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The Quantum-Mechanical Sensitive Na/K Pump Is a Key Mechanism for the Metabolic Control of Neuronal Membrane Function

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Abstract

At present, there are relevant scientific materials on the cellular and molecular mechanisms of electrogenic Na/K pump function and structure, as well as on the potential- and ligand-activated ionic channels in the membrane. However, the role of electrogenic Na/K pump in regulation of semipermeable properties of cell membrane has not been elucidated yet, which is due to the fact that our knowledge about the biophysical properties of cell membrane is based on the conductive membrane theory of Hodgkin-Huxley-Katz, which is developed on internally perfused squid axon and lacks intracellular metabolism. Thus, the accumulated abundance of data on the role of G-proteins-dependent intracellular signaling system in regulation of Na/K pump activity and biophysical properties of cell membrane presumes fundamental revision of some statements of membrane theory. The aim of the present review is to briefly demonstrate our and literature data on cell hydration-induced auto-regulation of Na/K pump as well as on its role in metabolic control of semipermeable properties and excitability of neuronal membrane, which are omitted in the study of internally perfused squid axon.

Keywords

Na/K Pump, Hydration, Ionic Channel, Membrane, Na/Ca Exchange, Cyclic Nucleotides

1. Introduction

According to the classical membrane theory, which is developed by Nobel laureates Hodgkin, Huxley and Katz and concerns the ionic mechanisms involved in excitation and inhibition of cell membrane, the signal transmission in cells is

realized by potential- and agonist-activated ionic channels in membrane, while the Na/K pump, functioning in electro-neutral regime, has a housekeeping role in controlling the intracellular ionic homeostasis [1] [2]. The main failure of this theory is that it does not evaluate the role of intracellular metabolism in regulation of membrane excitability, namely it is unable to explain what mechanism controls low Na^+ and high K^+ permeability of cell membrane in resting state of neurons. This omission is due to the fact that the theory was initially developed on the basis of experimental data obtained by the study of internally perfused squid axon [1].

In 1957 Jens Christian Skou disclosed Na/K-ATPase as an enzyme and a working molecule of Na/K pump and characterized its biochemical and pharmacological properties [3]. Thus, the Na/K pump became a subject of various biochemical and biophysical studies resulting in a discovery that it functions in stoichiometry of 3Na:2K, which identifies the electrogenic character of pump. The historical aspects of this discovery are presented in the excellent review by Rogers Thomas [4], who was the first to elucidate the electrogenic character of Na/K pump in neurons by measuring and characterizing the membrane current generated by Na/K pump by means of combination of "voltage-clamp" and "intracellular selective microelectrode" methods in Prof. Gerald Kerkut's laboratory [5] [6].

Although, at present the electrogenic character of Na/K pump can be considered as a proven fact, its multifunctional physiological role in regulation of biophysical properties of cell membrane, namely in controlling semipermeable properties of cell membrane, needs further evaluation. The problem of the functional role of Na/K pump in metabolic regulation of cell membrane function has served as one of the main research subjects of my group for more than 50 years. Thus, we have been able to solve some problems regarding the role of Na/K pump in metabolic regulation of semipermeable properties, excitability and chemosensitivity of neuronal membrane, which are briefly presented below.

2. The Na/K Pump Controls Membrane Potential in Neurons

The low and high permeability of cell membrane for Na^+ and K^+, respectively, are the main statements for membrane theory developed by Hodgkin-Huxley-Katz and are based on Goldman's constant field theory suggesting that the membrane potential (MP) is a sum of electrochemical potentials for K (E_k), Na (E_{Na}) and Cl (E_{Cl}) ions. This is known as Goldman-Hodgkin-Katz equation:

$$V_m = \frac{RT}{F}\ln\left(\frac{P_K\left[K^+\right]_o + P_{Na}\left[Na^+\right]_o + P_{Cl}\left[Cl^-\right]_i}{P_K\left[K^+\right]_i + P_{Na}\left[Na^+\right]_i + P_{Cl}\left[Cl^-\right]_o}\right)$$

V_m is the MP, R is the universal gas constant, T is the temperature, F is the Faraday's constant, P_K, P_{Na} and P_{Cl} are the membrane permeability for these ions. The symbols $[\ ]_i$ and $[\ ]_o$ refer to the thermodynamic activities of the ions depending on their being inside or outside of the cell, respectively.

However, the unreliability of this equation is shown by the study of MP dependence on $[K]_o$ and temperature. The studies on various objects have shown the absence of a linear dependence between MP and $[K]_o$ at low ranges up to 15 - 20 mM [4] [5].

