant.

Effect of extracellular pH

To establish a role for a possible proton pump in *D. discoideum*, measurements have been performed in cells bathed in Na^+/K^+-saline solution with different pH and different buffer types (Fig. 2). Measurements of the rapid potential transient upon impalement (Fig. 1) under various external pH revealed a dependency of the membrane potential on the extracellular pH. Fig. 2 shows, with E_p as an indicator of E_m, that the mem-

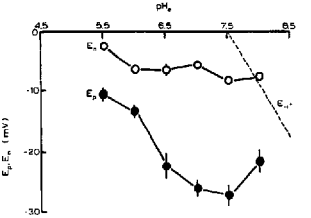

Fig. 2. The dependency of the membrane potential on the extracellular pH (pH_e). The biological buffers Hepes (pH 7.0, 7.5 and 8.0), ADA (pH 6.0 and 6.5), Mes (pH 5.5) as well as Na/K phosphate buffer (pH 7.0 and 6.0) in Na^+/K^+-saline solution were used. The peak-value E_p (see Fig. 1) serves as a good indicator of the true membrane potential. E_n is the semi-stationary potential of the impaled cell. The points represent the average of 33, 110, 29, 141, 60 and 36 measurements for pH_e 5.5 to 8.0, respectively. Bars represent S.E. of these values. The dashed line is the equilibrium potential (E_{H^+}) for protons (based on an intracellular pH of 7.5 [25]) as would be measured as E_p values (30% of the actual values).

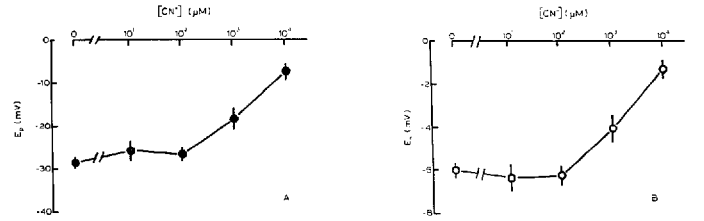

Fig. 3. Dependence of the resting membrane potential on the chain phosphorylation inhibiting agent cyanide (CN^-). Points represent the average value of 220, 32, 97, 22 and 10 measurements at 0 to 10^4 μM CN^- concentrations in Na^+/K^+-saline solution, respectively. Bars represent S.E. of these values. (A) The peak value (E_p) of the rapid impalement transient as a function of the CN^- concentration. (B) The semi-stationary potential (E_s) of the impaled cells as a function of the CN^- concentration.

brane potential depolarizes with decreasing pH below 7.5. Acid-induced changes in E_p yielded a response between 13 and 19 mV/$[pH^+]_e$ decade. External pH larger than 7.5 does not induce a further hyperpolarization but a depolarization. Fig. 2 shows the external pH effect on E_s as well, which is hardly detectable. This weak dependency of E_s, as compared with E_p, on the external pH is consistent with the earlier finding that the potential E_s is mainly determined by the microelectrode-induced shunt resistance [15].

To exclude buffer-type effects, biological as well as Na/K phosphate buffers have been used. Measurements with ADA-buffered solution (pH 6.0) did not give different potential values as compared with Na/K phosphate-buffered solution (pH 6.0) (Table I). This is also true for Hepes-buffered solution (pH 7.0) as compared with phosphate-buffered solution (pH 7.0) (Table I).

The effect of different external pH on the membrane potential was measured within 1 min after replacement of the extracellular solution. Measurements directly after changing extracellular solution showed no different E_p values as compared with measurements 15 min after solution change. The effect of changing membrane potentials with changing external pH, as shown in Fig. 2, is present for changes from high to low pH as well as for changes from low to high pH (data not shown).

Effect of metabolic inhibition and temperature

The effect of the respiratory chain blocking agent cyanide (CN^-) on the membrane potential was studied. Exposure of cells to CN^- rapidly decreases the intracellular ATP levels [16]. Therefore, CN^- can be used to illustrate the energy dependency of cellular processes. Fig. 3A shows the effect of various extracellular CN^- concentrations on E_p, reflecting the effect on the membrane potential. CN^- concentrations larger than 0.1 mM induce a depolarization of the membrane. This depolarization can be measured directly after perfusion with cyanide-containing Na^+/K^+-saline solution (i.e., within 1 min). Recovery from KCN-induced depolarization was present within 15 min after washing the cells with normal Na^+/K^+-saline solution. Additionally, the effect of CN^- on the membrane potential was also clearly visible in the semi-stationary potential E_s, as shown in Fig. 3B. Perfusion of the bath with 10 mM CN^- containing Na^+/K^+-saline solution caused a swelling of cells within 5 min.

