MODIFICATION MECHANISMS OF ELECTROPHYSIOLOGICAL CHARACTERISTICS OF *CHARA FRAGILIS* CELLS UNDER THE INFLUENCE OF GAMMA RAYS AND EXTRACTS OF PHYTOPROTECTORS

Hasanova A.E.
Institute of Botany ANAS
Badamdar shosse 40, Baku, Azerbaijan, AZ1004; e-mail: ahasanova89@gmail.com
Received: 20.07.2019

Abstract. The regularities of changes of not irradiated and irradiated with low doses of radioactive radiation *Chara fragilis* cells membrane potential (φ_m) and resistance (R_m) in the standard conditions and under the influence of extracts of sage, roots of licorice and danaya. has been studied with microelectrode technique. The average value of these parameters were -183 ± 4.9 mV and 9 ± 1.2 Ohm·m^2 under the standard conditions. For *Chara fragilis* cells irradiated with a low dose of γ - rays, these values were $\overline{\varphi}_m$ = -202 ± 9.4 mV and $\overline{R}_m$ = 11.7 ± 3.6 Ohm·m^2. The components of the primary active transport system of *Chara fragilis* cells, irradiated with a low dose of γ - rays, turned out to be sensitive to the action of phytoprotectors and the effective concentration, causing a reliable electrophysiological reaction was 1 mg/l. Activation ranges of K^+ - channels to the inward and outward rectification were -130 ÷ -50 and -300 ÷ -162 mV. The cytosolic activity of K^+ ions was 61.6 mM / l. Electrophysiological reactions of the PM were qualitatively and quantitatively determined by the initial level of φ_m and the concentration of phytoprotectors.
Key words: *plasma membrane, membrane potential, resistance, phytoprotector, γ-radiation, Chara fragilis.*

The large size of *Chara fragilis* interstitial cells provide wide opportunities for the identification of membrane processes in conditions of their intactness. One of the important aspects of research in this plan is the determination of the cellular mechanisms of the pathogenic and protective effect of some exogenous factors of a physicochemical nature, since the first phase of their influence occurs at the level of the cell membrane. Therefore, the main purpose of these researches was to identify the cellular mechanisms of such interactions by using the results of electrophysiological measurements. For this purpose, we studied the patterns of changes in the functional activity of the components of the primary active transport of plasma membrane (PM) of *Chara fragilis* cells under the influence of these factors. The components of primary active transport system of PM consist mainly of 2 types of K^+ - channels and H^+ - pumps. Control of the functional activity of these components was carried out by regular measurement of membrane potential (φ_m) and resistance (R_m) of *Chara* cells, both under standard environmental conditions and under the influence of the above mentioned exogenous factors.

MATERIAL AND METHODS

The experiments were conducted on internodal cells of *Chara fragilis* collected from a pond called "Katib Bulagy", which formed from sewage water of several mountain springs in the Tovuz region. The *Chara fragilis* cells were for the first time used in the electrophysiological research by us. The species and families of algae were determined by the Gollerbach determinant [1]. It turned out that, *Chara fragilis* belongs to the family of *Characeae Ag. Emand Hollerb* of *Charaphyceae* class [1]. The bioelectric parameters of *Chara fragilis* cells were measured by using standard microelectrode techniques [2]. The essence of this method is that the registration of the parameters φ_m and R_m is carried out by introducing 2 microelectrodes into the cylindrical interstitial cell *Chara* [2]. 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. 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. R_m of cells is calculated by the formula $R_m = \frac{\Delta U}{I}\pi dl$, where I – strength of current transmitted through the middle of the cell, l, d – the length and the diameter of the experimental cell.

The use of this method allows the measurement of the potential and resistance of the PM and tonoplast by introducing a measuring microelectrode into the cytoplasm and vacuole.