The issue on MP deviation from the theoretical size of electrochemical potential (E_K) (calculated by Nernst formula at low $[K]_o$) became a subject of special considerations and a number of hypotheses on the nature of such a non-linear dependence between the MP size and $[K]_o$ were suggested [4]. However, none of them could give a reliable explanation of this phenomenon as well as evaluate the nature of the metabolic mechanism controlling potassium-electrode properties of cell membrane. In 1940 Dean theoretically suggested that the non-linear dependence of MP on $[K]_o$ could be an active extrusion of Na^+ from the cells [4]. This prediction of Dean was experimentally proven by Grundfest's study of MP dependence on $[K]_i$ in squid axon, demonstrating that the increase of $[K]_i$ did not lead to elevation of MP as was assumed by Nernst's law. Therefore, he noted the contribution of Na/K pump in generation of MP in axon [7]. However, as squid axon has high electrical conductivity, shunting the Na/K pump current and generating low MP value, the validity of these data has not been adequately considered in literature. Later, Grundfest's results were proven by a number of works performed on muscles [4] and neurons [8] [9].

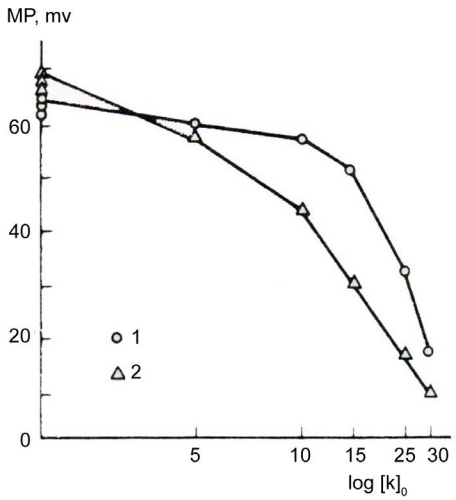

Figure 1. Membrane potential as a fraction of logarithm of external potassium ion concentration in "normal" Ringer's solution (1) and in presence of 0.2 mM dinitrophenol (2) [8].

Our study regarding the dependence of ionic composition of snail neuron on $[K]_0$ has shown that the $[K]_0$-induced decrease of $[Na]_i$, which takes place without changing $[K]_i$, as well as the activation of nerve ganglia respiration (O_2 uptake) are mostly expressed in the region of $[K]_0$ = 0 - 15 mM, which is blocked by cold (4˚C) and Na/K pump inhibitor-strophantin (**Figure 1**). This clearly indicates that in normal living state cell metabolism controls the level of MP through electrogenic Na/K pump [10].

By using the "voltage-clamp" and "concentration-clamp" methods we have shown that the 1mM $[K]_o$ with "0" effect on E_k, generates the potential-independent pump current in neurons, which proves that the $[K]_o$-induced activation of pump is not due to membrane depolarization but is a result of $[K]_o$-induced activation of Na/K-ATPase (**Figure 2**) [11].

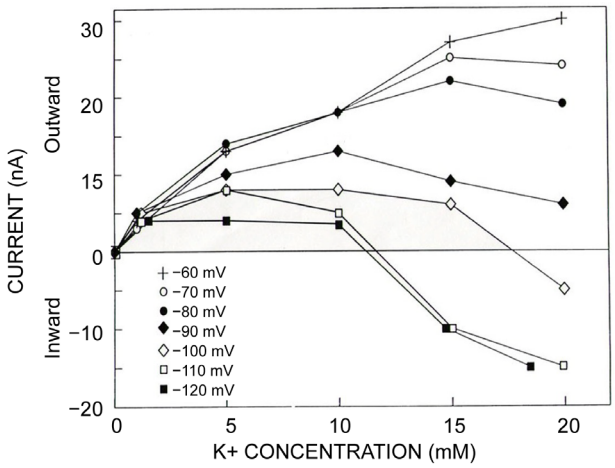

Figure 2. The MP-dependence of 1 mM $[K]_o$-activated Na/K pump currents, which was measured by "voltage-clamp" and "concentration-clamp" methods [11].

The existence of Na/K pump-dependent component of MP was also demonstrated by a number of studies on the temperature sensitivity of cells MP. The incomparably higher temperature sensitivity of Na/K pump-dependent component of MP compared with E_k predicted from Nernst's law (1.8 mV/10˚C), has been shown in molluscan neurons having strong modulation effects on endogenous (pacemaker) activity [9] [12] [13] [14]. It has been shown that the variations of metabolic components of MP are responsible for the endogenous generation of electrical activity and spontaneous inhibition of this activity [13] [15].

3. The Dependence of Cell Volume on Na/K Pump Electrogenicity

At present, when the electrogenic character of Na/K pump is a well-documented fact, its central role in cell volume regulation has become more obvious. The evolution of the studies on the role of Na/K pump in cell volume regulation has recently been presented in an excellent review by Alan R. Kay and Mordecai Blaustein [16]. The second author of this work, during 50 years of his research, has published a number of pioneer discoveries regarding the carrier-driven ion transporting mechanisms, such as Na/K pump and Na/Ca exchange in membrane and their physiological meaning. However, the feedback mechanisms between cell volume and Na/K pump activity, as well as the role of Na/K pump-dependence on cell volume in regulation of biophysical properties of cell membrane, such as membrane permeability, fluidity, excitability and chemosensitivity remain non-sufficiently evaluated.