To show a correlation between membrane hyperpolarization and H^+ efflux we measured external pH during recovery from CN^- poisoning in cells bathed in non-buffered Na^+/K^+-saline solution [31]. Fig. 4 shows that during recovery from cyanide poisoning protons are pumped out of the cells, which indicates the presence of an ATP-dependent proton pump in the plasma membrane of *Dictyostelium*.

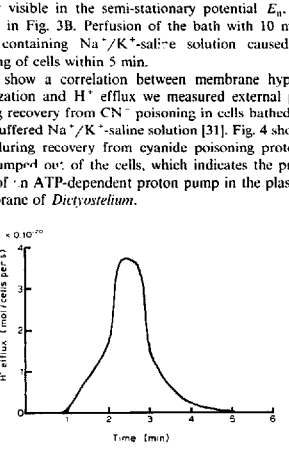

Fig. 4. H^+ efflux (mol/cell per s) during recovery from CN^- poisoning corrected for passive H^+ efflux (see Materials and Methods). After treatment with CN^- cells were resuspended at $t = 0$ in non-buffered Na^+/K^+-saline solution and the extracellular pH was measured. The experiment was performed in three different cell cultures with equal results. The figure shows one representative experiment.

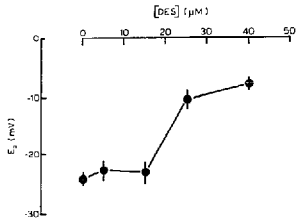

Fig. 5. The peak value (E_p) of the rapid impalement transient as a function of the DES concentration in Na^+/K^+-saline solution. Each point represent the average value of 144, 35, 45, 23 and 18 measurements for DES concentrations of 0 to 40 μM, respectively. Bars represent S.E. of these values.

Temperature decrease reduces the activity of most enzymatic reactions. Therefore, the ion fluxes generated by many ion pumps are temperature dependent. Decrease of the bath saline solution temperature from about 21 to about 3°C did result in a significant but not complete depolarization of the membrane potential, as indicated by the mean E_p value measured (Table I).

Effect of ion pump blocking agents

The effect of various ion pump blocking agents on the membrane potential was studied.

Fig. 5 shows the depolarizing effect of the proton pump blocker DES [21]. DES concentrations larger than 15 μM in Na^+/K^+-saline solution induce a depolarization of the membrane potential. Directly after addition of DES to the bath solution (i.e., within 1 min) the depolarizing effect was present. On a larger time scale (10–20 min) DES induces also swelling of cells.

Proton pumps which can be blocked by DES also are sensitive to vanadate [21,23,27], zearalenone [13] and miconazole [13,23]. Miconazole and zearalenone both depolarized the membrane potential (Table II). E_p values measured in cells bathed in 1 mM vanadate in Na^+/K^+-saline solution, however, were not different as compared to control measurements (Table II). Thimerosal inhibits some plasma membrane ATPases in *Dictyostelium* [24,26,27]. E_p values measured in cells bathed in Na^+/K^+-saline solution with 1 mM thimerosal, which is effective in biochemical studies, were not different from control measurements (Table II).

Ouabain, a well known blocker of the electrogenic Na^+/K^+ pump in animal cells, also had no effect on the membrane potential of *D. discoideum* (Table II) in concentrations which are effective in animal cells.

TABLE II

E_p and E_n values for D. discoideum cells bathed in Hepes-buffered Na^+/K^+-saline solution (pH 7.0; Control) at room temperature with different additions as indicated [a]

Additions	E_p (mV)	S.E. (mV)	E_n (mV)	S.E. (mV)	*n*
Control	−29.9	1.7	−5.2	0.4	89
30 μM miconazole	−8.3	1.2	−3.9	0.6	27
0.5 mM zearalenone	−8.0	1.3	−3.6	0.4	25
1 mM vanadate	−31.4	2.2	−6.2	0.6	47
1 mM thimerosal	−34.9	3.3	−7.2	0.8	35
2 mM ouabain	−28.3	2.8	−6.6	1.3	10

[a] Differences in E_p and E_n between control solutions and solutions with inhibitor are not significant, except for the E_p and E_n values of miconazole- and zearalenone-treated cells, and the E_n value of thimerosal-treated cells.

