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VOLTAGE DEPENDENT POTASSIUM FLUXES AND THE SIGNIFICANCE OF ACTION POTENTIALS IN *ACETABULARIA*

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SUMMARY

Membrane potential, V_m, and K^+ ($^{86}Rb^+$) fluxes have been measured simultaneously on individual cells of *Acetabularia mediterranea*. During resting state (resting potential approx. −170 mV) the K^+ influx amounts to 0.24–0.6 pmol · cm^{-2} · s^{-1} and the K^+ efflux to 0.2–1.5 pmol · cm^{-2} s^{-1}. According to the K^+ concentrations inside and outside the cell (40 : 1) the voltage dependent K^+ flux (zero at $V_m = E_K = -90$ mV) is stimulated approx. 40-fold for V_m more positive than E_K.

It is calculated that during one action potential (temporary depolarization to V_m more positive than E_K) a cell looses the same amount of K^+, which leaks in during 10–20 min in the resting state ($V_m = -170$ mV). Since action potentials occur spontaneously in *Acetabularia*, they are therefore suggested to have a significant function for the K^+ balance of this alga.

INTRODUCTION

The significance of electrogenic pumps with regard to the resting potential of plant cells has been shown by numerous electrophysiological studies (for review see ref. 1). In the giant green alga *Acetabularia mediterranea* an electrogenic Cl^- pump is suggested to give rise to the high resting potential of approx. −170 mV, which exceeds the potassium equilibrium potential E_K by about 80 mV ($E_K = -90$ mV; outside K^+ concentration $[K^+]_o$: 10 mM, measured inside K^+ concentration $[K^+]_i$: 400 mM [2–4]). This can be demonstrated by the blocking of the electrogenic pump. Inhibition of the energy metabolism with appropriate agents or by low temperatures causes the membrane potential V_m to depolarize to a stable level near E_K [2, 3]. Since the normal resting potential is significantly more negative than E_K, a net K^+ influx along its electrochemical gradient should be expected. The cells however, keep $[K^+]_i$ at 400 mM. Therefore, they somehow must be able to counteract an immoderate accumulation of K^+. This applies for *Acetabularia* as well as for other systems with a resting potential more negative than E_K, for instance *Nitella* [5], *Neurospora* [6], *Vallisneria* [7] and *Riccia* [8]. In principle, an active K^+ export might be suggested. A mechanism involving the action potentials is proposed in this paper.

Action potentials at biomembranes appear to be a universal phenomenon. Their function in systems where they obviously do not serve for signal transmission is still dubious. In *Acetabularia* action potentials have been suggested to be involved in morphogenesis [9]. The aim of this study is to demonstrate the significance of the action potential with respect to the K^+ balance of *Acetabularia*. This will be done on the basis of experiments where both membrane potential and K^+ flux were measured simultaneously.

MATERIALS AND METHODS

Cells of the unicellular giant marine alga *A. mediterranea* were cultured in Erdschreiber solution according to Hämmerling [10] and Beth [11]. Only cells that had not yet developed a cap were taken for experiments because of their nearly cylindrical geometry. ^{86}Rb (T1/2 : 18.7 d; Amersham-Buchler) was taken for K^+ flux measurements. The calculations of the K^+ fluxes were made on the supposition that $^{86}Rb^+$ fluxes reflect the K^+ fluxes with a 1 : 1 stoichiometry. However, deviations from this stoichiometry cannot be excluded, and a $^{86}Rb^+/K^+$ flux ratio of 1 : 2 might also be realistic [12, 13]. Radioactivity was determined in a liquid scintillation counter. All data are corrected for ^{86}Rb decay.

