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CHLORIDE EFFLUX DURING LIGHT-TRIGGERED ACTION POTENTIALS IN *ACETABULARIA MEDITERRANEA*

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SUMMARY

The electrical potential difference between the cytoplasm and the outer medium E_{co}, and Cl^- efflux have been measured simultaneously in individual cells of *Acetabularia mediterranea* under a light–dark regime.

1. Light–dark changes of E_{co} coincide with large, rather proportional changes of the Cl^- efflux, if no action potential is triggered.

2. During an action potential occurring under certain conditions after cessation of illumination, the initial small decrease of the Cl^- efflux reverses after about 20 s, when the depolarisation of E_{co} becomes steeper, into a short increase, exceeding the steady state efflux at least 3-fold.

3 The measured peak efflux of about 1.7 nmoles $Cl^- \cdot cm^{-2} \cdot s^{-1}$ compares well with an inward electric current of 90 $\mu A/cm^2$ during excitation, recorded under voltage clamp (Gradmann, D., (1970) *Planta* 93, 323–353).

4. We conclude that the depolarizing inward current during the action potential of *Acetabularia* is carried by a Cl^- efflux.

INTRODUCTION

Cl^- plays an important role in the electrical behaviour of the giant green algal cell of *Acetabularia*[1–3]. This cell exhibits a large electrogenic Cl^- influx[2,3], which is responsible for the high resting potential of about −170 mV. This is far more negative than the highest possible diffusion potential of about −90 mV in this alga.

The electrical potential difference between the cytoplasm and the outer medium, E_{co}, can be triggered to a drastic transient depolarisation by an electrical stimulus or cessation of illumination[1]. This response exhibits the basic features of an action potential, although it takes its course extremely slowly, *i.e.* in about 3 min. It even occurs, if the cell is bathed in pure choline chloride solution[1]. Hence no cation of normal sea water is essential for the inward current during the action potential, but an anion must leave the cell. This anion was assumed to be Cl^-. The aim of this study is to demonstrate directly the Cl^- efflux during the action potential. Since the action potentials in the experiments were triggered by preillumination, in addition, some data on the influence of the light on the Cl^- efflux were obtained.

MATERIALS AND METHODS

We used the fast growing species *Acetabularia mediterranea*, which does not seem to behave electrophysiologically significantly different from the somewhat larger cells of *A. crenulata*, used in previous studies[1,4,5]. Fig. 1 shows a diagram of the apparatus to measure simultaneously $^{36}Cl^-$ release and internal potential

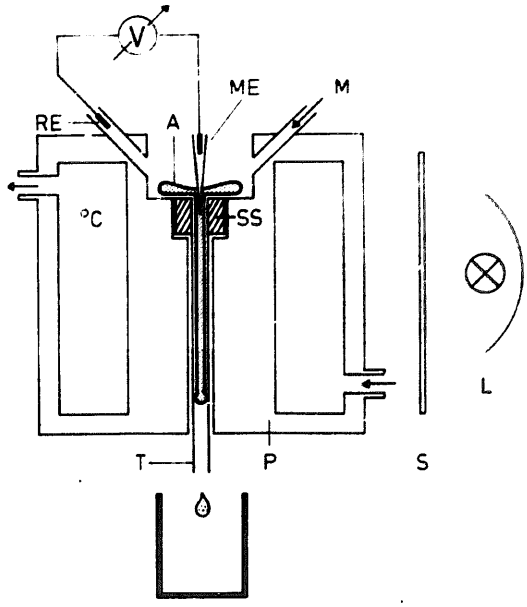

Fig. 1. Diagrammatical view of the apparatus for simultaneous measurement of internal potential E_{co} and Cl^- efflux. A, ^{36}Cl -labelled *Acetabularia* cell; L, light source; M, medium; ME, glass microelectrode; P, plexiglass vessel; RE, reference electrode; S, shutter; SS, silicone seal; T, plastic tube; °C, perfusion with thermostated water.

