*J. exp. Biol.* **126**, 111–117 (1986)
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# CHANGES IN MEMBRANE POTENTIAL DURING ADAPTATION TO EXTERNAL POTASSIUM IONS IN *PARAMECIUM CAUDATUM*

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*Accepted 1 July 1986*

## SUMMARY

When *Paramecium* are transferred from the solution to which they have been adapted to a solution of a different $K^+$ concentration, they initially change their swimming behaviour and then gradually recover normal swimming. This adaptation to the new solution takes 2–3 h. We found that in the process of adaptation there was a change in membrane potential: transfer of the cells to a lower $K^+$ concentration caused depolarization of the potential, whereas transfer to a higher concentration produced a hyperpolarization. In both cases, the change in potential was followed by a gradual repolarization to the original value in the control solution. During adaptation, the intracellular concentration of $K^+$ did not change much. It is probable that the cells change their membrane potential towards a constant level by controlling the membrane conductance for $K^+$.

## INTRODUCTION

A ciliated protozoan, *Paramecium*, can alter its swimming behaviour in response to a change in the ionic concentration of the environment (Jennings, 1906). When the $K^+$ concentration of the surrounding medium is lowered, the cells initially accelerate forward swimming and then gradually recover normal swimming (Nakaoka *et al.* 1983). However, when they are transferred to a solution of a higher $K^+$ concentration, they show transient backward swimming and then recover (Mast & Nadler, 1926). Within a limited range of $K^+$ concentration they can adapt to the new environment.

A close relationship between swimming behaviour and membrane potential has been found in *Paramecium* (Machemer, 1974; Machemer & Eckert, 1975; Naitoh & Eckert, 1974). Hyperpolarization of the membrane potential causes an increase in the

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Key words: *Paramecium*, adaptation, membrane potential.

beating frequency of cilia and depolarization causes ciliary reversal. We have therefore measured the change in potential produced by a change in $K^+$ concentration and examined the potential during adaptation.

## MATERIALS AND METHODS

### *Cells*

*Paramecium caudatum* (syngen 3, mating type V, stock Ksy1) was cultured at 25 °C in a hay infusion inoculated with *Klebsiella pneumoniae*. *Paramecium*, at a stationary growth phase, were collected by low speed centrifugation and incubated for about 3 h in various concentrations of KCl, in 0·25 mmol $l^{-1}$ $CaCl_2$ and 1 mmol $l^{-1}$ Tris-HCl, pH 7·2.

### *Determination of swimming velocity*

*Paramecium* were photographed while swimming in a rectangular glass vessel mounted on a copper plate whose temperature was controlled at 25 °C by water flow beneath it (Nakaoka & Oosawa, 1977). Swimming velocity was measured by averaging about 50 tracks on a photograph exposed for 2 s under dark-field illumination of the vessel.

### *Electrical recordings*

Membrane potential was measured by the method of Naitoh & Eckert (1972). *Paramecium* were placed in a small glass vessel mounted on the stage of an inverted microscope (Olympus, CK), and microelectrodes were inserted into the cell from above. The recording and current-passing electrodes were filled with 1 mol $l^{-1}$ KCl: their resistances were between 30 and 50 MΩ. A similar electrode was placed just outside the cell. The membrane potential was measured as the voltage difference between the internal and external electrodes after nulling the tip potentials in the bath. The potential was obtained 5–10 min after the experimental chamber had been filled with a test solution. The test solution usually contained various concentrations of KCl, 0·25 mmol $l^{-1}$ $CaCl_2$ and 1 mmol $l^{-1}$ Tris-HCl, pH 7·2.

### *Intracellular $K^+$ concentration*

Intracellular concentration of $K^+$ was measured by atomic absorption. After the cells had been adapted to a test solution for about 3 h, they were collected by centrifugation and washed twice with a solution containing 0·25 mmol $l^{-1}$ $CaCl_2$ and 1 mmol $l^{-1}$ Tris-HCl, pH 7·2. Part of the cell suspension was diluted 10 or 100 times and the cell density was counted by recording a video image at low magnification. This diluted suspension was then concentrated to $10^5$ cells $ml^{-1}$ and heated in a boiling water bath for 4 min. Disrupted cells were removed by centrifugation and the supernatant was used for the measurement. Cell volume was assumed to be $5·6 \times 10^{-7}$ ml (Fortner, 1925).

