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THE CHANGES OF STRUCTURE-FUNCTIONAL STATE OF PLASMA MEMBRANE IN *NITELLOPSIS OBTUSA* UNDER THE INFLUENCE OF DIMETHYLSULFOXIDE

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Abstract. The change regularity of potential (φ_m), resistance (R_m) and capacity (C_m) of the plasma membrane (PM) in *Nitellopsis obtusa* cells under the influence of dimethylsulfoxide (DMSO) have been studied. The investigated cells have φ_m=-171±3 mV, R_m=3,8±1,6 Om·m^2, C_m=0,92±0,04 mkF·sm^{-2} in the standard environmental conditions. The threshold concentration of DMSO 1÷4% caused the depolarization of the PM to 20-25mV and increase C_m to 22-26% in the R_m constant. The effective influence of DMSO concentration 1÷ 4% depolarizated PM to the level of -110mV. At first R_m decreased 40%, then occurres its restoration. The final stationary level of R_m was 20% higher than initial one. The value of PM depolarization depended on initial level of φ_m and the concentration of DMSO in the medium. In that case C_m only increased and its total value was 45% compared with the initial value. In the that work the effects of the solvent are discussed from the point of its influence on the structure state of lipid phase and the transport communications of PM.

Keywords: *Nitellopsis, plasma membrane, electrogenic activity, dimethylsulfoxide, potential, resistance, membrane capacity.*

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1. Introduction

Dimethylsulphoxide (DMSO) is a polar solvent with high dielectric permeability. That ensures it as an effective solution in an aqueous medium. Amphiphility of the DMSO molecule creates a condition for its rapid penetration into the organs and tissues of living systems (Ibrahimova *et al.*, 2002; Yu & Quinn, 1994). A high penetrating power of the solvent molecule in the lipid matrix of cell membranes can lead to a change in its electrical capacity and also subsequently electrogenic activity and permeability. To identify the expected effects of DMSO, it is necessary to study a complex of electrophysiological parameters of intact cells while interacting with a solvent. The investigation of such a plan allows to determine the interrelation between the changes of functional features and structurally-polarization properties of cellular membranes. Thereby, occurs the ability to regulate functional activity of cellular membranes by influencing on the structural features, in particular on the electrical capacity of the plasma membranes.

Based on the above, the aim of the present work is the comparison of change regularities of electrogenic activity, conductivity, and capacity as, indications of structural-polarization state of plasma membrane in *Nitellopsis obtusa* under the DMSO

influence. On the other side, in a significant part of the scientific-investigation work, in order to study the electrogenic and diffuse properties of DMSO cellular membranes were used polyene and other antibiotics as solvents (Yu & Quinn, 1994; Cheng *et al*., 2015). Consequently, for an accurate estimation of electrophysiological effects of polyene antibiotics, it is necessary to consider the DMSO contribution in the experimental values.

2. Materials and methods

As the object of our investigation the internodal cells of *Nitellopsis obtusa* plant have been used. The plant was grown in the laboratory condition in the artificial pond water (APW) at illumination of 20 W/m^2 and at 20-22ºC temperature. Ionic composition of APW was close to the composition of the lake water, which contained (mM/l): KH_2PO_4 - 0,1, $CaCl_2$-0,4, $NaHCO_3$-1, $Mg(NO_3)_2$-0,1, $MgSO_4$-0,1, pH=7-7,2. To ensure the measurement accuracy of the applied electrophysiological technique, the cells have been used no longer than 20 mm (Yermishkin & Zilbershtein, 1982). The diameter of such cells did not exceed 0,5 mm.

A complex of electrophysiological parameters was measured by using impedance spectroscopy made for the cells of cylindrical shape (Vorobyev & Musayev, 1979; Musayev & Vorobyev, 1981). It provided continuous and long-term recording of three electrophysiological parameters of experienced cells: membrane capacity (C_m), membrane potential (φ_m) and resistance (R_m) (Vorobyev & Musayev, 1979; Volkov, 2006). Because of low tonoplast resistance (Andrei *et al*., 2014; Cole, 1976), while introducing a measuring microelectrode into the vacuole of cells, the measured values of φ_m, C_m, R_m reflected the state of plasmatic membranes. The essence of the used methods is that, with the help of intercellular microelectrode (current microelectrode) and external *Ag-AgCl*-electrode the rectangular pulses of constant current are skipped through the middle of a cylindrical cell with density of 1mA/m^2, duration *1-2* seconds and alternating current with a frequency of *1-1000* Hz. The voltage drop across the direct and alternating currents is removed with the help of the second microelectrode (measuring microelectrode), introduced into the cell at a fixed distance from the current microelectrode. Capacitive resistance was separated from the total impedance with the help of vector diagram (Musayev & Vorobyev, 1981; Volkov, 2006). Electrical capacitance of unit area of the plasma membrane is calculated by formula:

$$C_m = \frac{1}{2\pi f x_c}$$

R_m was calculated by the magnitude of the electronically potential $\Delta\varphi_m$ (shift φ_m while current passes) and current power *I*, passing through the middle of the cell:

$$R_M = \frac{\Delta\varphi}{I} \cdot \pi dl$$

where d is a diameter, l is the the length of the experienced cells.

Membrane potential was measured as the potential difference with a measuring electrode and reference electrode. DMSO solutions were prepared for APW. Used DMSO concentrations didn't cause a shift of *pH* APW. Working DMSO solutions were introduced into the measuring chamber after establishing the stationary values of φ_m, R_m, C_m in APW.

3. Results

The variance values of φ_m plasmatic membrane in *Nitellopsis obtusa* cells were within -100÷ −258 mV, R_m- 1,1÷0,8 Om·м^2, C_m – 0,43÷1,33 mcF·sm^{-2}. The average values of these magnitudes were-171 ± 3,26 mV, 3,8 ± 1,6 Om · m^2, 0,92 ± 0,04 mcF·sm^{-2}. Between R_m and φ_m was determined linear dependence R_m=0, 0,22φ_m - 0,04 with a correlation coefficient r=0,465 (the number of cells-100). Correlation between C_m and φ_m doesn't exist.

Electrophysiological reactions of plasmatic membrane parameters in cells quantitatively and qualitatively depended on DMSO concentrations and initial φ_m level. Threshold DMSO concentration in APW content was 1% ($8\cdot10^{-4}$ m/l). With the appearance of the noted DMSO concentration in APW content, plasmatic membrane was hyperpolarized to 20-25 mV within 40 min (Fig.1). It happened at the constant level of membrane resistance R_m= 4,4Om·m^2. Here with the capacity of plasmatic membranes increased to 22-26%. The hyperpolarization of plasmatic membranes of 20-25 mV under the influence of 1% DMSO concentration on cells was characteristic for all the cells with the original φ_m ~ -150÷ -180mV. At the hyperpolarized state the cells can be hold within an hour and more. The washing of cells from the threshold concentration of DMSO also lasted enough longer -80÷ 90 min.

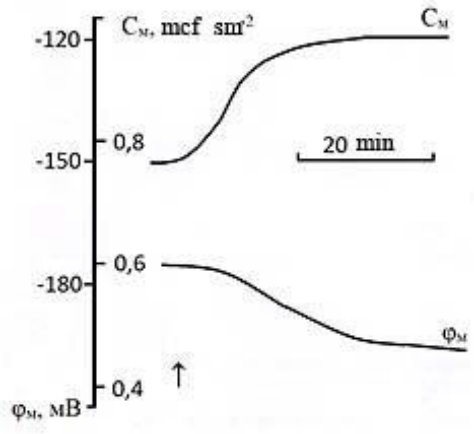

Figure 1. Kinetics of membrane potential change (φ_m) and membrane capacity (C_m) of *Nitellopsis obtusa* cells while including 1% dimethylsulphoxide (DMSO) into the nutrient medium. The moment of DMSO in corporation into the nutrient medium was shown by the arrow. The length, diameter and the resistance of the experimental cells relatively were 18, 0,4 mm and 4,4 Om·m^2

By appearing an increased concentration of DMSO in the nutrient medium was discovered a small transition of hyperpolarization only at the beginning of the exposure (Fig. 2). Further it was followed by the depolarization phase of plasmatic membranes to 60-70mV. The process was accompanied by a significant decrease in membrane resistance and an increase in membrane capacity (Fig. 2). Further increase of DMSO concentration in the nutrient medium content caused depolarization of the plasmalemma another 30 mV and an acute rise in resistance, slightly up to the original level. The increase of membrane resistance was accompanied by a further growth in the membrane capacity to 40% of the initial value. So, the increase of capacity under the DMSO influence had biphasic character. The first C_m increase phase occurred on a decreasing background, but the second - on a R_m increasing background. The total increase in the depolarization of the plasma membrane was 95 mV (Fig. 2).

