THE INFLUENCE OF PHYTOCOMPOSITIONS DANDELION-BURDOCK ON ELECTROPHYSIOLOGICAL CHARACTERISTICS OF *CHARA FRAGILIS* CELLS

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Abstract. By using of microelectrode techniques, patterns of change in the potential (φ_m) and resistance (R_m) of the plasma membrane (PM) of *Chara fragilis* cells under the influence of Dandelion–Burdock (DBC) phytocomposition solutions were studied. It is important to emphasize that the results of the analysis of the potential distribution, resistance of the plasma membrane by the number of cells of *Chara fragilis* under the standard conditions is being presented for the first time. The Hogg method allows simultaneous measuring of electrophysiological parameters such as φ_m and R_m. The average value of these parameters were -183±4.9 mV and 9±1.2 Ohm·m^2 under the standard conditions. For the action of low DBC concentrations (0.01 mg/ml) in the activation range of the KCOR was characterized by significant depolarization of PM. In this paper, the inhibition of ion transport through the PM is discussed from the point of view of oxidation, and hyperpolarization-changes in the physical state of its lipid phase.
Key words: *Chara fragilis, membrane potential, membrane resistance, phytoprotectors, H^+ - pumps, K^+ - channels.*

The search for effective ways to improve the radioresistance of bio-objects based on testing of synthetic-chemical and natural plant-origin products. In this aspect, plant-origin products are preferable in terms of their mild, long-lasting effect, without any toxic side effects. On the other hand, they are widespread in nature. *Tarakacum officinale* (Dandelion ordinary), *Arctium lappa*, the roots of *Radix Baradanae* (Burdock root) can be attributed to these plants. Both of these remedies have long been used for a therapy and improvement of radioresistance [6, 13]. These composites are also successfully used in the medical practice as a tonic and known as a radioprotectors [1, 2, 7, 14]. The PM is a primary target of the study when extracts of these plants are used. Detection of interaction mechanisms of these preparations with the PM could stimulate the establishment of new effective methods for radioprotection and improvement of radioresistance. In this study, we attempt to investigate the mechanisms of action of water solutions of the Burdock + Dandelion composition on individual components of the primary active transport system of the PM, particularly on the electrogenic activity of the H^+-pumps, conductivity (resistance) of selective and non-selective ion transport paths on the basis of electrophysiological analysis of the behavior patterns of membrane potential φ_m and resistance R_m.

MATERIALS AND METHODS

The experiments were conducted with internodal cells of *Chara fragilis* (Fig. 1). *Chara fragilis* plants were collected from a pond called "Katib Bulagy", which formed from sewage water of several mountain springs in the Tovuz region. *Chara fragilis* belongs to the family of *Characeae Ag. Emand Hollerb* of *Charaphyceae* class. The *Chara fragilis* internodal cells were for the first time used in the electrophysiological research by us [9]. The length of mature internodal cells of *Chara fragilis* reached 5–8 cm with 0.6–1 mm diameter. The large size of the internodal cells of *Chara fragilis* allows them to be used for several days in microelectrode studies. The mineral composition of the "Katib Bulagy" water was determined by atomic absorption spectroscopy AAS-1 spectrophotometer company Bruker, Germany, corresponded to the mineral composition of artificial pond water (APW), containing 0.1M K^+, 1M Na^+, 0.4M Ca^{2+}, 0.3 M Mg^{2+} cations and Cl^-, HCO^{3-}, NO^{3-}, PO_4^{3-}, SO_4^{2-} anions at pH - 6.9 ÷ 7.2 [9]. Plants were grown on APW, in aquariums with 0.3 × 0.4 × 0.5 m dimension under room light conditions (with intensity 6 W/m^2) 12-14 hours per day. The optimal temperature ranges for growing *Chara fragilis* was 18-23 ºC.To ensure the high accuracy of membrane resistance measurements, cells no longer than 20 mm were used [11]. This value corresponded to the length of the second cell from the top part of the *Chara fragilis* plants. To measure the main electrophysiological parameters, we used the two-electrode Hogg method developed for cells having cylindrical shapes [10, 11]. The Hogg method allowed simultaneous and many hours recording of membrane potential φ_m and resistance R_m of experimental cells. Through one microelectrode introduced into the center of the cell, the direct current pulses with a density of 10^{-4} A/m^2 in a duration of 2–3 seconds was transmitted (Fig. 2). Via the second microelectrode was recorded the φ_m of cell and the electrotonic potential ΔU generated by passing a direct current through the cell.

