Influence of ouabain on the resting potential of plant cell and isolated protopasmic droplet

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INFLUENCE OF OUABAIN ON THE RESTING POTENTIAL OF PLANT CELL AND ISOLATED PROTOPLASMIC DROPLET

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The influence of ouabain on membrane potentials of plant cell and of protoplasmic droplets have been investigated.

The reduction of resting potential both in the cell and in protoplasmic droplets were detected, but at different rates. We assume that the reason for this might be a specific structure of membrane of droplets.

It is known, that ouabain (strofantin G) acts as an inhibitor of Na^+,K^+-ATPase /7/. Many authors investigated the effects of ouabain on the action potentials of the animal exitable cells and tissues and established a relationship between the action potentials and Na^+,K^+-ATPase activity /6,7/. Some authors showed the existance of an active transport of cations in some organs of higher plant and green algae /4,5/.

In this work the effect of ouabain on the resting potentials of plant cell and isolated protoplasmic droplets have been investigated, and the obtained results compared.

The experiments were performed on freshwater algae *Nitella*. The algae were collected late in autumn and kept in tap water, under standard room conditions.

All experiments were done on cells constituting the second internodus from the top. The lenght of the cells varied from 9 to 30 mm, and their diameter from 0,35 mm to

0,45 mm. The internodal cell was immersed into the basal solution containing 90 mM KCl, 50 mM NaCl and 6mM $CaCl_2$, and amputated. The protoplasm was traped and the protoplasmic droplets were formed using an apparatus previously described /2,6/. The diameter of the droplets varied from 0,3 mm to 0,5 mm.

The membrane potential was measured using glass microelectrodes filled with 3M KCl solution. The microelectrode was inserted either into the vacuola of the cells, or into the protoplasmic droplet, with reference electrode immersed into the surrounding medium. The investigation were performed in *experimental E* solution of 0,1 mM KCl and 1,0 mM NaCl with various concentrations of ouabain (up to 0,01 M). The control potential was measured in ouabain free solution. All experiments were performed at room temperature in the dark.

The difference of the membrane potentials was tested by the "difference method" for small dependent samples and by analysis of variance /1/.

The membrane potentials of the cell and of the droplets were measured first in the basal solution, and afterwards in experimental solution. The time required to reach a stable membrane potential in experimental solution was measured too. The values of the membrane potentials, and of the gradient of increase in membrane potential in experimental solution are shown in Tab. 1.

The resting potential is found to be lower in droplets then in the cells. The data obtained are in accordance with the published ones /3,8/.

TAB. 1 Membrane potentials and gradient of increase of the potential in solution E

	U_E /mV	$\frac{\Delta U_E}{\Delta t}$/mV min^{-1}
CELL	(90.2 + 0.8)^{24}	(1.9 + 0.20)^{24}
DROPLET	(16.5 + 1.6)^{25}	(0.4 + 0.03)^{25}

Further measurements were carried on in experimental solutions containing various concentrations of ouabain. The values of resting potential (U_{EC}) were expressed in percentage of control potential, and are shown in Fig. 1.