In this study plant extracts from the flora of Azerbaijan which are rich with antioxidants are used as a phytoprotector. As raw materials for the extracts were used sage leaves (*Folia Salvia officinalis*), liquorice roots (*Radix glycyrrhizae*) and danae leaves (*Danae racemosa*). These phytoprotectors revealed antioxidant, antimicrobial and antimutagenic, hypoglycemic and hypolipidemic features [3-8]. The screening of alcoholic extracts of dried plant: sage leaves with predominant content of saponins (ursolic oleanine acid, carotene, vitamin C), roots of liquorice with saponine – glycyrrhizin and leaves of *Danae racemosa* with predominant content of carotenoids [9, 10] was performed. The extraction of crumble material was undertaken with 95% ethanol during 7 days in the dark and at room temperature. To clarify the extracts, they were filtered. The concentration of the extracts, which caused the most effective electrophysiological reaction, was obtained by diluting the alcohol extract 200 times with artificial pond water (APW). This concentration can be expressed as 2 mg/10·200 ml=10^{-3} mcg/ml=1 mg/l.

For the testing of the action of small doses of γ - radiation, *Chara fragilis* cells are located in a glass test tube with a 100 mm length and 25 mm diameter inside a steel sleeve in the γ - device "URI". The exposure time in our experiments was 2 min 55 s, the average radiation dose rate was 19 rad/s, and the dose rate of ^{60}Co isotopes was 33.25 Gy/s. It should be noted here that the concept of "low dose", the dose causing a statistically significant bioelectric "effect" may not coincide [4].

The laws of variation statistics, the computer programs Excel-2016 and SigmaPlot12.0 were used for plotting of graphs.

RESULTS AND DISCUSSIONS

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. According to these values, the average values of the potential and the resistance of the membrane were $\overline{\varphi}_m$= -183±4,9 mV и $\overline{R}_m$= 9±1,2 Ohm·m^2. Measurements of φ_m and R_m were held in 106 and 45 cells, respectively. A detailed description of the statistical analysis of the electrophysiological parameters of these cells are given in our previous publications [12, 13].

The average values of membrane potential and resistance of *Chara fragilis* cells irradiated with a low dose of γ - rays were $\overline{\varphi}_m$= -202±9,4 mV (n=17) и $\overline{R}_m$= 11,7±3,6 Ohm·m^2 (n=15). Thus, as a result of irradiation, the absolute value of φ_m of *Chara fragilis* cells increased by 10,4%, and R_m - by 18,2% (Fig. 1). These figures show that the ion - transport system of cells irradiated with a low dose of γ - rays is represented mainly by K^+ -channels of inward rectification (KCİR). As seen, the adaptive response of *Chara fragilis* cells to a low dose of γ - rays consists of the inactivation of the K^+ -channels of outward rectification (KCOR) and the activation of the KCIR PM, which is accompanied by its hyperpolarization. Membrane potential is the main source of free energy of PM. Consequently, the hyperpolarizing effect of the γ - rays dose is a manifestation of the energization of the PM. On the other hand, it is also a manifestation of radiation hormesis in plant objects. The exact mechanisms of these effects on plant objects have not been established. Obviously, in this case, the energization of the PM occurs due to the restriction of the leakage of ions through the small resistance of the KCOR. In all likelihood, an increase in the productivity of some agricultural crops and the stimulation of cellular processes [11] under the influence of low doses of radioactive radiation occurs due to the restructuring of the primary active transport system leading to the energization of the PM.

The components of the primary active transport system of *Chara fragilis* cells, irradiated with a low dose of γ - rays, turned out to be sensitive to the action of phytoprotectors. Their effective concentration, causing a reliable electrophysiological reaction was 1 mg/l. For an objective quantitative representation of the kinetics of the electrophysiological effects of phytoprotectors, the results of our measurements obtained for 10 cells were averaged (by using the computer program SigmaPlot 14.0).