E_{co} of single cells of *Acetabularia*. Large adult cells with a cap and stalk which was still dark green, were incubated for a least 12 h in 4 ml of artifical sea water containing 100 μCi $^{36}Cl^-$ in the 2.2 mmoles Cl^-. The labelled cells were gently sucked into a 1-mm wide plastic tube leaving the cap free. The tube was tightly inserted in a thermostat-controlled (23 °C) lucite vessel with inlets for fresh medium, a thermometer and the reference electrode. The inserted tube was closed on the lower end and some artificial sea water introduced into the vessel, The cell was then punctured in the middle of the cap, using conventional microelectrode techniques. About 15 min later the recorded voltage E_{co} reached its steady level of around -170 mV. The tube was then opened, and the medium washed the cell at a final rate of about 1 drop per s; the medium was sampled in fractions of 30 s. The accuracy of this procedure was better than ± 2 s.

The experiments were performed under normal daylight of about 100 erg/cm^2 s, referred to as D, compared to L, that is illumination by 10^6 erg·cm^{-2}·s^{-1} white light from a 250-W quartz–iodide projector. Due to the perfusion system, the radioactivity of the samples lagged some seconds behind the recorded voltage. The data have not been corrected for this small lag. An aliquot of Unisolve 1 scintillation gel was added to the samples and their radioactivity (cpm) determined in a Beckman liquid scintillation counter.

RESULTS

In order to obtain absolute values for the Cl^- efflux, the radioactivity released at a particular time is related to the release at time zero, at which the loaded cell is switched to cold medium. A typical washout is shown in Fig. 2. The biphasic kinetics reflect the washout of two compartments. Apparently, the fast phase ($t_{1/2}$=19 min) corresponds to the $^{36}Cl^-$ release from the cytoplasm, the slow one ($t_{1/2}$=56 h) from the vacuole[6]. This experiment took place during illumination. Under complete darkness the cytoplasmic phase was slowed down to $t_{1/2}$ of approx. 200 min, indicating that the steady state Cl^- permeability rises under illumination.

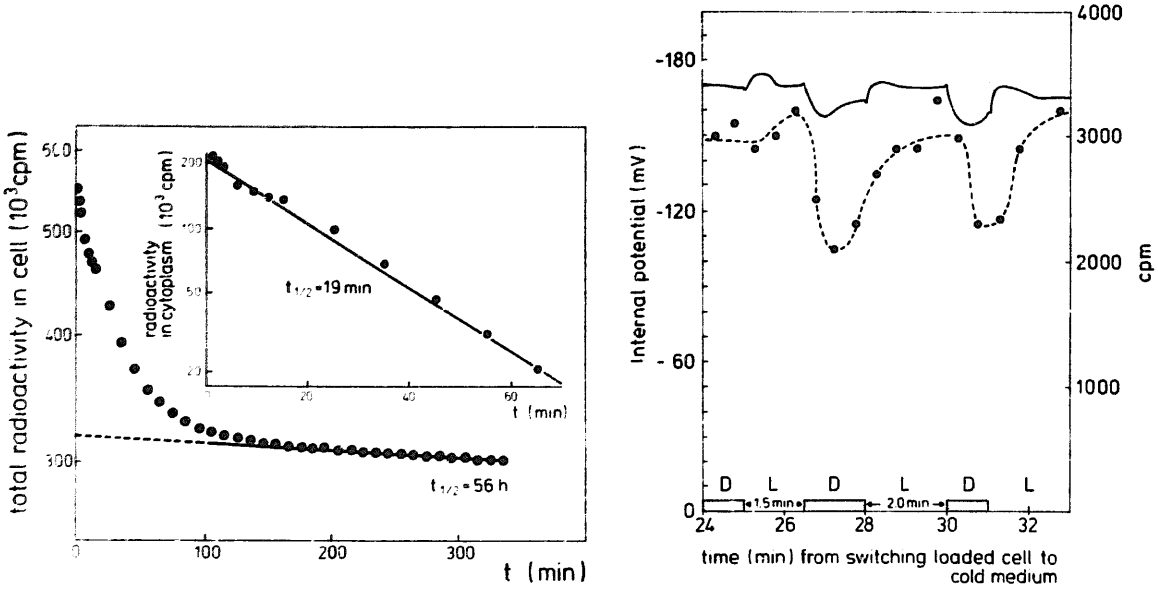

Fig. 2. Semilogarithmic plot of the $^{36}Cl^-$ washout kinetics of an *Acetabularia* cell, after, switching from the incubation medium (100 μCi $^{36}Cl^-$ in 4 ml artificial sea water) to cold medium. ——, radioactivity of the vacuolar compartment. Inset: same experiment, $^{36}Cl^-$ washout kinetics of the cytoplasm = radioactivity of the total cell *minus* radioactivity of the vacuole.