## RESULTS

### *Behavioural adaptation to various $K^+$ concentrations*

The time taken for adaptation to different concentrations of $K^+$ was determined by measuring swimming velocity at intervals in solutions of various $K^+$ concentrations after initial adaptation to 4 mmol $l^{-1}$ $K^+$. When external $K^+$ concentration was lowered, the velocity increased between two- and three-fold, and then decreased to a stationary value which was nearly equal to that before the change. This adaptation took 3–4 h (Fig. 1). However, when the cells were transferred to solutions of higher $K^+$ concentration, the swimming velocity did not change, but initially there was a short period of backward swimming.

### *Membrane potential during adaptation to various $K^+$ concentrations*

Membrane potential was measured at intervals after transfer of adapted cells into solutions of different $K^+$ concentrations. Cells transferred to 2 mmol $l^{-1}$ $K^+$ after adaptation to 8 mmol $l^{-1}$ $K^+$ showed a membrane potential that gradually increased from its initial low value (Fig. 2). In cells adapted to 2 mmol $l^{-1}$ $K^+$ and then transferred to 8 mmol $l^{-1}$ $K^+$, the membrane potential (measured during brief intervals in 2 mmol $l^{-1}$ $K^+$) gradually decreased to the same value as that of the cells adapted to 8 mmol $l^{-1}$ $K^+$ (Fig. 2).

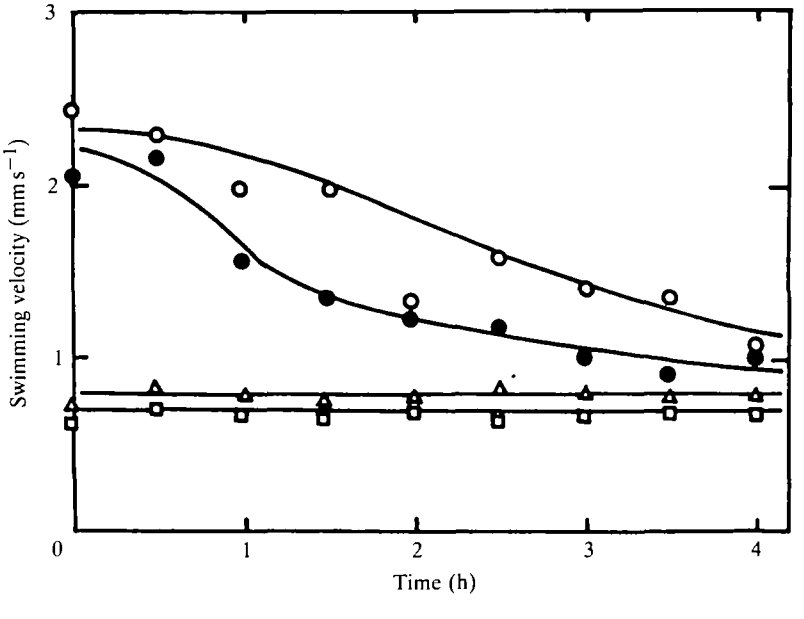


Fig. 1. Time courses of behavioural adaptation to various $K^+$ concentrations. *Paramecium* adapted to a solution of 0·25 mmol $l^{-1}$ $CaCl_2$ and 4 mmol $l^{-1}$ KCl were transferred to a solution containing 0·25 mmol $l^{-1}$ $CaCl_2$ and 1 (○), 2 (●), 4 (□) or 16 mmol $l^{-1}$ (△) KCl, after which, at the time indicated on the abscissa, a small sample of the cell suspension was pipetted out and dropped into a freshly prepared solution of the same composition. The swimming velocity was measured 1 min later.

*Membrane potential after adaptation to various $K^+$ concentrations*

The dependence of membrane potential upon external $K^+$ concentration was measured after 3 h adaptation to various $K^+$ concentrations (Fig. 3). The potential was proportional to $K^+$ concentration over the range 0·5–16 mmol $l^{-1}$. More negative potentials were obtained for a given $K^+$ concentration after adaptation to higher $K^+$ concentrations.

*Membrane resistance of the cells adapted to various $K^+$ concentrations*

Membrane resistance was measured by injecting a small hyperpolarizing current through the recording microelectrode, at $K^+$ concentrations of 2 and 8 mmol $l^{-1}$, after adaptation to either 2 or 8 mmol $l^{-1}$ $K^+$ (Table 1). Resistance was reduced both when measured at 8 mmol $l^{-1}$ $K^+$ and in cells adapted to 8 mmol $l^{-1}$ $K^+$.

*Intracellular $K^+$ concentration*

Intracellular $K^+$ concentration, measured by atomic absorption, was found to be 21 ± 3 mmol $l^{-1}$ (mean of three different samples) in cells adapted to 2 mmol $l^{-1}$ $K^+$ for 3 h, and 23 ± 2 mmol $l^{-1}$ (mean of three different samples) after adaptation to 8 mmol $l^{-1}$ $K^+$.