Since the rigidity of animal cell membrane is not so high to be able to stand to considerable gradients of both hydrostatic and osmotic pressures they behave as an osmometer: cell swells or shrinks by taking in or giving out water through the cell membrane. Therefore, for studying the dependence of Na/K pump activity on cell volume as well as the role of Na/K pump-dependent cell volume in regulation of membrane biophysical properties we have studied cell swelling and shrinkage in hypotonic and hypertonic saline, respectively. Neurons were chosen as experimental models because they have weak, expressed and fast cell volume recovery (CVR) systems to response of osmolality changes in cell bathing solution like non-excitable cells [17].

The studies on the osmotic properties of isolated giant neurons of Helix and Aplysia mollusks have shown that the cell volume changes (swelling and shrinkage) to osmotic pressure have a discreet character [18] [19]. Such a non-gradual dependence of cell volume on osmotic gradients on the membrane has been explained by the existence of invaginations (caveolae) in cell membrane surface, which open by swelling and close by cell shrinking. The existence of caveolae in plasma membrane was identified by electron microscopy in 1953 [20], but their functions became a subject for investigations only at the end of the last century [21].

We have developed a system using cultured Aplysia neurons and confocal scanning laser microscopy to directly monitor cell volume when the osmolality of the perfusion solution is altered and when sodium transport is blocked. Volume changes of greater than 30% were observed, accompanied by changes in surface area of greater than 15%. The volume increases secondary to sodium pump inhibition and hypotonic solutions and the volume decrease secondary to hypertonic solutions were reversible. These results demonstrate that neuronal volume may change dramatically and raise the possibility that dynamic changes in neuronal cell volume may have physiological importance (**Figure 3**) [19].

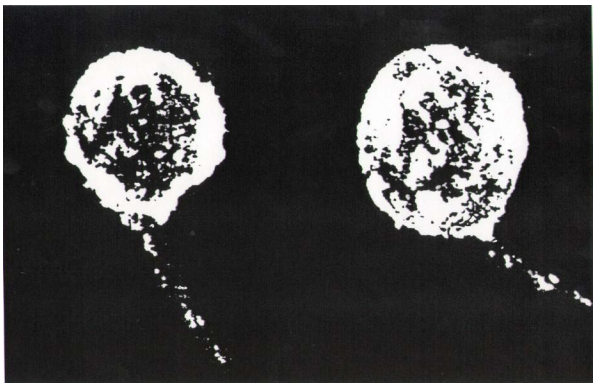

Figure 3. The effect of 10^{-5} M ouabain containing ASW on medium neuron of Aplysia. The left neuron was in normal state, the right one was subjected to ouabain containing ASW for 5 min. Changes of neuronal volume of 30% or more were regularly observed with this manipulation and corresponding changes of surface are of at least 15% [19].

Although the electrogenic character of Na/K pump predicts its crucial role in cell volume regulation and it has been reported that such swelling occurs when Na/K pump is inhibited with cardiac glycosides and K-free solution, the existing data on this question are, to some extent, conflicting. The ouabain-induced pump inactivation in *in vitro* experiments isn't often accompanied by neuron swelling as it is predicted by pump hypothesis [16].

The detailed investigation of ouabain effect on water and ion contents of freshly prepared rabbit and rat renal cortical slices [22] [23] and isolated single neurons of mollusk [24] shows that the ouabain-induced pump inhibition is accompanied by cell swelling. The result is explained by the fact that cell membrane in fresh preparation has comparably higher electrical resistance, which brings to higher Na/K pump electrogenicity, while cells in "non-fresh" preparations are in their swelling state and pump electrogenicity is shunted, thus the ouabain-induced cell swelling is absent [23] [24]. It has been shown that the cell volume dependence on electrogenic Na/K pump activity is depressed by high membrane permeability for Cl ions, which increases as a result of the impairments of metabolic water efflux from the cell [24].

The normal Ringer used had the following composition: NaCl, 80 mM; KC1, 4 mM:CaCl;, 7 mM; $MgCl_2$, 13 mM; Tris-chloride (pH 7.8), 10 mM; and glucose, 10mM. The potassium-free solution had an excess of 4 mM NaCl above normal. In order to change the tonicity (T;) without altering the ionic strength, the NaCl content of the standard solution was reduced to 40 mM ($[Na]_o$ = 40 mM/) and sucrose was used to obtain media of different tonicities, from *T,* - 0.5 (0 mMsucrose) to *T,* - 2 (189 mM sucrose). In some experiments *T,* was equal to 2.5 (252 mM sucrose). In replacing NaCl by sucrose, 1 mM of NaCl was taken as osmotically equivalent to 1.57 mM of sucrose [25].

The fact that the electrogenicity of Na/K pump strongly depends on cell membrane resistance is indicated in the data presented in **Figure 4** on the membrane I-V characteristics and Na/K pump current (Ip) depending on osmolality of cell bathing PS, where the peak of Ip after the transfer of neurons from K-free to normal PS is increased with a rise in tonicity, which is accompanied by the increase of membrane resistance.

Thus, the obtained data indicate that there is a negative feedback between Na/K pump-generated currents and membrane permeability that is realized by Na/K pump-activated cell shrinkage, which has a crucial role for the quick recovery of the factor-induced increase of membrane permeability [18].