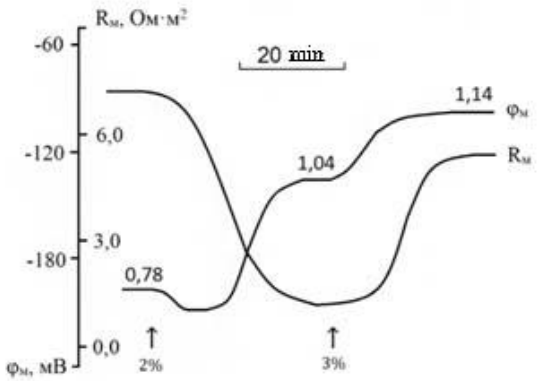

Figure 2. The kinetics of membrane potential change φ_m and resistance R_m of *Nitellopsis obtusa* cells during sequentially inclusion of 2% and 3% concentrations of dimethylsulphoxide (DMSO) in the nutrient medium. The moment of DMSO incorporation into the nutrient medium was shown by the arrow. By figures on the kinetic curve of the membrane potential were shown the values of membrane capacity in plasma membranes, corresponding to the moments of registration φ_m

The depolarization values of plasmatic membranes depended both on the initial level and also on the DMSO concentration (Fig. 3). Initial φ_m cells, which were exposed to the depolarization under the influence of DMSO were in the range of -110÷230 mV. For a more objective assessment, the depolarization values of the initial φ_m are divided into 4 sub ranges with a width of 30 mV and are determined their average values. The depolarization values, under the influence of various DMSO concentrations were estimated by displacing the average φ_m values in the graph (Fig. 3). The largest amount of depolarization was detected in cells with a large initial φ_m under the action of relatively high concentrations of the solvent in the environment of the experimental cells (Fig. 3). After the depolarization of plasmatic membranes, final φ_m levels of investigated cells located in the band of membrane potential with width 20 mV about φ_m ~-100 mV.

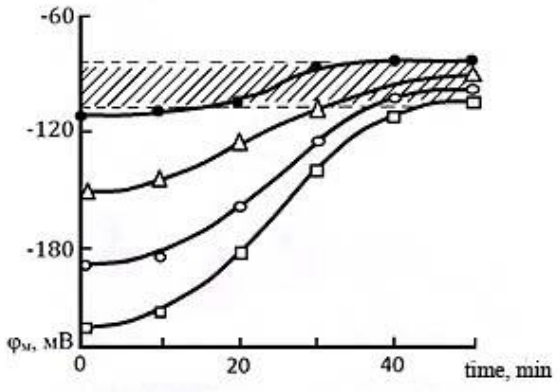

Figure 3. The depolarization of plasmatic membranes in *Nitellopsis obtusa* cells under the influence of various DMSO concentrations in APW: □-2%;○ -3%; Δ-4%;• -5%. By points on the graph are presented the average values, the standard deviation which did not exceed the vertical 10%. Each point was obtained as a result of 7-8 measurements. The point of origin corresponds to the moment of DMSO addition into the medium, surrounding the experimental cells

4. Discussion

When considering the course of electrophysiological reactions of the plasma membranes in *Nitellopsis obtuse* on appearance in an environment washing the experimental cell appearing in a medium, washing the experimental cells, DMSO, the attention is drawn to the following features of its operation: a significant increase in the electrical capacity (Fig. 1, 2), hyperpolarization under the influence of low doses and its depolarization under the influence of relatively high concentrations of solvent (Fig. 1, 2), two-phase change in membrane resistance (Fig. 2), irreversibility of electrophysiological reactions under the influence of relatively high concentrations of solvent.

