EFFECT OF DECREASING ELECTRICAL RESISTANCE IN *CHARACEAE* CELL MEMBRANES CAUSED BY THE FLOW OF ALTERNATING CURRENT

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ABSTRACT

By means of the techniques of external electrodes and microelectrodes, it was found that evanescent flow of an alternating current through plasmalemma of *Characeae* cells neutralises oscillatory change in their electrical resistance and reversibly diminishes its value. This effect is particularly significant in the case of "high resistance cells", but it weakens with increasing temperature. The value of the estimated activation energy indicates that, after flow of the alternating current through the membrane, a rapid increase in the conductivity may be caused by an increase in conductivity of potassium channels. This result seems to support the hypothesis of electroconformational feedback.

KEY WORDS: *Characeae*, resistance changes, oscillations, Arrhenius plots.

INTRODUCTION

During our study of oscillatory changes in electrical parameters of plant membranes we found that an alternating current modifies and partly neutralises the oscillatory behaviour, e.g., their electrical resistance. Identification of membrane reaction in the flow of alternating current of low intensity through it, not causing depolarisation, was recognised to be important for explanation of the mechanism of generation of oscillation behaviour (Śpiewla and Tokarska 1990, Fisahn and Lucas 1991, Tokarska and Śpiewla 1994).

This problem seemed to be important also in context of the results of our previous work (Skierczyńska et al. 1973) in which it was shown that quasi-parallel measurements of the membrane conductivity of *Chara brauni*, *Nitellopsis obtusa* and *Nitella flexilis* cells, by methods which used direct and alternating currents, gave different results. The resistance values obtained by the direct current method were relatively high and unstable in time, but those obtained by the alternating-current method were low and stable. In both methods, very small current densities were used, which excluded changes in the resistance caused by depolarisation or pseudoinduction effects described by Homblé and Jenard (1984). In order to explain this effect, we advanced the hypothesis of existence of two high resistance layers in the vicinity of the cell wall. One of the two layers, the interior one of high electrical capacity, is, responsible for the unstable value of the resistance. The results presented in this work seem to confirm this hypothesis.

MATERIALS AND METHOD

The measurements were made on internodal cells of two plant species from the *Characeae* family: *Nitellopsis obtusa*

(Desv. in Lois) J. Gr. and *Nitella flexilis* f. *Atkahensis* R.D.W. The plants, collected from Lake Zaglebocze, were cultured in the laboratory in aquarium with natural pond water at the temperature 16.5-19°C (under natural day/night light cycle). One day prior to the experiment, the single internodal cells were isolated from the associated branches and stored until the beginning of the experiment. Cells 2-10 cm long and about 0.45 mm in diameter were investigated.

As experimental solutions we used natural lake water (NPW; natural pond-water ingredients: 0.06 mM K⁺, 0.28 mM Cl⁻, 0.29 mM Na⁺, 0.01 mM Mg²⁺; pH 7.0 ± 0.2) and the standard artificial solution (APW; artificial pond water from distilled water containing 0.1 mM KCl, 0.1 mM NaCl and 0.1 mM CaCl₂; pH was adjusted to 7.0 with 2mM HEPES and 10% NaOH).

The measurements of membrane resistance were made by external and microelectrode techniques described by Skierczyńska et al. (1972).

RESULTS

A series of experiments was conducted, in which the values of the membrane resistance measured by means of direct current, before and after the flow of an alternating current through the cell, were compared to the effect of alternating current, and a transient decrease in the membrane electrical resistance with a rapid return to the initial value was observed. For a series of eight *Nitellopsis obtusa* cells, a 57 ± 8% decrease in the average electrical resistance of their membranes was found.

Figure 1 presents the course of changes in the resistance of a randomly chosen cell of *Nitellopsis obtusa*. The membrane resistance was measured every 15 minutes, twice before and after the flow of alternating current for 60 s at a frequency of

Fig. 1. Membrane resistance as a function of time for *Nitellopsis obtusa* cell. Measurements were made twice, every 15 minutes: before (circles connected by solid line) and after (squares connected by solid line) passing of alternating current for 60 s (23 Hz 0.1 μ A). In the right top corner is the kinetics of resistance recovery to the initial value (resistance value after exposure to alternating current expressed as a fraction of the value of resistance measured before passing of alternating current).

23 Hz and an amplitude of 0.1 μ A. It may be seen that the resistance values obtained before the flow of the alternating current are much higher (average value 13.7 ± 4.1 k Ω cm 2) than those measured after the flow (average value 2.3 ± 0.31 k Ω cm 2).