The dried powder extract of Dandelion root and Burdock is used as a phytocomposite. The powder extract was bought from the pharmaceutical company "Herba-Flora", Azerbaijan. The composite mixture of these plants was extracted from their powders in an equal ratio with 70% ethanol. The raw materials and extractant in relation to 1:15 were stirred for 10 minutes on a magnetic stirrer at room temperature and filtered. The extraction with the following filtration was repeated three times. Then the filtrates were evaporated using a rotary evaporator in a vacuum to dryness [7, 14]. The working solutions of the extract were prepared on APW. Statistical processing of experimental results was carried out according to the laws of variation statistics [4] by the using of computer programs Excel-2016 and SigmaPlot12.0.

Figure 1. Isolated *Chara fragilis* internodal cells in the Petri dish

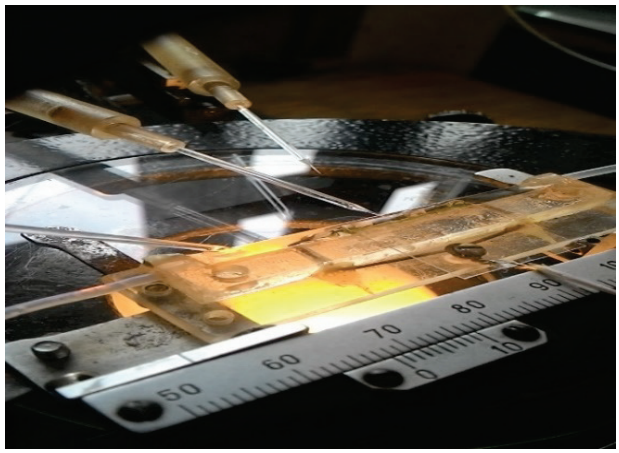

Figure 2. The working part of the measuring installation with vernier to determine the length of the experimental cells. The diameter of the test cell was measured using a micrometer installed in the microscope ocular. The accuracy of measurement of these values was 0,1 mm and 5 μm, respectively

RESULTS AND DISCUSSION

Since the *Chara fragilis* cells were first introduced by us in the practice of electrophysiological research, we needed to conduct a statistical analysis of the distribution of electrophysiological parameters by the number of cells in standard conditions. It was found that the stationary values of φ_m and R_m scattered in a rather wide range: -90 ÷ -300 mV and 1 ÷ 32.6 Ohm·m^2, respectively. The average value of φ_m was -183± 4.9 mV. The sample size m of the experimental material was 106. The distribution of φ_m obeyed to the Gauss – Moivre – Laplace law of normal distribution. The value of the membrane resistance, which is calculated from the resistance level of the cell membrane, was 9 ± 1.2 Ohm·m^2 (n=45). The distribution of the set of values of R_m did not obey the law of normal distribution.

The contact of the measuring microelectrode tip with the cell membrane under standard environmental conditions caused the appearance of a signal which value was φ_0 = -30 ± 2,5 mV (the potential of the cell wall) and the value of resistance was R_0 = 3,8 ± 0.7 Ohm·m^2 (n=28).

The number of classes was determined by the formula proposed by Brooks and Caruzer, which was 10. The sample size m of the experimental material was 106, the class interval i = 21 mV. Therefore, we presented the variational series φ_m as a distribution of the total volume between 11 classes (Fig.3). The variational series φ_m, represented as a histogram, had a maximum at -174 mV. The estimation of this value, according to our empirical data, yielded χ^2 = 16,75. And the theoretical value of critical value for the 0,5% significance level was 16,75, i.e. $\chi^2 = \chi^2_{kr}$. This means that the distribution of φ_m in classes corresponds to the normal distribution. Similar calculations were made for the membrane resistance. The distribution of the set of values of R_m did not obey the law of normal distribution. There was no correlation between φ_m and R_m. The correlation coefficient between these values was r = -0.019878. The distribution of the potential and resistance by the number of cells indicates that a significant part of the total number is composed of cells with high electrogenic activity.