4. The Na/K Pump Controls Membrane Semipermeability and Excitability

One of the essential omissions of membrane theory of Hodgkin-Huxley-Katz on nerve excitation is the disregard of water fluxes through the membrane, which are potential-dependent and lead to cell volume changes. Although, from the thermodynamic point of view, it is predicted that the MP variation leads to respective changes of osmotic gradient on the membrane by generating water

fluxes through the membrane and cell volume changes. It is worth noting that one of the main postulates of membrane theory is that "membrane conductance increases by membrane depolarization", but the role of water influx through the membrane in determining membrane depolarization-induced increase of membrane conductance has not been considered [1]. Meanwhile, Tasaki and coworkers [26], as well as Terakawa [27] have shown that membrane depolarization and hyperpolarization lead to axon swelling and shrinkage, respectively, even during generation of single action potential by using the elegant experimental methods for detection of squid axon diameter changes.

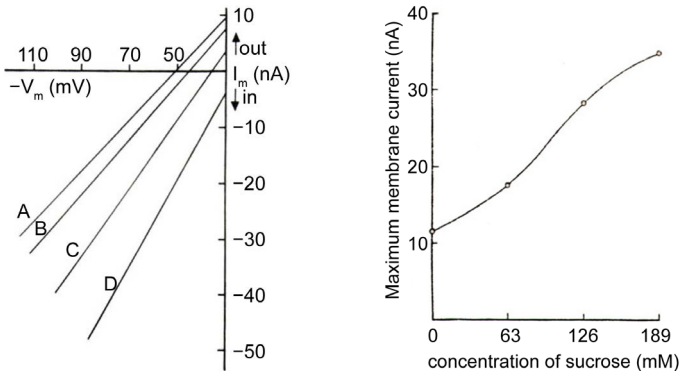

Figure 4. The I-V characteristics of membrane and peak of pump-induced current in normal Ringer's solutions with different tonicities after preliminary incubation of neurons in potassium-free solution. The cell was clamped at the resting potential level in normal Ringer's Motion (46 mV). The curve was drawn by eye [18].

Although at present the role of electrogenic Na/K pump as a powerful mechanism in controlling water fluxes through the membrane and the cell volume can be considered as a well-documented fact, the physiological meaning of such fundamental properties of Na/K pump has not been adequately considered in literature. Therefore, this question was the subject of our study [28].

It is known that Na/K pump generates water efflux from the cells due to its function in stoichiometry of 3N:2k and, being the highest APT utilizing machine in membrane, it stimulates endogenous water molecules during intracellular oxidative-phosphorylation.

Traditionally, the role of electrogenic Na/K pump in regulation of membrane excitability is explained by pump-induced membrane hyperpolarization. However, as can be seen in the studies on the neuronal endogenous activity of Japanese land snail presented in **Figure 5**, in case of the Na/K pump inactivation by K-free physiological saline the membrane hyperpolarization is accompanied by activation of electrical activity of neuron, while at 5mM $[K]_o$-induced activation of pump, the membrane depolarization is accompanied by inhibition of its activity [28].

The potential-independent and Na/K pump-induced inhibition of neuronal activity is more pronounced when $[K]_o$ is replaced by $[Rb]_o$. The analogical depression has been obtained by noradrenalin-induced activation of Na/K pump as

well as by applying hypertonic solution [28].

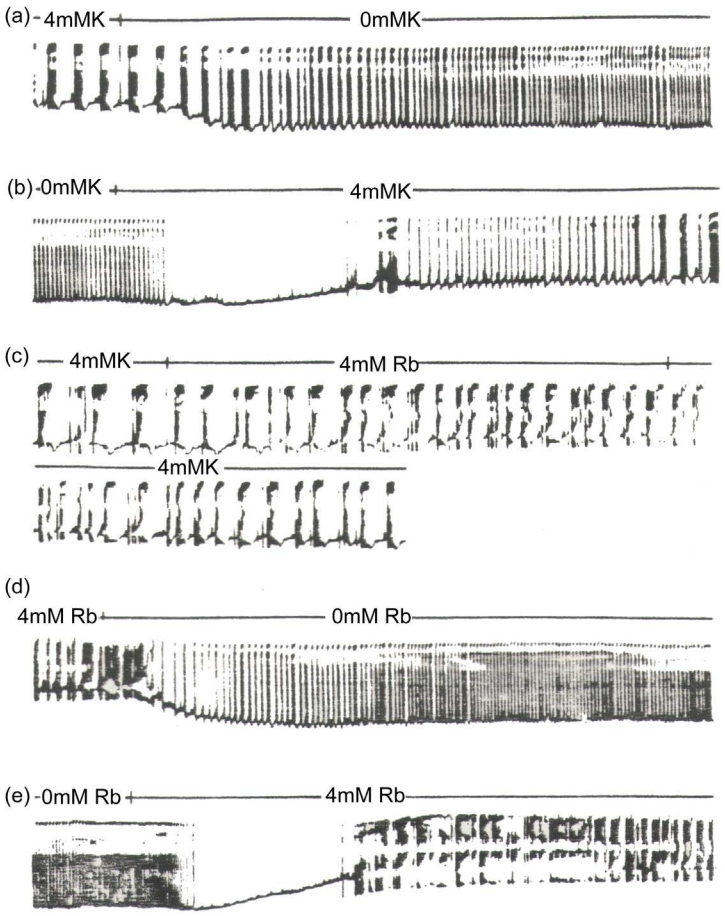

Figure 5. The effect of O mM K (a), 4 mM K (b), O RbmM (d) and 4 mM Rb (e) induced hyperpolarization and 4 mM K replacement by 4 mM Rb (c) in external solution on bursting activity of pacemaker neurons [28].