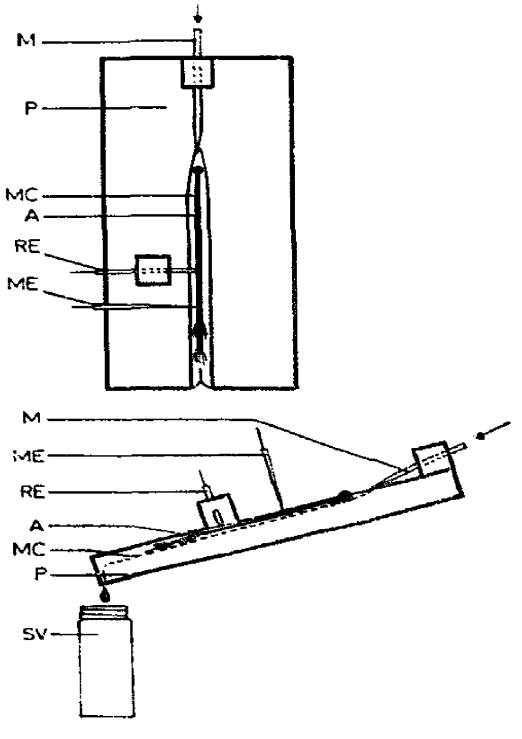

Fig. 1. Diagrammatic view of the apparatus for simultaneous measurement of intracellular potential (V_m) and tracer ion ($^{86}Rb^+$) efflux. A, ^{86}Rb labelled *Acetabularia* cell; M, medium supply; MCl, medium conduit; ME, glass microelectrode; P, plexiglass base plate; RE, reference electrode; SV, scintillation vial.

The algae were incubated in artificial sea-water according to Bentrup [14], containing the following component: 461 mM Na^+, 10 mM K^+, 53 mM Mg^{2+}, 10 mM Ca^{2+}, 529 mM, Cl^- 28 mM, SO_4^{2-}, 2 mM HCO_3^-, 10 mM Tris · HCl buffer. For labelling, part of the potassium (0.1–10 %) was substituted by $^{86}Rb^+$.

Algae with a length of approx. 15 mm and a diameter of approx. 0.15 mm were taken for uptake measurements. For each assay, 15–20 cells were incubated in 10 ml medium containing a radioactivity of 0.1 $\mu Ci \cdot ml^{-1}$. In order to measure $K^+(^{86}Rb^+)$ uptake, samples of 3–4 cells were taken out of the medium at different times after the beginning of the incubation. The radioactivity of the cells was determined.

Cells 30–40 mm in length and approx. 0.3 mm in diameter were taken for efflux experiments. Samples of 10 algae were incubated in 5 ml medium containing a radioactivity of 0.2 $mCi \cdot ml^{-1}$ for each assay.

Efflux experiments were carried out on the device shown in Fig. 1, which allowed a continuous washout of tracer ions with non-radioactive artificial sea-water. Time intervals between drops were set by the height of the medium reservoir. Simultaneously the intracellular potential V_m was recorded by conventional microelectrode techniques. Since V_m is light-dependent [2, 3], changes of the membrane potential were accomplished by a light-dark regime.

In order to obtain $K^+(^{86}Rb^+)$ flux as a function of V_m, the amount of radioactivity released at certain V_m values during the time course of an experiment was read in the original recordings (compare Fig. 4). The average release of radioactivity at the respective potential values was calculated as pmol $K^+ \cdot cm^{-2} \cdot s^{-1}$ and plotted against the particular membrane potential (see Fig. 5).

RESULTS

Influx

The time course of $K^+(^{86}Rb^+)$ uptake into *Acetabularia* cells in light ($5.2 \cdot 10^2 \cdot erg \cdot cm^{-2} \cdot s^{-1}$) is illustrated in Fig. 2. There is a fast increase (time constant τ