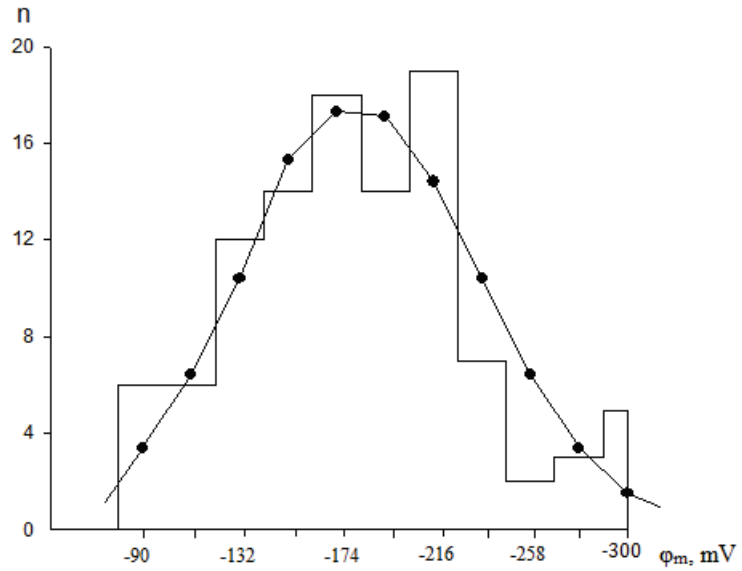

Figure 3. Histogram of the membrane potential φ_m distribution of the *Chara fragilis* by the number of cells n. The figure shows the theoretical frequencies calculated from the variation statistics of the dotted variants. A continuous line shows the course of the theoretical dependence of the normal distribution

The dependences of φ_m and R_m from the K^+ concentration in the medium were illustrated by three-phase curves (Fig. 4). The first phase of the dependence in the range of φ_m -300 ÷ -165 mV probably shows the activation of K^+ -channels of inward rectification (KCİR), and the third phase in the range of φ_m -120 ÷ -50 mV – K^+ -channels of outward rectification (KCOR). The second phase can probably be called a transition phase because assumedly the partial inactivation of the K^+-channels of the inward and the activation of the K^+-channels of the outward rectification occur in this phase. The K^+-equilibrium potential in a medium with 10^{-4} M K^+ for the other species of *Chara* algal corresponded to the limit range of the activation for two kinds of K^+-channels. According to it, φ_m for *Chara fragilis* is -162 mV (Fig. 4). In view of this value, the intracellular activity of a_{K+} ions can be calculated from the Nernst formula, which was 61.6 mM/l.

In our experiments, we compared the electrophysiological characteristics of *Chara fragilis* interstitial cells under the influence of the composite solution of *Taraxacum officinale* and *Arctium Lappa L* roots. Electrophysiological effects of Dandelion-Burdock composite mixture (DBC) on membrane potential φ_m and resistance R_m depended on both the concentration of radioprotectors and the physiological state of cells. The lowest concentration of composites that caused the electrophysiological reaction of the PM (threshold concentration) was 10^{-2} mg/ml (10^{-5} kg/l). And besides, the