Fig. 3. Internal potential E_{co} (——) and $^{36}Cl^-$ release (– – –) of a non-excitable *Acetabularia* cell in light/dark regime.

During the experiments of Figs 3 and 4 the cells were exposed to a light–dark regime. The release of $^{36}Cl^-$ took place with a $t_{1/2}$ of 42, and 22 min, respectively. According to the specific radioactivity of the incubation medium, 1 cpm of $^{36}Cl^-$ represents a release of 10^{-11} mole Cl^- at time zero, when the specific radioactivity in the cytoplasm is still the same as in the incubation medium. We disregard the small error, arising from the influence of the vacuole on the kinetics. A detailed compartment analysis will be the subject of a separate study.

In the experiment of Fig. 4, extrapolation of the radioactivity data to time zero yields a release of 2800 cpm in 30 s, which corresponds to a steady state Cl^- efflux of 0.6 nmole·cm^{-2}·s^{-1}, the cell surface being taken as 1.5 cm^2. This efflux value agrees well with the range of 290–855 pmoles·cm^{-2}·s^{-1}, reported earlier[2].

Continuous illumination with L stimulates the steady-state Cl^- efflux by only 5–10% (Figs 3 and 4). Light-on and -off elicits transient changes of both, E_{co} and

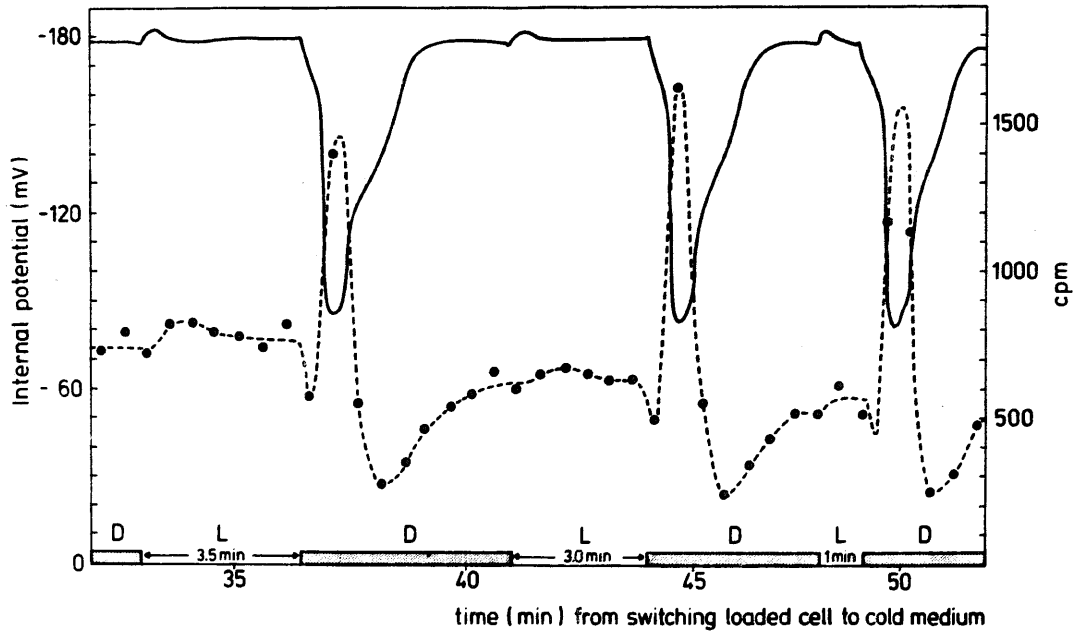

Fig. 4. Internal potential E_{co} (——) and $^{36}Cl^-$ release (- - -) of an excitable *Acetabularia* cell in a light/dark regime.

the Cl^- efflux. About 30 s after light-on E_{co} passes a maximum which is about 5 mV (3%) above the steady-state value. During this time the Cl^- efflux is raised about 10% over the control value (Figs 3 and 4).