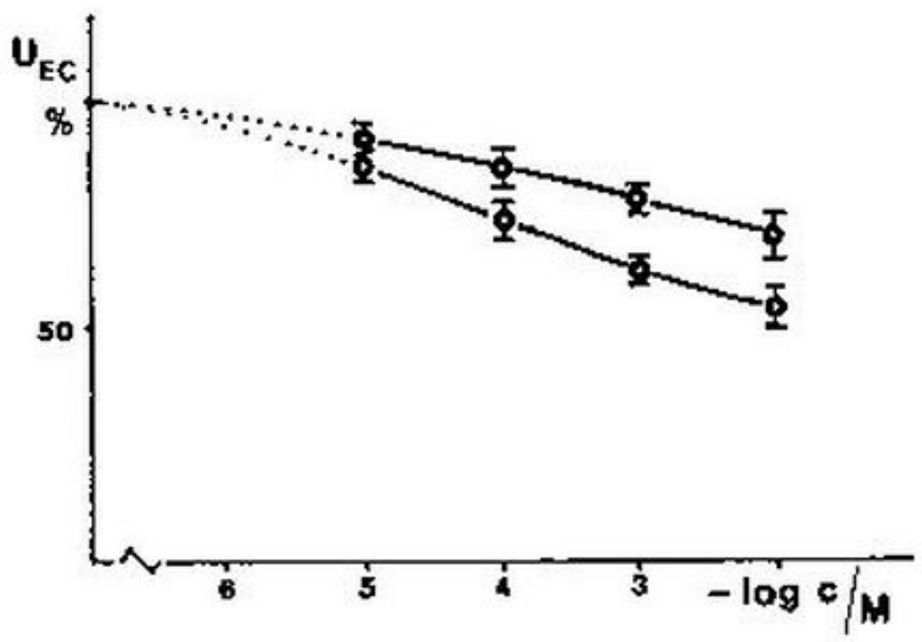

FIG. 1 The effect of ouabain on membrane potential of *Nitella* cell (full line) and of drop (dotted line)

The increase in the concentration of ouabain causes the reduction of resting potential both in the cell and in protoplasmic droplets. These reductions were of a different type and depended upon the concentration of ouabain. At higher concentrations the differences became statistically

significant.

Gradient of the increase in membrane potential of protoplasmic droplets was lower then that of the cell, as presented in Tab. 1.

The effect of ouabain on the gradient of increase of resting potential of the droplets is shown in Fig. 2. The results are expressed in percentage of control gradient.

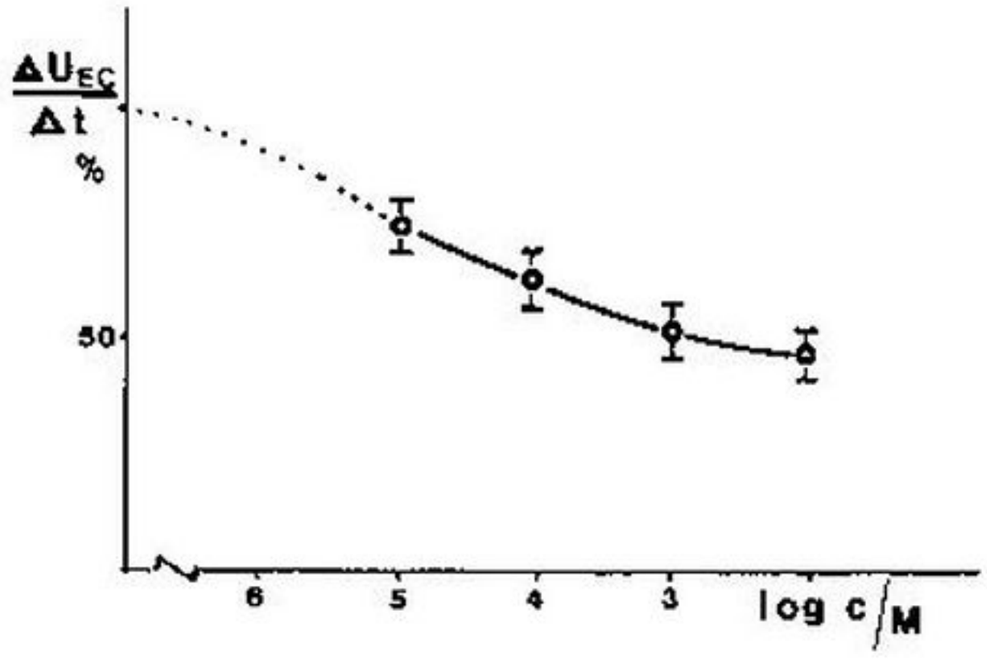

FIG. 2 The effect of ouabain on gradient of the increase of the membrane potentials of the drop

The decrement of gradient caused by higher concentrations of ouabain is statistically significant.

From the results obtained on the intact cell of *Nitella* it was possible to conclude that active transport of cations takes part in formation of its "summary" resting potential. This corresponds to early published data of inhibition of cations transport in *Nitella* /4/.

Results obtained on the isolated protoplasmic droplet

indicate that there is an active transport in droplet membrane. The differences between the responses of cell and droplets on the ouabain may be connected with reduced active transport in droplet membrane. We assume that the reason for this might be a specific structure of membrane of droplets.

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