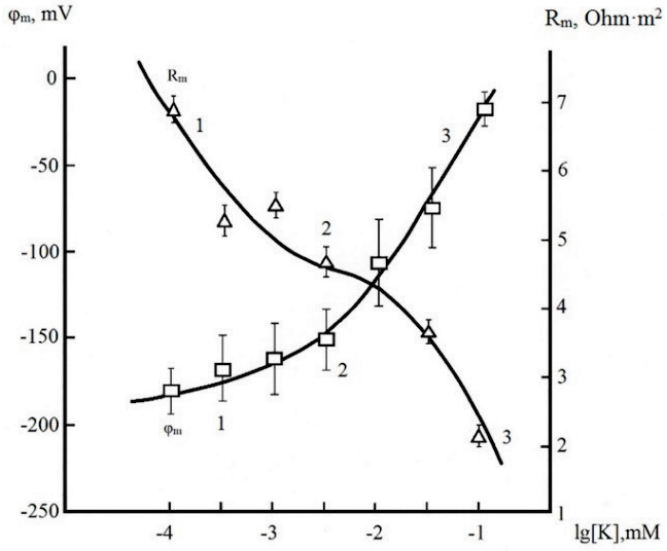

Figure 4. Dependence of stationary values of φ_m and R_m of the PM of *Chara fragilis* cells from the concentration of KCl in the APW. The numbers 1, 2, 3 on the curves indicate the particular phases of change of the corresponding values. Each point reflects the average value of the measured magnitude of φ_m and R_m for 9-10 cells. Vertical lines indicate the standard deviation of the mean of φ_m and R_m

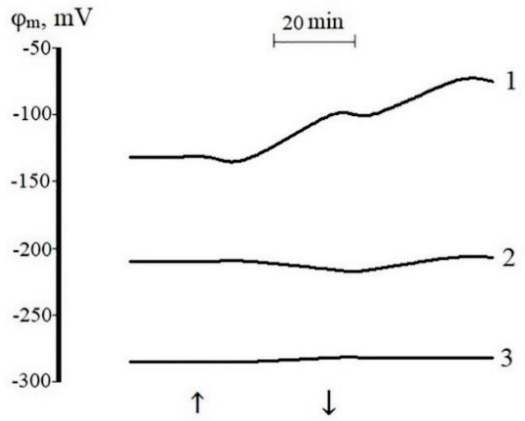

Figure 5. The kinetics of the membrane potential φ_m of the different *Chara fragilis* cells during the introducing of 10^{-2} mg /ml concentration of the Dandelion and Burdock composites into artificial pond water. The up arrow indicates the addition of composites into the APW, and the down arrow indicates when 10^{-2} mg /ml of the radioprotector was removed from the composition. The numbers next to the kinetic curves indicate the numeration of the experimental cells.

electrophysiological effects of DBC, such as depolarization of the PM at the constant level of R_m, were found only in cells, φ_m which was in the range of activation of the K^+ - channels of outward rectification (KCOR) (Fig. 5). The magnitude of the depolarization varied from -75 to -129 mV with an average value of $\overline{\varphi}_m$ = -103 ± 2.5 mV. The recovery of $\overline{\varphi}_m$ was not detected during the removing of radioprotectors from the external environment within 60-80 minutes. In other words, the electrophysiological effect of 10^{-2} mg/ml of composites of the mixture of Dandelion and Burdock on *Chara fragilis* cells, $\overline{\varphi}_m$ which was in the activation range of KCOR, were not reversible. The analysis of electrophysiological characteristics of plant cells shows, that Ca^{2+}- channels of the PM can also be in the conducting state in the same range of KCOR activation [5]. This review provides a detailed analysis of the calcium signaling system, where the structural-functional organization of Ca^{2+} - channels of various plants is analyzed in detail. The activation of Ca^{2+}- channels begins when the membrane potential is -140 mV [5]. And the activation of Ca^{2+}- channels reaches the maximum when the absolute potential of the membrane drops to -60 ÷ -100 mV [12]. φ_m of cells turned up exactly in this range after the appearance of the 10^{-2}mg/ml Burdock + Dandelion composites in the medium. Here, the role of the phytoprotector in all probabilities consists of initiating of Ca^{2+} - channels into the open state. And the increase of intracellular Ca^{2+} concentration causes the intracellular enzymatic processes leading to irreversible oxidation of the PM lipids [3, 8], which in our study is reflected as irreversible depolarization of *Chara fragilis* cells (Fig. 6). Hence, the same thing occurs during the appearance of 10^{-1} mg/ml Burdock + Dandelion composites in the external medium.