Thus, the obtained data clearly indicate that the Na/K pump-induced inactivation of membrane excitability is also realized by potential-independent mechanism, which can be due to water efflux and membrane surface decrease as a result of cell shrinkage. For estimation of the role of water fluxes through membrane in regulation of membrane excitability, the effect of hypertonic solution-induced water efflux on membrane inward sodium (I_{Na}) and outward potassium (I_K) currents in internally perfused squid axon as well as in intracellular perfused and intact neurons has been studied [29] [30] [31].

The method of internal perfusion of giant axon is similar to the method of Baker *et al.* [32]. The control isotonic solution for the axons contains (in mM): NaCI 517, KCI 5; $CaCI_2$ 50; Na-HEPES (pH 7.8) 12.7. A solution with low Na concentration is prepared by replacing 300 mM NaCI by 500 mM glucose. The isotonic internal solution contains (in mM) KF 100, glucose 888, Na-HEPES 12. Hypertonic and hypotonic solutions are prepared by adding or reducing 500 mM glucose in corresponding isotonic solutions.

As can be seen in Figure 6, water efflux has time-dependent activation of potassium currents (I_k) and depressed tail currents are due to I_k [33].

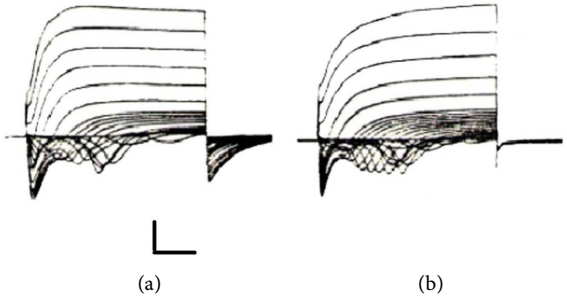

Figure 6. (a) Transmembrane ionic currents in internally perfused squid axon when in both sides of the membrane there were isotonic solutions; (b) Transmembrane ionic currents in internally perfused axon when it was internally hypotonic and externally isotonic solutions (outward water fluxes). The calibrations are 25 nA and 10 msec [33].

It is known that TTX (specific inhibitor for Na channels) in 10^{-9}M fully inhibits Na channels which are in active state. In order to evaluate the inactivation and activation effects of water efflux and influx on inward Na current, respectively, we have studied the effects of water influx on excitability of the axon poisoned by 10^{-8} M TTX, *i.e.* where all active Na channels are in blocked states.

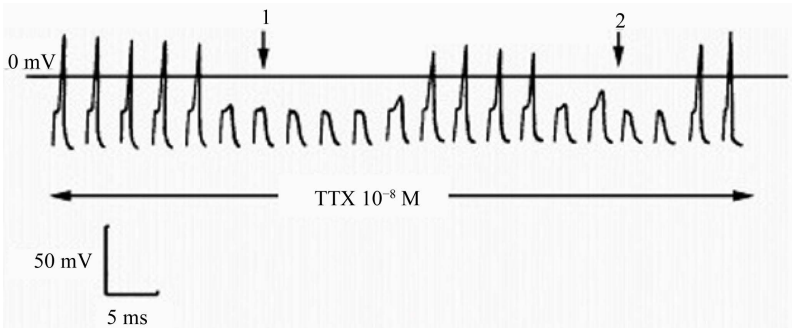

Figure 7. The transient recovery of the action potentials in squid giant axon in 10^{-8} M TTX containing solution at changes of the tonicity of the external solution. Originally the axon was perfused by the hypertonic external and isotonic internal solutions (outward transmembrane water flow was present). At the first arrow the outside hypertonic solution was replaced by isotonic one (transmembrane water flow was stopped). At the second arrow, external isotonic solution was replaced by the hypotonic one and inward transmembrane water flow was present. Outward and inward transmembrane water flows were produced by adding or removing 500 mM glucose in external isotonic solution [29].

As can be seen in Figure 7, the transient recovery of the action potentials was observed in 10^{-8} M TTX-containing solution, when water influx through the membrane was applied. These data clearly indicate that there are Na channels in axon with different energy activations and water influx has activation effects on

"reserve" channels, which are poisoned only by their activation. These suggestions are supported by the data of "voltage-clamp" study on water inward andoutward water fluxes effects on inward I_{Na} and outward I_K in internally perfused squid axon in normal (517 mM NaCI) and 217 mM NaCI external solution (Figure 8).