Upon light-off the cell can respond in two clearly distinct ways; Case A, the preceding light dose is insufficient to trigger an action potential by the light-off stimulus; this case is represented by the results displayed in Fig. 3. Case B: the stimulus suffices to trigger and action potential; the results of this case are shown by the examples in Fig. 4. In case A the voltage passes a minimum of about 15 mV (12%) below the control value. Simultaneously, the Cl^- efflux is diminished by 20–30% during the minimum (in other cases up to 70%, when E_{co} drops below the steady-state value by more than 20 mV).

In Case B, during the first 20 s a depolarisation as in Case A occurs; now, if E_{co} reaches a certain threshold, at about -165 mV, the depolarisation accelerates due to an action potential. Then E_{co} turns at about -85 mV or lower towards repolarisation which finishes about 2 min later. The corresponding time course of the Cl^- efflux shows an appreciable decrease like in case A during the first 20 s after light-off. However, in the following seconds the Cl^- efflux rises drastically to at least the 3-fold of the control value. With finer resolution of time, the sharp peaks are assumed to appear even higher and narrower, according to the short time of fast depolarisation. The peaks are strictly correlated with the steep depolarisation of the action potential. After its maximum the Cl^- efflux again passes a minimum of about 30–40% of the steady state value, which is reached about a min later as is E_{co}.

DISCUSSION

Before we treat the changes in Cl^- efflux associated with the action potential, we want to discuss another interesting question which arises already from the results

of the control experiment (Fig. 3). The measured Cl^- efflux, down its electrochemical gradient, is probably strictly passive. However, its relative changes are too big to be caused only by the corresponding change of E_{co} (concentration changes can be ignored), because an EMF can change an electrophoretical flux not more than proportionally. On the other hand, these changes in E_{co} are explained as a direct effect of changes in the electogenic Cl^- influx[1,3]. The striking conformity between the changes of the Cl^- influx, which are visible as changes of the E_{co}, and the measured changes of the Cl^- efflux, indicate a close linkage between the active Cl^- influx and the passive Cl^- efflux. The fact, that the time constant for the Cl^- release from the cytoplasm is larger in darkness than in light, leads to the same conclusion.

Now, in the case of an action potential, there is an obvious correlation between the fast depolarisation of E_{co} and the significant increase in the Cl^- efflux .The event can be discussed quantitatively by using the data of Fig. 4. The steady-state Cl^- efflux is 0.6 nmole$\cdot$cm$^{-2}\cdot$s^{-1}. During excitation it is raised by the factor of three or more to about 1.8 nmoles$\cdot$cm$^{-2}\cdot$s^{-1}. Subtraction of 0.6 nmole$\cdot$cm$^{-2}\cdot$s^{-1} Cl^- influx (the balancing equivalent to the measured steady state Cl^- efflux) yields a net Cl^- efflux of about 1.2 nmoles$\cdot$cm$^{-2}\cdot$s^{-1} for the peak. This value corresponds to an electrical inward current of 1.2 nmoles$\cdot$cm$^{-2}\cdot$s$^{-1}\times F=115\mu$A/cm^2, F being the Faraday constant $=96500$ A$\cdot$s$\cdot$mole^{-1}.

For comparison, in a previous study[1] E_{co} was kept at -120 mV and the changes of the current to hold E_{co} were measured upon light-off, also after 10^6 ergs$\cdot$cm$^{-2}\cdot$s^{-1}. The peak current yielded about 90 μA/cm^2. (Probably this value is also somewhat too small, because point clamp was applied.) This value agrees nicely with the calculated net 115 μA/cm^2 Cl^- mediated inward current during excitation.

If E_{co} goes below the K^+ equilibrium potential, E_k of about -90 mV, an enhanced K^+ efflux is also expected[1,5]. However, the main part of the repolarizing current is probably also due to Cl^- rather than K^+, because E_{co} soon becomes more negative than E_k, and E_{co} exceeding E_k is shown to be due to the electrogenic Cl^- influx[1,3]. The apparent low Cl^- efflux during repolarisation (Fig. 4) confirms this idea.

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