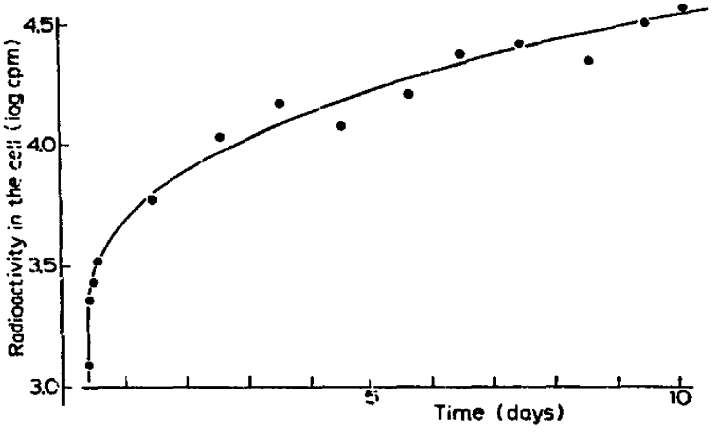

Fig. 2. Uptake of $K^+(^{86}Rb^+)$ into *Acetabularia* cells (length, 15 mm; diameter, 0.15 mm) in light ($5.2 \cdot 10^2 \cdot erg \cdot cm^{-2} \cdot s^{-1}$); ordinate: total radioactivity in the cell (log cpm), abscissa: time after beginning of incubation (days).

TABLE I

$K^+(^{86}Rb^+)$ FLUXES OF *ACETABULARIA*

	Influx	Efflux
Flux (pmol · cm^{-2} · s^{-1})	0.24–0.6	0.2–1.5
Rate constant (s^{-1})	$3.1 \pm 0.2 \cdot 10^{-7}$	$1.0 \pm 0.2 \cdot 10^{-7}$
Light (W m^{-2})	0.52	84

in the range of minutes) of radioactivity in the cells followed by a slow increase with $\tau = 34$ days. The fast increase is probably due to $K^+(^{86}Rb^+)$ uptake into the cell wall, whereas the slow component is likely to represent $K^+(^{86}Rb^+)$ uptake into the protoplast without further discrimination of the compartments "cytoplasm" and "vacuole". Table I shows $K^+(^{86}Rb^+)$ uptake data in pmol $K^+ \cdot cm^{-2} \cdot s^{-1}$, calculated according to Hope [15] for a single compartment system.

Efflux

Fig. 3 represents continuous $K^+(^{86}Rb^+)$ washout kinetics of *Acetabularia* cells in light ($8.4 \cdot 10^4 \cdot$ erg · cm^{-2} · [illegible], which displays two phases with different time constants. The fast component at the beginning proves to be non-linear in a semi-logarithmic plot, thus not fitting the presumptions to be demanded for tracer ion efflux from a 2-compartment system in series [15, 16]. A formal mathematical treatment as a system of two compartments in series would yield a K^+ concentration in the cytoplasm of about 8 mM, which is much less than the measured concentration of 400–500 mM [3, 4]. The fast component of the kinetics is therefore concluded to represent loss of residual tracer from the cell wall. The $K^+(^{86}Rb^+)$ efflux kinetics remain single-phased for at least 10 h, thus indicating $K^+(^{86}Rb^+)$ efflux from one compartment. This finding suggests the $K^+(^{86}Rb^+)$ permeability of the tonoplast to

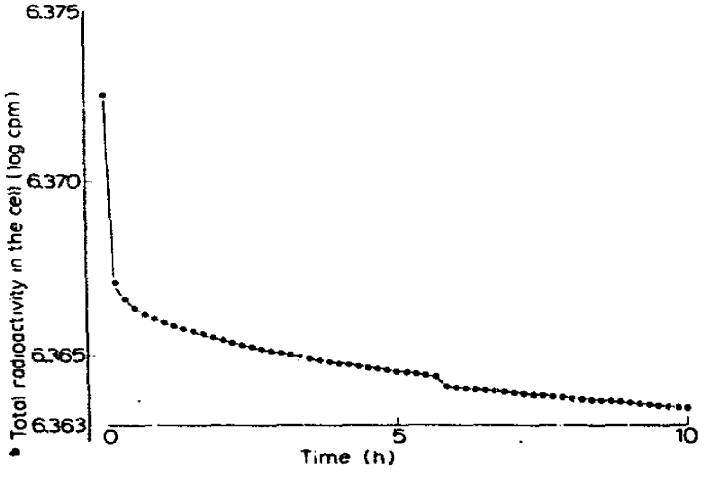

Fig. 3. Example of $K^+(^{86}Rb^+)$ washout kinetics of an *Acetabularia* cell; ordinate: time after switching from the incubation medium (1 mCi $^{86}Rb^+$ in 5 ml artificial sea-water) to cold medium. Light: $8.4 \cdot 10^4 \cdot$ erg · $cm^{-2} \cdot s^{-1}$.