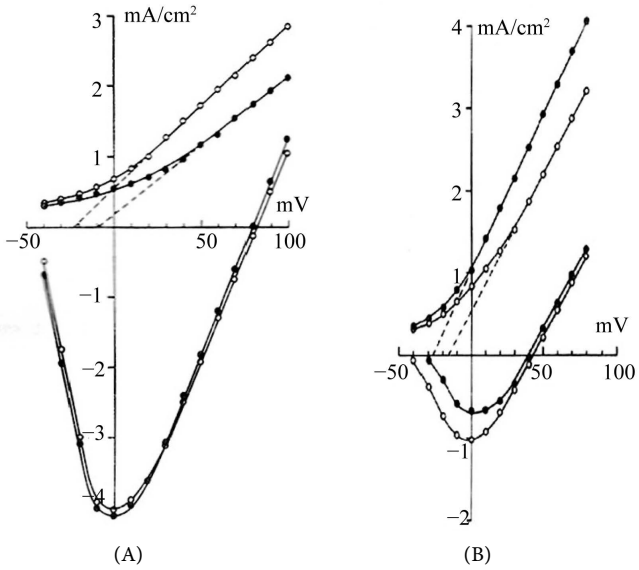

Figure 8. (A) Voltage of the membrane with (A) and without (B) inward water flow at the normal $[Na]_o$ = 517 mM NaCI. The peak of inward and steady outward currents at the end of 25-msec depolarizing voltage pulses with (o) and without (.) the water flow as plotted against the membrane potential. The holding potential was −100 mV. (B) Current-Voltage relations the membrane currents with and without outward water flow at the $[Na]_o$ = 217 mM NaCI [29].

As can be seen in Figure 8, water efflux has activation effect on potassium outward current (I_k) but has no significant effect on inward current (I_{Na}) at external medium containing 517 mM NaCI, *i.e.*, where Na gradient has stronger effect on channel than water efflux from the axon (A), while at low (217 mM) NaCI external solution the water influx has inactivation effect on I_k and significant activation effect on I_{Na} (B). These data indicate that water fluxes have activation effect on ionic currents having the same direction and inactivation effect on the ionic currents with opposite directions [29]. The fact that the number of functionally active channels depends on the membrane surface changes is clearly demonstrated by the study of ionic currents in snail intact neurons incubated in physiological solution (PS) with different osmolality [29].

As can be seen in Figure 9, upon the addition of 252 mM sucrose to the external solution, the volume of snail neurons gradually diminishes for 10 - 20 min and then stabilizes, *i.e.* for about 10 min the outward water flow through the membrane takes place and then stops after stabilization of the cell volume. Upon the returning of the neuron to the isotonic medium, the opposite process, in-

ward water flow through the membrane takes place [18] [29]. The study of the same time-dependent changes of amplitudes of outward K currents shows that, beginning from the 30th sec after placing the neuron in the hypertonic solution, the K outward current increases despite the fact that the amplitude of the command pulse remains constant. After 2.5 min, the outward current reaches its maximum value and then begins to decrease. Such a decrease of the K current lasts up to the 10th min, *i.e.* until stabilization of the neuronal volume in the hypertonic solution [18] [29].

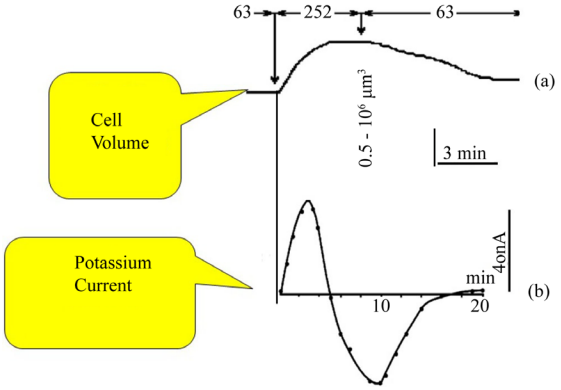

Figure 9. Time-dependent changes of cell volume and amplitudes of potassium currents after replacing isotonic PS by hypertonic (252 mM sucrose) and after returning of the neuron to the isotonic medium [18] [29].

The theoretical analysis of the experimental data on the effect of the outward and inward water flows on the outward potassium currents showed the maximum potassium conductance (G_{kmax}) increases by outward water efflux, while the inward water flow has the opposite effect. These data suggest that the changes of transmembrane currents during transmembrane water flow in dialyzed neurons are mainly due to the changes in single-channel conductance and the time constant of current activation [31].

Thus, the presented data firstly elucidate the nature of the metabolic mechanism controlling low permeability of cell membrane for Na and high permeability for K ions: osmotic water influx precedes the activation of sodium ionic channels for inward currents. Therefore, the metabolic water efflux from the cell, in which Na/K pump has a central role, inactivates membrane permeability for Na influx and activates membrane permeability for K efflux. Thus, on the basis of the aforementioned data, the impairment of Na/K pump-induced water efflux from the neurons, leading to the increase of membrane permeability for Na and membrane excitability, is suggested as a common consequence of nerve disorders.