TABLE III

E_p and E_n values for D. discoideum cells bathed in Hepes (pH 7.0)- and Mes (pH 5.5)-buffered K^+-free saline solution.

pH and additions	E_p (mV)	S.E. (mV)	E_n (mV)	S.E. (mV)	*n*
pH 7.0	−20.9	1.0	−6.5	0.4	87
pH 5.5	−17.9	0.9	−6.5	0.5	63
pH 7.0 + 40 μM DES	−4.2	1.0	−2.1	0.7	11

Role of external potassium

The experiments show that the membrane of *D. discoideum* can be almost completely depolarized by proton pump inhibition. Other experiments, however, indicate a role for potassium ions originating the membrane potential [15]. In *Neurospora* cells [31,32], and probably also in other non-animal eukaryotic cells, a H^+ pump and a high-affinity transport system, that carries K^+ inward in cotransport with H^+, work together to maintain the intracellular pH near neutrality. Some experiments in K^+-free saline solution have been done to investigate a possible role for potassium in the function of proton pumping in *Dictyostelium*.

Results of the experiments with cells bathed in K^+-free saline solution deviated from the results obtained from cells bathed in normal Na^+/K^+-saline solution with respect to the acid-induced membrane depolarization. Cells bathed in K^+-free saline solution did not show a large membrane depolarization when the extracellular pH was changed from 7.0 to 5.5 (Table III).

Apparently, extracellular potassium is required for the acid-induced depolarization. However, cells bathed in K^+-free saline solution (pH 7.0) still showed a membrane depolarizing response upon addition of 40 μM DES (Table III). This indicates that the membrane potential in K^+-free saline solution still is generated by an electrogenic proton pump.

Discussion

In the present study rapid impalement potential transient measurements were used to establish that an electrogenic proton pump largely contributes to the membrane potential of *D. discoideum* cells. The mean peak value of the impalement transient, E_p, measured in cells bathed in Na^+/K^+-saline solution (pH 7.0) is more negative than the previously reported value (about −19 mV) [15]. The use of electrodes with a smaller rise time (rise time in previous report about 45 μs [15]) which cause a smaller microelectrode-induced shunt as well (as indicated by a more negative E_a value) will be the main reason for the more negative E_p values in the present report.

Analysis of membrane potential measurements [15] indicates that in the present experiments E_p is about 30% of the true membrane potential, E_m (i.e., E_m is about −90 mV in Na^+/K^+-saline solution).

The membrane potential measurements in cells with different extracellular pH values reveal that the membrane potential is strongly pH dependent (Fig. 2). Because E_p is about 30% of E_m, a dashed line has been drawn in Fig. 2 showing the 30% value of the equilibrium potential for H^+ ions, based on an intracellular pH of 7.5 [25]. The membrane potential over the pH range of 5 to 7.5 is close to the Nernstian behavior. The acid-induced changes in membrane potential are consistent with outward-proton pumping. The decreased potential at alkaline external pH may be due to a drop in pump activity [18]. The overall behavior (including the pH value at which the potential is most negative) is consistent with characteristics of *Chara* [17], *Neurospora* [20], yeast cells [22] and *Vicia faba* and *Commelina communis* stomatal guard cells [18] proton pumps.

Further evidence for a role of an electrogenic proton pump is provided by the membrane potential response to the metabolic inhibitor CN^- (Fig. 3), the presence of H^+-efflux during recovery from CN^- poisoning (i.e., during hyperpolarization) (Fig. 4), and by the response to the addition of the proton pump inhibitors DES (Fig. 5), zearalenone and miconazole (Table II). These responses closely resemble the results reported for *Neurospora* [19,21,31], *Chara* [17] and *P. polycephalum* [16]. In addition, the proton pump demonstrated biochemically in *Dictyostelium* is inhibited by DES, zearalenone and miconazole [13,23,24]. CN^-- and DES-induced depolarization due to K^+ gradient dissipation is very unlikely because cells bathed in high extracellular potassium concentrations (making the potassium equilibrium potential zero) still have a large negative membrane potential [15]. Furthermore, the swelling of cells, which could be associated with dissipated ion gradients, occur much later than depolarization (larger DES concentrations) or only at very high concentrations (CN^-). Uncoupling of oxidative phosphorylation by DES is unlikely since much higher concentrations are required for this DES effect [21].