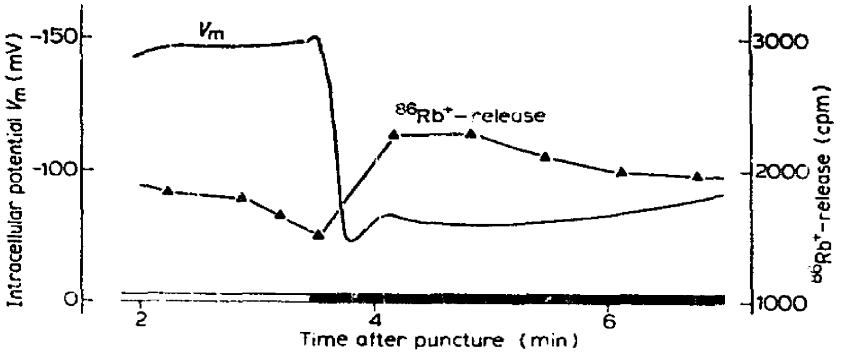

Fig. 4. Example of the time course of membrane potential V_m (—) and $^{86}Rb^+$ release (▲— ▲) of an *Acetabularia* cell in a light-dark regime.

be higher than the $K^+(^{86}Rb^+)$ permeability of the plasmalemma. The results of the corresponding analysis for a single-compartment system according to Hope [15] and Cram [16] are compiled in Table I.

Between 5 h 40 min and 5 h 50 min after the beginning of the washout experiment (Fig. 3) a transposition of the efflux kinetics appears. Within this period, K^+ ($^{86}Rb^+$) efflux appears to be increased by the factor 45. An even higher increase could have happened in a shorter period of time, since the measured efflux only represents an average of a 10 min interval. After this transposition the kinetics continue with the same slope as before. This phenomenon will be discussed below.

Voltage dependent efflux

Fig. 4 shows a $K^+(^{86}Rb^+)$ washout experiment with simultaneous recording of the membrane potential V_m. In Fig. 5A and B the measured $K^+(^{86}Rb^+)$ flux is plotted versus the actual voltage (Φ/V_m-diagrams). They prove the $K^+(^{86}Rb^+)$ efflux to be significantly correlated with the membrane potential.

Shifting V_m by 40 mV from −130 mV to −90 mV ($\approx E_K$) causes the K^+ ($^{86}Rb^+$) efflux to increase only by approx. 6.17 pmol $K^+ \cdot cm^{-2} \cdot s^{-1} \cdot V^{-1}$. Further depolarization of V_m by another 40 mV results in a 42-fold increase of $K^+(^{86}Rb^+)$ efflux to about 260 pmol $K^+ \cdot cm^{-2} \cdot s^{-1} \cdot V^{-1}$.

The transposition of the efflux kinetics demonstrated in Fig. 3 is therefore suggested to be due to a temporary depolarization beyond E_K, which is shown to give rise to a drastic increase of $K^+(^{86}Rb^+)$ efflux.

As mentioned before, changes of $K^+(^{86}Rb^+)$ efflux in the membrane potential range of −90 mV to −130 mV are little. However, Fig. 5A shows a further decrease of $K^+(^{86}Rb^+)$ efflux of about 19 pmol $K^+ \cdot cm^{-2} \cdot s^{-1} \cdot V^{-1}$ if V_m becomes more negative than −130 mV. The cause of this additional decrease of $K^+(^{86}Rb^+)$ efflux at high V_m is not clear yet.