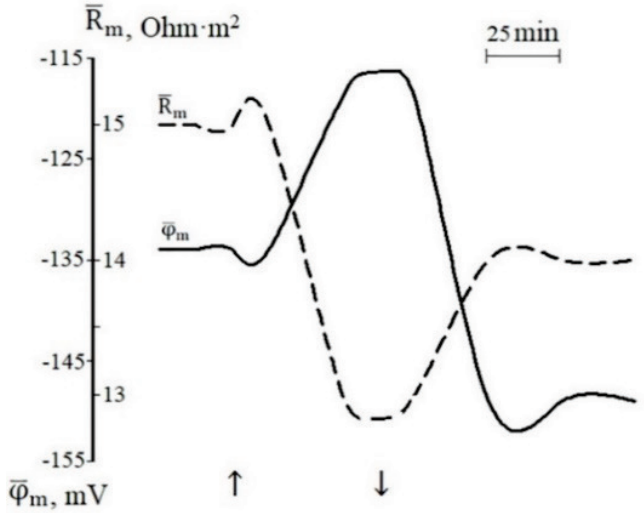

Figure 6. The changes of the average values of the potential $\overline{\varphi}_m$ and PM resistance $\overline{R}_m$ of *Chara fragilis* cells, φ_m which was in the range of KCOR, during the including of 1 mg/ml concentration of Burdock and Dandelion composites into the composition of APW. The arrows indicate the moments of the appropriate concentrations (molar) addition in the nutrient medium and removing from the nutrient medium (APW). The standard deviation of the average values of $\overline{\varphi}_m$ and $\overline{R}_m$ did not exceed 7-8%. The curve is drawn by using "Sigma-Plot12.0".

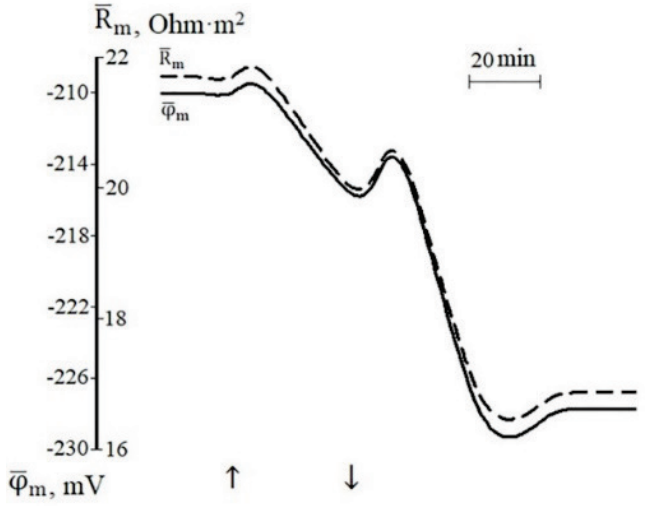

Figure 7. The record of changes of the average values of the potential $\overline{\varphi}_m$ and PM resistance $\overline{R}_m$ of *Chara fragilis* cells, φ_m which were in the range of KCOR, during the including of 1 mg/ml concentration of Burdock and Dandelion composites into the composition of APW. The arrows indicate the moments of the appropriate concentrations (molar) addition in the nutrient medium and removing from the nutrient medium (APW). The standard deviation of the average values of $\overline{\varphi}_m$ and $\overline{R}_m$ did not exceed 7-8%. The curve is drawn by using "Sigma-Plot12.0".

A variety of electrophysiological reactions of *Chara fragilis* cells was observed with the addition of 1 mg/ml concentration of Burdock and Dandelion composites into the composition of artificial pond water (Fig. 6, 7). Thus, the presence of 1 mg/ml concentration of Burdock and Dandelion composites caused a slight transient hyperpolarization, which was accompanied by an increase of the membrane resistance by several percents in cells, $\overline{\varphi}_m$which was in the range of activation of KCOR (n=7) (Fig. 6). When the radioprotector composites were removed from the medium, the electrophysiological parameters of *Chara fragilis* cells were restored (Fig. 6).