5. The Na/K Pump Dependence on Cell Volume: The Auto-Regulation of Na/K Pump

As the electrogenic Na/K pump has a pivotal role in cell volume regulation, it is

predicted to be a negative feedback between cell volume and Na/K pump activity through which the auto-regulation of Na/K pump takes place. To check this suggestion we have studied the dependence of Na/K pump activity on cell volume by changing the osmolality of cell surrounding medium by means of measuring ^{22}Na efflux from the cells and counting pump units (^{3}H-ouabain receptors) in membrane [18].

The information about the osmolality effects on Na/K pump activity in the literature is contradictory: Keynes (1965) has observed that an increase in external tonicity stimulates the active ^{22}Na efflux from muscle [34], Mullins and Awad [35] haven't observed significant effect of tonicity on ^{22}Na efflux, while Venosa has shown that the tonicity leads to stimulation of ^{22}Na from muscle [36]. We have studied the dependence of cell bathing medium tonicity on ^{22}Na efflux from neurons with low and high $[Na]_i$. For this purpose, before ^{22}Na efflux measurement in normal saline with different osmolality, the neurons were incubated in K-free, ^{22}Na containing medium for 15 min (in case of low $[Na]_i$) and for 6 hours (in case of high $[Na]_i$). As can be seen in **Figure 10**, cell shrinkage activates the ^{22}Na efflux at low $[Na]_i$ and inhibits it at high $[Na]_i$.

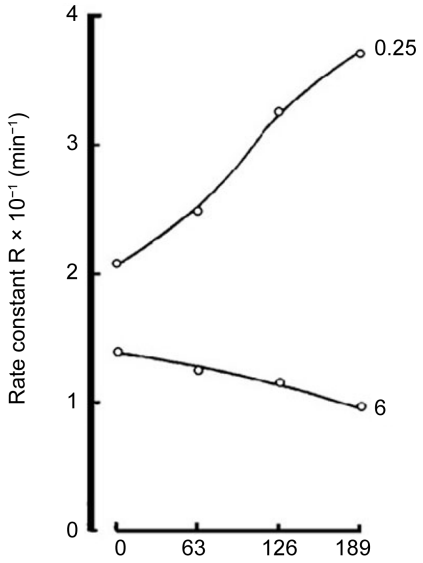

Figure 10. The initial rate constant of ^{22}Na efflux from the cells and the number of [^{3}H]-ouabain molecules binding with membrane as a function of the tonicity of the surrounding medium for cells exposures for 25 min and 6 hours to cold K-free solution containing of ^{22}Na. Numbers on the right-hand side of the curves represent the incubation times (hours). Curves are drawn by eye [18].

By this study it becomes clear that osmolality increase of cell bathing solution has double effects on Na/K pump activity: on one hand it activates Na/K pump by shrinkage-induced increase of $[Na]_i$ and on the other hand it decreases pump activity by an unknown mechanism in case of high $[Na]_i$. The double effect of osmolality on cell surrounding medium on Na/K pump activity is more clearly

demonstrated by the study on the dependence of the rate constant of Na efflux from neurons on time in solutions with different tonicities.

In order to determine whether the number of functioning pump units in the-membrane is changed in response to dependent changes of cell volume the inhibitory effect of ouabain on Na/K pump activity as well as [^{3}H]-ouabain binding with cell membrane of bathing neurons with different osmolality have been studied.

Table 1. Binding of [^{3}H]-ouabain to Helix pomatia cell membrane as a function of concentration of glycoside in solutions with different tonicities ($\times 10^8$ molecules/mg dry weight) [18].

Ouabain Content (Mol)	Incubation Medium		
	Hypotonic	Isotonic	Hypertonic
10×10^{-10}	4.59 ± 0.32	3.23 ± 0.24	2.03 ± 0.16
3×10^{-10}	18.3 ± 1.4	11.7 ± 0.87	6.29 ± 0.41
6×10^{-10}	28.9 ± 20	17.9 ± 1.2	10.0 ± 0.67
1×10^{-9}	32.0 ± 2.2	21.1 ± 1.4	12.2 ± 0.9
3×10^{-9}	144 ± 29.4	90.5 ± 5.7	53.8 ± 3.1
6×10^{-9}	431 ± 29.4	266 ± 15.8	147 ± 9.7
1×10^{-8}	793 ± 45.6	508 ± 30.1	283 ± 19.4

The data presented in Table 1, clearly indicate that cell swelling in hypotonic medium increases the number of ouabain receptors in membrane, while the shrinkage in hypertonic solution leads to opposite effects, *i.e.* to the decrease of the number of ouabain receptors compared with the number of ouabain receptors of neurons bathing in isotonic medium.

To elucidate whether the number of functioning pump units is changed under normal conditions in response to the increased passive membrane permeability, the binding of [^{3}H]-ouabain to the membrane was studied in the presence of synaptic transmitters [18].

Table 2. The effects of Ach, GABA and PTZ on [^{3}H]-ouabain binding to neuronal membrane [18].