Drastic lowering of the temperature (from 22 to about 4°C) causes only a slight depolarization of the membrane potential (Table I). A similar response is reported for *P. polycephalum* [16]. From our measurements it is clear that the membrane potential of *Dictyostelium* is not strongly temperature dependent.

DES-sensitive plasma membrane bound ion-dependent ATPases identified in *Dictyostelium* are vanadate sensitive [23,24,27]. Proton pumps in *Neurospora* and yeast cells are vanadate sensitive as well [21]. Although in the present study a larger vanadate concentration was used than reported in the biochemical studies [21,23,24] no effect of vanadate on the membrane potential was found. This can be due to the fact that vanadate might not enter the cells, as reported in other studies [21].

Assuming that thimerosal enters the cells we conclude that the Ca^{2+}/Mg^{2+}-dependent membrane ATPases, which have been reported to exist [24,26,27], are not strong electrogenic ion pumps or are not active in non-stimulated cells.

Ouabain, which blocks the electrogenic Na^+/K^+ pump in animal cells had no effect on the membrane potential of *D. discoideum* cells (Table II). Ouabain did not affect isolated plasma membrane bound ATPases as reported by others [23,24,26,28]. Some effects of ouabain on differentiation, however, are known [14]. From our measurements we conclude that no animal-like electrogenic Na^+/K^+-pump contributes largely to the membrane potential in vegetative *Dictyostelium* cells.

All results together of the pH, pump blocking, metabolic inhibition and H^+-efflux experiments allow us to conclude that the membrane potential of *D. discoideum* cells is mainly generated by an electrogenic proton pump. The experiments with different extracellular pH, CN^- and DES indicate that the contribution of this pump to the membrane potential is at least 60% (Figs. 2, 3 and 5). Since inhibition of the electrogenic proton pump almost completely depolarizes the membrane and various other membrane ATPase inhibitors showed no effect on the membrane potential we conclude that no other electrogenic ion pumps, different from the electrogenic proton pump, have a large contribution to the membrane potential.

Experiments reported previously indicate a role for potassium in the origin of the membrane potential [15]. The present measurements in K^+-free saline solution, indeed, support a role for potassium in the generation of the membrane potential (Table III). The acid-induced depolarization requires the presence of external potassium. In the absence of external K^+ the diffusion potential of potassium increases and can, probably, dominate E_m, and the pH_e effects on E_m are not so apparent, as is described for *Chara* with the linear equivalent-circuit

model for the plasmamembrane [32]. However, the less negative E_p value (as compared with E_p in Na^+/K^+-saline solution) and the presence of membrane depolarization upon addition of DES in cells bathed in K^+-free saline solution argue against this hypothesis for *Dictyostelium* (Table III). An other possible explanation for the effect of potassium can be found in that the (hyperpolarizing) pump current is dependent on the external and internal pH difference [17], i.e. more acid outside reduces the current. The pH difference between the intra- and extracellular fluid might not be maintained in cells bathed in K^+-free saline solution. Due to this, pump activity (current) will not decline with decreasing extracellular pH, and no acid-induced depolarization will be present. In *Neurospora* cells it has been shown that H^+-pumping alone is not very effective in maintaining the intracellular pH near neutrality, which is probably also true for other non-animal cell types [33]. The presence of an outward directed proton pump in combination with an inward directed K^+/H^+ cotransporter provides a net H^+ export, which efficacy is restricted without external potassium ions [33,34]. The absence of acid-induced depolarization in cells bathed in K^+-free saline solution suggests that such a system might also be present in *D. discoideum* cells. Future experiments in which the intracellular pH is measured for different extracellular potassium concentrations are necessary to achieve more information of the intracellular pH regulating system.

Knowledge about the presence of such systems, including cotransporters, might result in a better understanding of the as yet rather confusing data on the effects of monovalent ions, extracellular and intracellular pH on differentiation of *D. discoideum* [9,11–14,35].

Our experiments seem to indicate that *Dictyostelium* has electrophysiological properties similar to plants and other fungi. Future experiments should be directed towards a further electrophysiological characterization of the ion transporting mechanisms in *Dictyostelium* and the role of these systems in signal transduction and differentiation.

Acknowledgements

The authors wish to thank G.A. Telkamp (Department of Physiology) for assistance, Mei Wang (Cell Biology and Genetics Unit) for experimental assistance, and D.L. Ypey (Department of Physiology), T.M. Konijn, L.G. Van der Molen and P.J.M. Van Haastert (Cell Biology and Genetics Unit) for their stimulating support.