DISCUSSION

Voltage-dependence of $K^+(^{86}Rb^+)$ fluxes

According to the different K^+ concentrations at both sides of the membrane ($[K^+]_o = 10$ mM, $[K^+]_i \approx 400$ mM) the limiting conductances (g_K) of the K^+

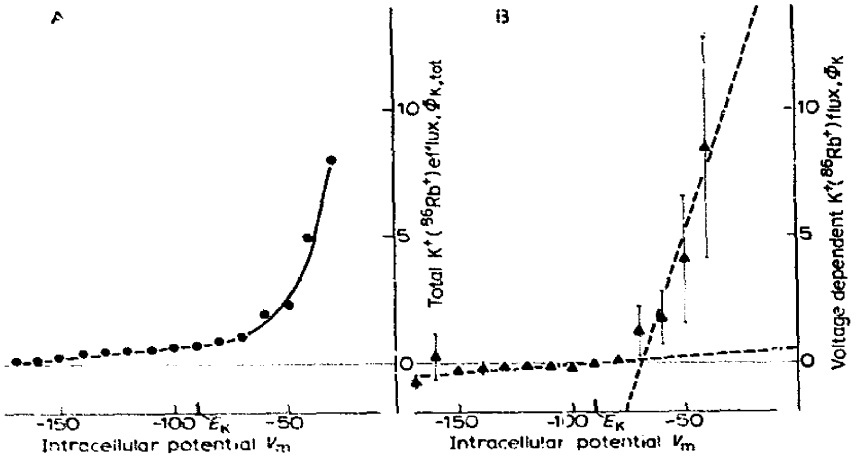

Fig. 5. $K^+(^{86}Rb^+)$ flux, Φ_K (pmol $K^+ \cdot cm^{-2} \cdot s^{-1}$), versus V_m (mV). A: total efflux including voltage-dependent and voltage independent components; B: voltage-dependent net flux, which is zero at $V_m = E_K$. Vertical bars indicate S.E. Dashed lines indicate linear slope of net Φ_K for V_m more negative and V_m more positive than E_K (slope ratio, 1 : 42, see text).

diffusion system yield a ratio of 40 : 1 for large depolarization (g_{K_i}) and hyperpolarization (g_{K_o}), respectively [17]. This implies low K^+ conductance for K^+ uptake and high K^+ conductance for K^+ efflux. This diode characteristic of the K^+ channel has been supported by electrical measurements [3, 18]. In fact, the Φ/V_m-diagram in Fig. 5B shows a 42-fold increase of $K^+(^{86}Rb^+)$ efflux for membrane voltages more positive than E_K. At $V_m = E_K$ no net K^+ flux is expected according to the electrochemical equilibrium. The still measured $K^+(^{86}Rb^+)$ efflux (Fig. 5A) can be interpreted by electroneutral $K^+(^{86}Rb^+)$ exchange. Subtraction of this amount from the entire measured $K^+(^{86}Rb^+)$ efflux leads to the net $K^+(^{86}Rb^+)$ flux as a function of V_m (Fig. 5B and Fig. 6): low $K^+(^{86}Rb^+)$ influx at V_m more negative than E_K, no net $K^+(^{86}Rb^+)$ flux at $V_m = E_K$, and high $K^+(^{86}Rb^+)$ efflux at V_m more positive than E_K.

Obviously a net $K^+(^{86}Rb^+)$ influx occurs at the resting potential (Fig. 5B and Fig. 6). Since $[K^+]_i$ is kept below the electrochemical equilibrium, however, a balancing K^+ export has to be postulated.

$K^+(^{86}Rb^+)$ fluxes during action potentials

Among the different possible ways to accomplish K^+ balance (like active K^+ export for example) one attractive mechanism seems to apply for *Acetabularia*. Temporary depolarizations to membrane voltages more positive than E_K give rise to an increased K^+ efflux due to the electrochemical gradient and the high g_{K_o}. During this period there is a considerable loss of K^+. It has to be shown now, whether such depolarizations occur and what significance they might have with respect to the K^+ balance of the cell.