Particularly notable is the hyperpolarization of plasma membranes on the background of a significant increase in its electrical capacity. Electrical capacity of cell membranes is its most informative characteristics. By measuring electrical capacity can get the information about physical state, polarized features, thickness, dielectric constant of cell membranes (Volkov, 2006; Cole, 1976). In the existing literature there are accents that the electrical capacity of biomembranes is an indicator of the lipid phase (Andrei *et al.*, 2014; Cole, 1976; Lüttge & Pitman, 2012). But in investigations, carried out in *Nitellopsis obtusa* cells, attempt to differentiate the electrical capacity of the plasma membranes on its structural phases clearly showed the fairness of the made accents. The modification of the content of the protein phase in the plasma membrane did not cause a change in the electrical capacitance (Lüttge & Pitman, 2012). This fact can be the confirmation of the provision that, electro-capacity is really the indication of physical state of lipid phase in biological membranes. On the other side, the functional activity of membrane proteins a resterically regulated by their lipid phases (Lüttge & Pitman, 2012; Adrian & Almers, 1976). Consequently, the observed enhancement of electrogenic activity, accompanying the increase of electrical capacitance in membranes, is the result of change in structural-polarized state in its lipid phase under the influence of DMSO. Amphiphilicity and high penetrating ability of the solvent molecules (Ibrahimova *et al.*, 2002; Yu & Quinn, 1994). allow it to bein corporated into the lipid membrane phase, as evidenced by the increase in capacity of plasma membranes (Fig. 1). Marked position was proved by the thin physical method with the use of neutron and synchrotron radiation (Mahmudova & Musayev, 2017a; Musayev & Ismailov, 2007).

Enhancement of electrogenic activity of plasmatic membranes under the influence of relatively low DMSO doses, apparently is a characteristic feature of *Chara* cells, as the depolarization of plasmatic membranes when R_m is constant, is identified under the action of the same DMSO concentrations in *Chara gymnophyla* (Mahmudova & Musayev, 2017b) Double increase of DMSO content in the composition of APW caused suppression of electrogenic activity in plasmatic membranes. Herewith occur the gradient realization of electro-chemical potential in plasmatic membranes by Na ($\Delta\mu_{Na+}=ZF\Delta\varphi_m\approx$ 13,5 kC/mol), directed inside of cells. In this case considerable decrease of membrane resistance agrees with the facts, established in the early works Mahmudova & Musayev, 2017b). We suppose that, it could be due to the loss of Na^+-K^+ selectivity of K^+-channels because of conformational rearrangements, in result K^+-channels are also permeable for Na^+. These events resulted in the depolarization of plasmatic membranes. And the increase of Na^+ permeability in plasmatic membranes of

cells under the influence of DMSO has clearly been demonstrated at work (Bodo *et al*., 2014).

The increase of R_m with increasing DMSO concentration on the background of continuous membrane depolarization was accompanied by the considerable increase of membrane capacity (Fig. 2). As noted above, every change of membrane capacity is the reflection of a change in structural-polarized state of membrane lipid phase, which could lead to the destruction of functionally active conformation in channel proteins, also electrogenic pumps. And probably, under the influence of increased DMSO concentration happens not destruction of membrane structure, but rearrangement of functionally active conformation in transport communication components. This can be evidenced by the fact established in *Chara gymnophyla* cells, while replacing the APW measurement chamber with pure DMSO occurs φ_m decrease to zero, however, the membrane resistance was established at a level higher than the R_m level in the APW for several times (Musayev & Ismailov, 2007). This indicates that even in the presence of the largest concentration of DMSO, the cell membrane retains its integrity, which is provided mainly by the lipid matrix and the structural proteins of the plasma membrane (Fig. 2, 3).

One can't by pass the fact, that effective influencing concentration of DMSO on the transport and structure-polarization properties of the plasma membrane is compared with its concentration in working solutions of polyene antibiotics (Cheng *et al*., 2015; Yu & Quinn, 2000; Kiselyov, 2007). Therefore, to evaluate the modifying effects of polyenes on the permeability of the lipid phase of membranes, it is necessary to differentiate the total effect on the antibiotic and the solvent. Having conducting the results of the analyses of electrophysiological effects of DMSO on plasma membrane, one can see that, the first stage of action of the solvent is a change of structure-polarization properties of lipid phase, which is accompanied by an increase in the electrogenic activity of cells. After these events, conformational restructuring of transport communications takes place, which leads to their inactivation.

Experimental and theoretical analyses carried out by us of the regularities of resistance behavior potential and capacity under the influence of Dimethylsulphoxide allowed to set up the following conclusions:

- Dimethylsulphoxide can be used as the effective regulator of transport functions in biological membranes;
- Totality of established data indicates the existence of the relationship between the functional activity and structural lability in cellular membranes;
- The first stage of dimethylsulphoxide influence on the cellular membranes is the change of their electrocapacity.

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