References

1 Devreotes, P.N. (1983) Adv. Cycl. Nucleotide Res. 15, 55–96.
2 Van Haastert, P.J.M. and Konijn, T.M. (1982) Mol. Cell. Endocrinol. 26, 1–17.
3 Janssens, P.M.W. and Van Haastert, P.J.M. (1987) Microbiol. Rev. 51, 396–418.
4 Newell, P.C., Europe-Finner, G.N. and Small, N.V. (1987) Microbiol. Sci. 4, 5–11.
5 Schaap, P. (1986) Differentiation 323, 1–16.
6 Aeckerle, S., Wurster, B. and Malchow, D. (1985) EMBO J. 4, 39–43.
7 Bumann, J., Wurster, B. and Malchow, D. (1984) J. Cell Biol. 98, 173–178.
8 Bumann, J., Malchow, D. and Wurster, B. (1986) Differentiation 31, 85–91.
9 Maeda, M. (1983) Bot. Mag. (Tokyo) 96, 193–210.
10 Malchow, D., Nanjundiah, V., Wurster, B., Eckstein, F. and Gerisch, G. (1978) Biochim. Biophys. Acta 538, 473–480.
11 Marin, F.T. and Rothman, F.G. (1980) J. Cell Biol. 87, 823–827.
12 Gross, J.D., Bradbury, J., Kay, R.R. and Peacey, M.J. (1983) Nature (London) 303, 244–245.
13 Gross, J.D., Peacey, M.J. and Pogge von Strandmann, R. (1988) Differentiation 38, 91–98.
14 Town, C.D. (1984) Differentiation 27, 29–35.
15 Van Duijn, B., Ypey, D.L. and Van der Molen, L.G. (1988) J. Membr. Biol. 106, 123–134.
16 Kuroda, H. and Kuroda, R. (1981) J. Gen. Physiol. 78, 637–655.
17 Beilby, M.J. (1984) J. Membr. Biol. 81, 113–125.
18 Blatt, M.R. (1987) Planta 170, 272–287.
19 Slayman, C.L. (1965) J. Gen.Physiol. 49, 69–92.
20 Slayman, C.L. and Sanders, D. (1984) in Electrogenic transport: fundamental principles and physiological implications (Bäumstein, M. and Lieberman, M., eds.), pp. 307–322, Raven, New York.
21 Goffeau, A. and Slayman, C.W. (1981) Biochim. Biophys. Acta 639, 197–223.
22 Lichtenberg, H.C., Giebeler, H. and Hofer, M. (1988) J.Membr. Biol. 103, 255–261.
23 Pogge von Strandmann, R., Kay, R.R. and Dufour, J.-P. (1984) FEBS Lett. 175, 422–428.
24 Serrano, R., Cano, A. and Pestana, A. (1985) Biochim. Biophys. Acta 812, 553–560.
25 Aerts, R.J., De Wit, R.J.W. and Van Lookeren Campagne, M.M. (1987) FEBS Lett. 220, 366–370.
26 Blanco, M. (1982) Biochim. Biophys. Acta 687, 94–96.
27 MacDonald, J.I.S. and Weeks, G. (1984) Arch. Biochem. Biophys. 235, 1–7.
28 Milne, J.L. and Coukell, M.B. (1988) Biochem. J. 249, 223–230.
29 Ince, C., Van Dissel, J.T. and Diesselhoff-den Dulk, M.M.C. (1985) Pflügers Arch. (Eur. J. Physiol.) 403, 240–244.
30 Ince, C., Van Bavel, E., Van Duijn, B., Donkersloot, K., Coremans, A., Ypey, D.L. and Verveen, A.A. (1986) Biophys. J. 50, 1203–1209.
31 Slayman, C.L. (1970) Am. Zool. 10, 377–392.
32 Tazawa, M. and Shimmen, T. (1982) Curr. Top. Membrane Transp. 16, 49–67.
33 Blatt, M.R. and Slayman, C.L. (1987) Proc. Natl. Acad. Sci. USA 84, 2737–2741.
34 Rodriguez-Navarro, A., Blatt, M.R. and Slayman, C.L. (1986) J. Gen. Physiol. 87, 649–674.
35 Van Lookeren Campagne, M.M., Aerts, R.J., Spek, W., Firtel, R.A. and Schaap, P. (1988) Development 105, 401–406.