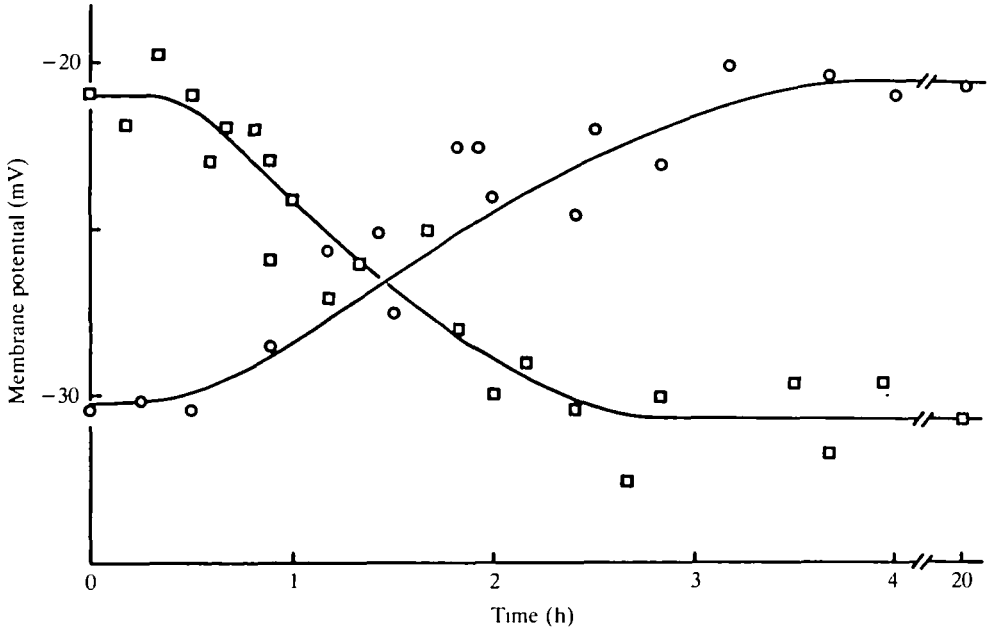


Fig. 2. Change in membrane potential during adaptation to $K^+$. Cells adapted to a solution containing 2 mmol $l^{-1}$ KCl (□) were transferred to a solution containing 8 mmol $l^{-1}$ KCl. Cells adapted to a solution containing 8 mmol $l^{-1}$ KCl (○) were transferred to a solution containing 2 mmol $l^{-1}$ KCl at time zero. Membrane potentials were measured in a solution of 2 mmol $l^{-1}$ KCl.

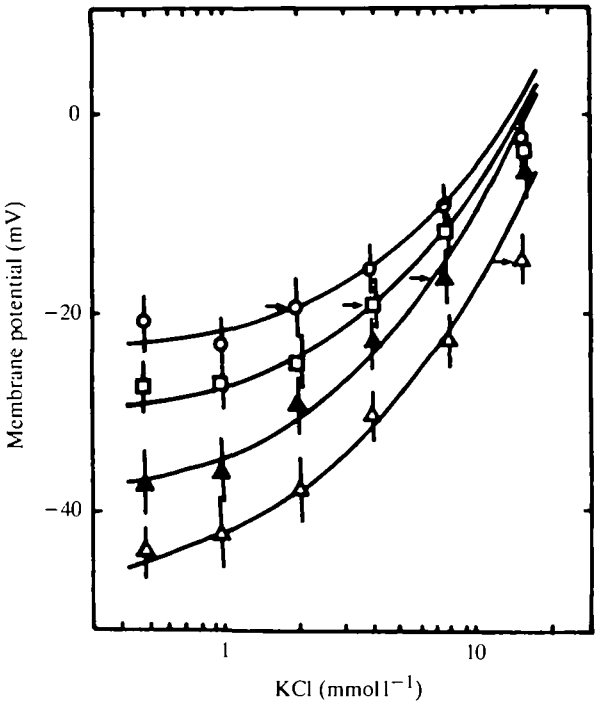


Fig. 3. Effect of $[K^+]_o$ on membrane potentials of cells adapted to various concentrations of $K^+$. Cells were adapted to a solution of 2 (○), 4 (□), 8 (▲) or 16 mmol l$^{-1}$ (△) KCl, and membrane potentials were measured in a solution containing the KCl concentration indicated on the abscissa. Arrows show the membrane potentials at the condition in which the cells were adapted. The curves were drawn to fit measured points using equation 1 in the Discussion with $[K^+]_i = 20$ (○, □), 22 (▲) and 27 mmol l$^{-1}$ (△) and $p_K/p_{Ca} = 0{\cdot}09$ (○), 0·13 (□), 0·17 (▲) and 0·25 (△). $N = 3$–5. Bars represent standard deviations.