Spontaneous depolarizations to membrane voltages more positive than E_K are observed and described as action potentials [2, 3, 17] (Fig. 6). The amount of K^+ released during one action potential has been calculated from the Φ/V_m diagrams

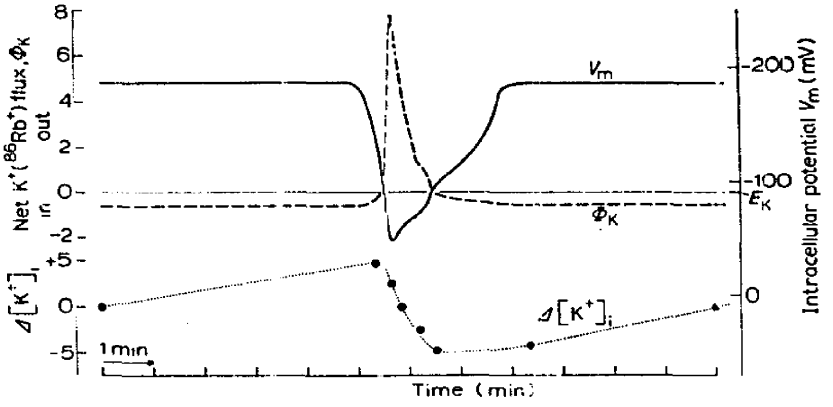

Fig. 6. Example of the time course of net $K^+(^{86}Rb^+)$ flux, Φ_K (pmol $K^+ \cdot cm^{-2} \cdot s^{-1}$), (– – –); and change of internal K^+ concentration, $\Delta[K^+]_i$ (μM), (. . .); during a spontaneous action potential (—); horizontal line indicates E_K.

(Fig. 5) by integrating the voltage dependent $K^+(^{86}Rb^+)$ fluxes in the time during the course of an action potential. The results illustrated by Fig. 6 give the time course of V_m (solid line) and net $K^+(^{86}Rb^+)$ flux (dashed line) during a spontaneous action potential. The corresponding change of the intracellular K^+ concentration, $\Delta[K^+]_i$, can be calculated now from the geometry of the cell. This is also plotted in Fig. 6 (dotted line).

During an action potential, the voltage-dependent $K^+(^{86}Rb^+)$ efflux is about 0.2 pmol $K^+ \cdot cm^{-2} \cdot s^{-1}$ at −80 mV, and already 2 pmol $K^+ \cdot cm^{-2} \cdot s^{-1}$ at −60 mV (compare Fig. 5B). Since V_m is between −65 mV and −55 mV for about 5 s during the depolarization phase, 10 pmol K^+ leave the cell within this period. The action potential in our example reaches a voltage minimum at approx. −50 mV. This occurs about 16 s after V_m had passed E_K. During this time, the cell has lost 80–100 pmol K^+. Including major loss of K^+ during the slow repolarization phase to E_K, the total loss of K^+ during one action potential is calculated to be 300–400 pmol K^+. Taking a K^+ influx of 0.6 pmol $\cdot cm^{-2} \cdot s^{-1}$ (Table 1), 8–11 min suffice to accumulate 300–400 pmol K^+. Therefore, the K^+ level of the cell would be reset with one action potential occurring every 8–11 min.

Acetabularia reveals spontaneous action potentials with a frequency of 6 action potentials per h and more [9, 19]. In this case, the suggested mechanism alone might suffice to balance $[K^+]_i$. However, spontaneous action potentials can occur less frequently (every 15–25 min or more) and with smaller amplitude, so that we cannot exclude additional mechanisms for K^+ balance of the *Acetabularia* cell.

Since some other systems with a resting potential more negative than E_K, like *Nitella* or *Neurospora* display spontaneous action potentials [5, 20], the suggested mode of control of $[K^+]_i$ by means of action potentials might be no special feature of *Acetabularia*.

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