The process of stimulation of the PM transport processes under the influence of 1 mg/ml of Burdock and Dandelion composites were more clearly revealed when protectors were added into APW, when φ_m of cells was in the range of activation of KCIR (Fig. 7). The sample size m of the experimental material was 7. Moreover, the stimulation of the transport processes of the PM was occurred in two-steps (Fig. 7).

The first step took place at the beginning of the impact of the composites of these radioprotectors. The second step of stimulation took place when cells were washed out from composites (with the exclusion of composites from the composition of APW). On the first step of stimulation, the PM hyperpolarized by several mV, and its resistance decreased by 10% (Fig. 7). On the second step of stimulation of the PM transport processes further hyperpolarization was revealed and a significant increase in its resistance (increase of conductivity). The φ_m of cells at the hyperpolarization level could last for hours. And the magnitude of the overall hyperpolarization depended from the initial level of φ_m under standard conditions (Fig. 8). By using regression analyses between these values, a linear dependence was established $\Delta\varphi_m=14+0.308|\varphi_m|$ (n=7) with a correlation coefficient r = 0.9358 (Fig. 8). Thus, it can be seen that the second step of stimulation of the PM is a result of the impact of DBC. Probably, this property of the effect of composites provides a radioprotective feature of the composites of the above-mentioned plants.

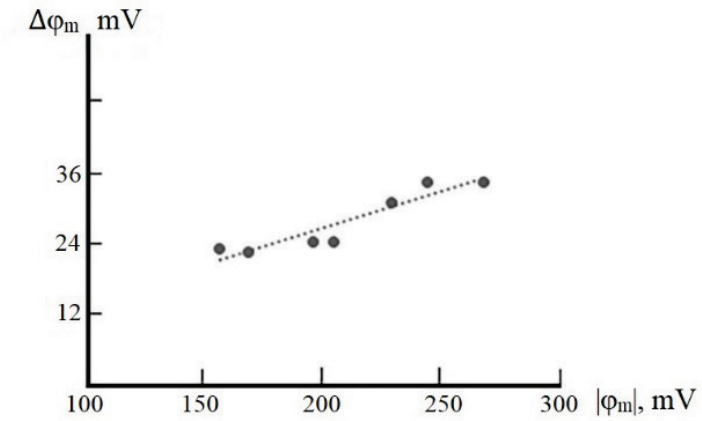

Figure 8. Dependence of the hyperpolarization of the PM $\Delta\varphi_m$ under the influence of 1 mg/ml concentration of DBC to *Chara fragilis* cells from the absolute value of membrane potential $|\varphi_m|$ under the standard conditions.

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ВЛИЯНИЕ ФИТОКОМПОЗИЦИЙ ОДУВАНЧИК-ЛОПУХ НА ЭЛЕКТРОФИЗИОЛОГИЧЕСКИЕ ХАРАКТЕРИСТИКИ КЛЕТОК *CHARA FRAGILIS*

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Аннотация. С использованием микроэлектродных методов были изучены закономерности изменения потенциала (φ_m) и сопротивления (R_m) плазматической мембраны (ПМ) клеток *Chara fragilis* под влиянием растворов фитокомпозиции Одуванчик – Лопух (КОЛ). Представлены результаты анализа распределения потенциала φ_m, сопротивления R_m плазматической мембраны по количеству клеток *Chara fragilis* в стандартных условиях. Среднее значение этих параметров в стандартных условиях составило -183 ± 4,9 мВ и 9 ± 1,2 Ом·м^2.Для действия низких концентраций КОЛ (0,01 мг / мл) в диапазоне активации K^+- каналов наружного выпрямления φ_m была характерна значительная деполяризация ПМ.В работе обсуждается ингибирование транспорта ионов через ПМ с точки зрения окисления и изменений в физическом состоянии его липидной фазы.

Ключевые слова: *Chara fragilis, мембранный потенциал, мембранное сопротивление, фитопротекторы, H^+- насосы, K^+- каналы.*