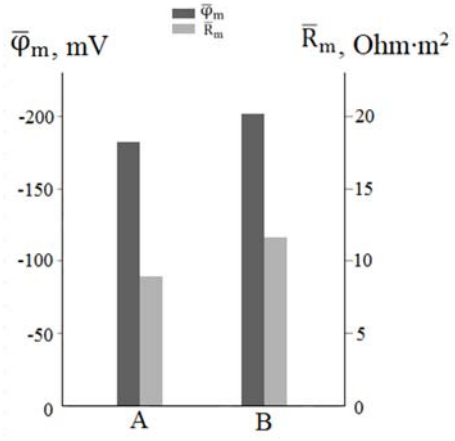

Figure 1. The histogram of average values ($\overline{\varphi}_m$ and $\overline{R}_m$) of electrophysiological parameters of *Chara fragilis* algal cells under standard environmental conditions (A) and irradiated with a low dose of γ - rays (B)

During the introduction of 1 mg/l the concentration of sage extract into the medium, in *Chara fragilis* cells, which φ_m was in the activation range of KCIR, $\overline{\varphi}_m$ did not change, while $\overline{R}_m$ decreased by 17% (fig. 2). In other words, the adaptive response of the PM of *Chara fragilis* cells apparently reflects the repair of the KCOR channel protein under the influence of the protector. At the same time, the PM remains in the energized state. The exact identification of this case can be established by using bioinformatic methods.

The introduction of liquorice root extract into the medium after washing the sage extract led to depolarization of PM by 11 mV and an increase in $\overline{R}_m$ by 25%. Undoubtedly, this electrophysiological reaction of PM is the result of the combined action of both protectors. Introduction to the medium of the mentioned concentration of the *Danae racemosa* extract hyperpolarized *Chara fragilis* cells by 12 mV and reduced $\overline{R}_m$ by 15%. Changes of $\overline{\varphi}_m$ and $\overline{R}_m$ are signs of partial

activation of H^+ - pumps of PM. Therefore, it can be established that in the range of KCIR of membrane potential the resistance of H^+ - pump and KCIR are comparable.

If the hyperpolarization of the PM is accompanied by a decrease in its resistance, then it can only be associated with a decrease in the internal resistance of H^+ - pump. The decrease of the KCIR resistance would be accompanied by depolarization of the PM. Thus, the activation of H^+ - pump should be considered as a proton flux gain, through the PM. It can be assumed that the stimulating effect of phytoprotectors is due to an increase in fluidity (decrease in viscosity) of the lipid phase of PM, leading to an increase in the mechanical frequency of the hydrophobic part of the H^+ - pump, accompanied by an increase in the turnover of the protein complex as an enzyme.

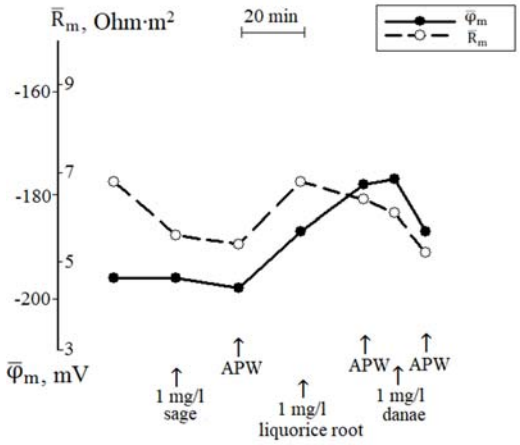

Figure 2. Changes in the kinetics of average values of membrane potential $\bar{\varphi}_m$ and resistance $\bar{R}_m$ of *Chara fragilis* cells under the influence of 1 mg/l of concentrations of extracts of sage, roots of licorice and danaya. The dots in the figure show the average values of the experimental quantity for 10 cells. The standard deviation of their values did not exceed 8-9% of the main value

In conclusion, we note that the stimulation of transport processes under the influence of phytoprotectors at the PM level was also found on cells that were not exposed to radioactive radiation [14, 15].

Data on the effect of phytoprotectors on PM cells irradiated with a low dose of γ - rays confirmed the previously established position that the initial phase of phytoprotectors influence at the cellular level can be considered as a stimulating effect on the primary active transport system. The established stimulating effect consists in increasing the functional activity of the H^+ - pump, which increases the mobility of the electromotive force of the primary active transport system and in improving the perfection of the membrane in relation to selectivity and conductivity.