## DISCUSSION

When *Paramecium* were transferred to media of different $K^+$ concentrations, they changed swimming behaviour and then recovered (Fig. 1). During the period of adaptation, they changed the resting potential to make it almost independent of the new $K^+$ concentration (Figs 2, 3).

In the process of adaptation, the $K^+$ concentration inside the cell changed only slightly, but membrane resistance showed significant changes (Table 1). According

Table 1. *Membrane resistance (MΩ)* of cells adapted to $K^+$*

| $[K^+]$ for adaptation (mmol l$^{-1}$) | $[K^+]$ for measurement (mmol l$^{-1}$) 2 | 8 |
|---|---|---|
| 2 | 58 ± 8 | 32 ± 8 |
| 8 | 35 ± 5 | 21 ± 3 |

* Resistances were measured by steady-state displacements in membrane potential following small square pulses (0·1 nA, 200 ms) of inward current.
Average ± S.D., $N = 5$–8.

Table 2. *$p_K/p_{Ca}$ and membrane conductance*

| $[K^+]$ for adaptation ($mmol\,l^{-1}$) | $p_K/p_{Ca}$* | Conductance (nS)† $[K^+]$ ($mmol\,l^{-1}$) for measurement | |
|---|---|---|---|
| | | 2 | 8 |
| 2 | 0·09 | 17 | 29 |
| 8 | 0·17 | 31 | 48 |

* Values used for curves in Fig. 3.
† Reciprocal of the mean of membrane resistance in Table 1.

to the data in Fig. 3, the increase of the membrane potential ($E_m$) with increasing $K^+$ concentration ($dE_m/d\log[K^+]_o$) was raised by an increase in the $K^+$ concentration of the adaptation medium. Therefore, it is probable that the $K^+$ conductance of the membrane changed during adaptation.

If the Goldman–Hodgkin–Katz equation (Goldman, 1943; Hodgkin & Katz, 1949) is applied to the membrane potential of *Paramecium*, assuming that the membrane current is mainly carried by $K^+$ and $Ca^{2+}$, then

$$E_m = (RT/F)\ln[(p_K[K^+]_o - p_K[K^+]_i + A^{1/2})/(2p_K[K^+]_i + 8p_{Ca}[Ca^{2+}]_i)] \quad (1)$$

and

$$A = p_K{}^2([K^+]_i - [K^+]_o)^2 + 4(p_K[K^+]_o + 4p_{Ca}[Ca^{2+}]_o)\,(p_K[K^+]_i + 4p_{Ca}[Ca^{2+}]_i),$$

where $p_K$ and $p_{Ca}$ are ion permeabilities of the membrane for $K^+$ and $Ca^{2+}$; $[K^+]_o$ and $[K^+]_i$, $[Ca^{2+}]_o$ and $[Ca^{2+}]_i$ are concentrations of $K^+$ and $Ca^{2+}$ outside and inside the cell, respectively. $[Ca^{2+}]_i$ was lower than $10^{-7}\,mol\,l^{-1}$ and $[K^+]_i$ was measured (see Results). $[K^+]_o$ and $[Ca^{2+}]_o$ were known in each experiment. Thus, the best fitting of the calculated value of $E_m$ to the experimental data in Fig. 3 was found by adjusting the value of the ratio $p_K/p_{Ca}$. The continuous curves in Fig. 3 are obtained from the equation by using the value of $p_K/p_{Ca}$ shown in the legend to Fig. 3. The agreement with the data was satisfactory. $p_K/p_{Ca}$ was greatest at the highest $K^+$ concentrations in the adaptation medium.

Table 2 summarizes the $p_K/p_{Ca}$ values and the membrane conductances of cells adapted to 2 and 8 $mmol\,l^{-1}$ $K^+$. Since the $K^+$ conductance ($g_K$) is much larger than the $Ca^{2+}$ conductance ($g_{Ca}$) in the present experimental conditions (Naitoh & Eckert, 1974), the membrane conductances in Table 2 almost correspond to $g_K$. The $p_K/p_{Ca}$ values in the two groups of cells agree well with the ratio of membrane conductances, supporting the hypothesis that the change in membrane potential is mainly caused by the change in $g_K$.

Thus, it is very probable that, during adaptation, *Paramecium* change the membrane conductance or permeability to $K^+$, resulting in maintenance of the resting potential at a constant level. It is not known, however, whether the change of conductance is due to a change in the number of channels or a change in the permeability of single channels.

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