The effect of diminution and stabilisation of resistance was especially visible in cells of high resistance. This confirms the observations made by Skierczyńska et al. (1973) who separated "high resistance cells of unstable resistance". The de-

pendence of this effect on the applied intensity of alternating current and on the time of its flow is presented in Fig. 2A and B. As seen from this figure, the value of this effect increases with the applied intensity as well as the time of flow of alternating current.

It was also found, that this effect depends on the frequency of the applied alternating current. Two kinds of reaction may be separated:

Fig. 2. Proposed courses (dashed lines) of dependence of the effect of decreasing membrane resistance after the action of alternating current: (A) on intensity of alternating current used (frequency 23 Hz, time of current flow 60 s), and (B) on time of passing alternating current (frequency 23 Hz, intensity 0.1 μ A). The degree of decrease of membrane resistance is expressed as fraction of maximal value of the decrease.

Fig. 3. Proposed courses (dashed lines) of dependencies of the effect of decreasing membrane resistance after the action of alternating current on the frequency of used current (time of flowing 60 s intensity 0.1 μA). Examples of 2 kinds of observed dependencies: (A) effect became pronounced at frequencies of several Hz and remained at constant level when frequency was increased to hundreds of Hz, and (B) at frequency of 10 Hz the effect reached maximum. The degree of decrease of membrane resistance is expressed as fraction of maximal value of decrease.

1. The effect becomes significant at a frequency of several Hz and becomes stabilised at a constant level in spite of increasing frequency up to several hundred Hz (Fig. 4A);
2. The effect reached a significant maximum at about 10 Hz (Fig. 3B).

It should be emphasised that, in all kinds of the experiments described above, substitution of the interaction of alternating current by direct current of the same density does not lead to any effect.

In further experiments it was established that the effect of interaction of alternating current on the resistance of the membrane decreases with an increase in the environment temperature. Figure 4 shows the dependence of membrane resistance, before and after the flow of alternating current through the cell and also their differences on temperature changes. Figure 5 illustrates the dependence of the difference in these resistances on absolute temperature in the form of the so-called Arrhenius plot. The activation energy value estimated from the figure (42 kJ/mol) is very close to the value reported

Fig. 4. Temperature dependence of the decreasing membrane resistance after the action of alternating current (frequency 23 Hz, time of the flow 60 s, intensity 0.1 μA). Membrane resistance is before (circles connected by solid line) and after the flow of alternating current (squares connected by solid line). The dependence of these resistances on temperature is shown in the right top corner.

Fig. 5. Arrhenius plot for the dependence presented in right top corner of Fig. 4. Δr_m – changes of membran resistance.

by Beilby and Coster (1976) for equilibrium conductivity, and is mainly associated with the motion of potassium ions.

DISCUSSION

The results obtained here enable us to assume that a fast increase in membrane resistance observed after passing of alternating current may be attributed to the maximal increase in potassium conductivity. It is known that, depending on the voltage, potassium channels occur in *Characeae* cells, but in the experiments reported here, the current density used was too small to cause a depolarisation of the membrane. Thus it seems that some factor other than a decrease in the transmembrane potential is responsible for the opening of potassium channels.

In the light of a series of theoretical and experimental works (Astumian et al. 1989, Markin and Tsong 1991, Fuliński 1992 and others), dealing with the possibility of activation of transmembrane transport by external alternating electromagnetic field, causing modulation of conformational state of membrane lipids, it seems very probable that the effect described here is caused by electroconformational feedback. The results obtained by Tsong (1990), who stimulated the transport of K^+ and Rb^+ realised by $Na^+/K^+ - ATPase$ in human red blood cells by an alternating field, confirm the validity of this concept.

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EFEKT ZMNIEJSZANIA SIĘ OPORU ELEKTRYCZNEGO BŁON KOMÓREK *CHARACEAE*
WYWOŁANY PRZEPŁYWEM PRĄDU ZMIENNEGO

STRESZCZENIE

Stosując techniki elektrod zewnętrznych i mikroelektrod stwierdzono, że krótkotrwałe przepuszczanie prądu zmiennego przez plazmalemę komórek *Characeae*, znosi częściowo oscylacyjną zmienność jej oporu elektrycznego oraz wyraźnie obniża odwracalnie jego wartość. Efekt ten jest szczególnie wyraźny w przypadku komórek „wysokooporowych”, słabnie natomiast wraz ze wzrostem temperatury. Wartość wyliczonej energii aktywacji wskazuje, że szybki wzrost przewodnictwa po przepuszczeniu przez błonę prądu zmiennego, może być spowodowany wzrostem przewodnictwa kanałów potasowych. Wynik ten wydaje się potwierdzać hipotezę zwrotnego sprzężenia elektrokonformacyjnego.

SŁOWA KLUCZOWE: *Characeae*, zmiany oporu, oscylacje, pętle Arrheniusa.