Along with all the above, it is easy to see that plant extracts used as a phytoprotector can be successfully applied to the stimulation of the transfer of ions to PM, and their protective effects can be successfully applied to radiation protection of plants not irradiated and irradiated with low doses of radioactive radiation.

References:

1. Gollerbakh V.I., Krasavina L.K. *Qualifier of freshwater algae of the USSR*. Leningrad.: Science, 1983, 185 p. (In Russ.)

2. Hogg J., Williams E.J., Jhonston R.I. A simplified method for measuring membrane resistances Nitella translucens. *Biochim. et Biophys. Acta*, 1968, vol. 150, p. 518.

3. Ahmad Ghorbani Mahdi Esmaeilizadeh, Pharmacological properties of *Salvia officinalis* and its components. *Journal of Traditional and Complementary Medicine*, 2017, vol. 7, iss. 4, pp. 433-440.

4. Jedinák A., Mucková M., Kost'álová D., Maliar T., Masterova I. Antiprotease and antimetastatic activity of ursolic acid isolated from *Salvia officinalis*. *Z. Naturforsch.*, 2006, vol. 61, pp. 777-782.

5. Russo A., Formisano C., Rigano D. *et al.* Chemical composition and anticancer activity of essential oils of Mediterranean sage (*Salvia officinalis* L.) grown in different environmental conditions. *Food ChemToxicol*, 2013, vol. 55, pp. 42-47.

6. Asl M.N., Hosseinzadeh H. Review of pharmacological effects of *Glycyrrhiza sp.* and its bioactive compounds. *Phytother Res.*, 2008, vol. 22, no. 6, pp. 709-24. DOİ: 10.1002/ ptr.2362.

7. Shahreari Sh., Khaki Arash, Ahmadi-Ashtiani H.R., Rezazadeh Sh. Effects of *Danae racemosa* on testosterone hormone in experimental diabetic rats. *Journal of Medicinal Plants*, 2010, pp. 114-119.

8. Zhongguo Zhong Yao Za Zhi. Review of pharmacological effects of Glycyrrhiza radix and its bioactive compounds. *China journal of Chinese materia medica*, 2009, vol. 34, no. 21, pp. 2695-700.

9. Dadasheva S.B., Kurbanova I.M., Varfolomeev S.D., Gasanov R.A. Photodestruction of isolated chlorophyll – protein complexes of thylakoids. *Photosyntetica*, 1990, vol. 24, no. 2, pp. 280-282.

10. Kerimov Yu.V. The useful properties of some plants from flora of Azerbaijan. *Proceedings of the Azerbaijan National Academy of Sciences, biology series*, 1982, vol. 1, pp. 16-21. (In Russ.)

11. Ivanovsky Y.A. Radiation hormesis. Are low doses of ionizing radiation beneficial? *Bulletin of the Far Eastern Branch of the Russian Academy of Sciences*, 2006, no. 6, p. 86. (In Russ.)

12. Hasanova A., Najafaliyeva Sh., Aliyeva P., Musayev N. Electrophysiological parametres of the cell membrane and plasma membrane of *Chara fragilis* cells. *Advances in Biology & Earth Sciences, Jomard Publishing*, 2018, vol. 3, no. 1, pp. 60-68. (In Russ.)

13. Hasanova A., Najafaliyeva Sh., Aliyeva P., Musayev N. *Chara fragilis* cells – a new object for electrophysiology researsch. *Baku Engineering University. Chemistry and Biology*, 2018, vol. 2, no. 1, pp. 8-14.

14. Hasanova A.E., Shamilov E.N., Abdullayev A.S., Musayev N.A. The influence of dandelion and burdock phytocomposition on the potential of the plasma membrane of *Chara fragilis* cells. *New and non-traditional plants and prospects for their use, materials of the XI International Symposium*, June 15-19, 2015, Pushino, Moscow, pp. 203-206. (In Russ.)

15. Hasanova A.E., Najafaliyeva Sh.A. The fitoprotectors influence on electrophysiological parameters of *Chara fragilis* cells. *Conference of young scientists and students Innovations in biology and agriculture to solve global challenges*, Baku, Azerbaijan, October 31, 2018, p.145.