Ouabain Content in the medium (Mol)	Normal Ringer	Normal Ringer Containing 10^{-4} M Ach	Normal Ringer Containing 10^{-4} MGABA	Isotonic + 5×10^{-2} M PTZ
10×10^{-20}	3.16 ± 0.48	5.13 ± 0.62	4.24 ± 0.21	5.21 ± 0.28
1×10^{-9}	20.56 ± 0.55	30.54 ± 1.55	27.63 ± 3.17	17.18 ± 1.1
5×10^{-9}	109.40 ± 10.47	170.29 ± 13.36	139.62 ± 11.43	26.79 ± 1.8
1×10^{-8}	143.54 ± 8.91	270.93 ± 28.53	174.48 ± 13.54	29.0 ± 2.3
1×10^{-7}	3254.47 ± 74.20	3944.33 ± 107.23	-	-
1×10^{-6}	23938.20 ± 852.41	3944.33 ± 107.23	-	-

From Table 2, it can be clearly seen that ACh and GABA increase ouabain binding significantly. Therefore, we suggest that the increased membrane permeability brought about by exposure to synaptic transmitters is accompanied by a corresponding alteration in the number of functioning pump units in the-membrane.

Thus, the facts that water influx leads to the increase of membrane permeability for ions leading to cell swelling, which stimulates the pump activity by the increase of the number of pump units in membrane, while the pump activation-induced cell shrinkage decreases membrane permeability (membrane conductance) and the number of pump units in membrane, indicate that pump-dependent cell volume serves as an auto-regulatory system for pump. By this mechanism the metabolic regulation of membrane functions, such as excitability [37], chemo-sensitivity [38] [39] and second exchanger systems are realized. Therefore, the dysfunction of the auto-regulation of Na/K pump is suggested as a common consequence of cell pathology.

6. The Ouabain Inactivates Na/K Pump and Activates Na/Ca Exchange

Baker *et al.* have identified two components of ^{22}Na efflux in intra-perfused squid axon. They are ouabain-sensitive and insensitive components, which are due to Na/K pump and Na/Ca exchange in reverse (R) mode, respectively [40]. These authors, have explained the activation of R Na/Ca exchange by Na/K pump inactivation-induced increase of $[Na]_i$. Our study of dose-dependent ouabain effect on ^{22}Na efflux from intact neurons and rat brain and heart muscle tissues has shown that ouabain inactivates Na/K pump only at high ($>10^{-7}$ M) concentrations, while the low concentrations ($<10^{-7}$ M) of ouabain have activation effects on ^{22}Na efflux from the cells (Figure 11), which does not have effect on Na/K pump activity measured by ^{86}Rb uptake (Figure 12).

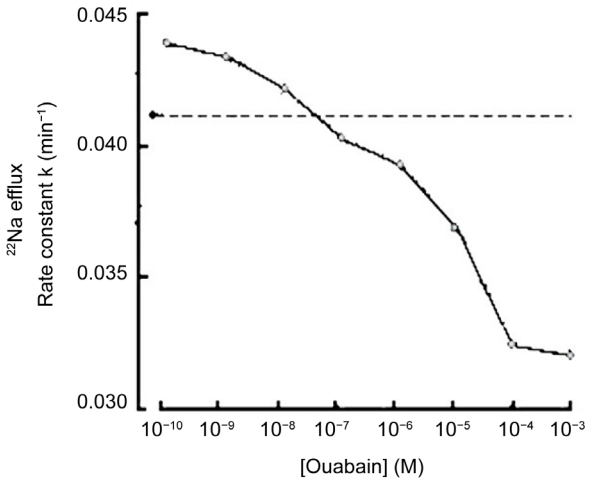

Figure 11. The dose-dependent (10^{-10} to 10^{-3} M) ouabain effect on the rate constant of ^{22}Na efflux from the neurons [18].

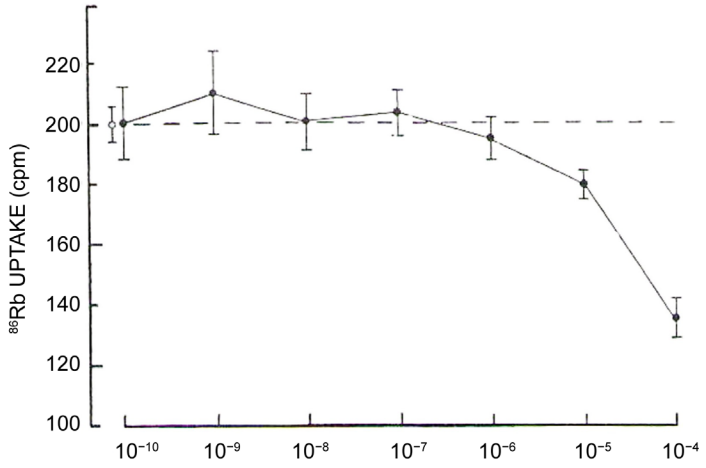

Figure 12. The dose-dependent (10^{-10} to 10^{-3} M) ouabain effect on the rate constant of ^{86}Rb uptake in neurons [43].

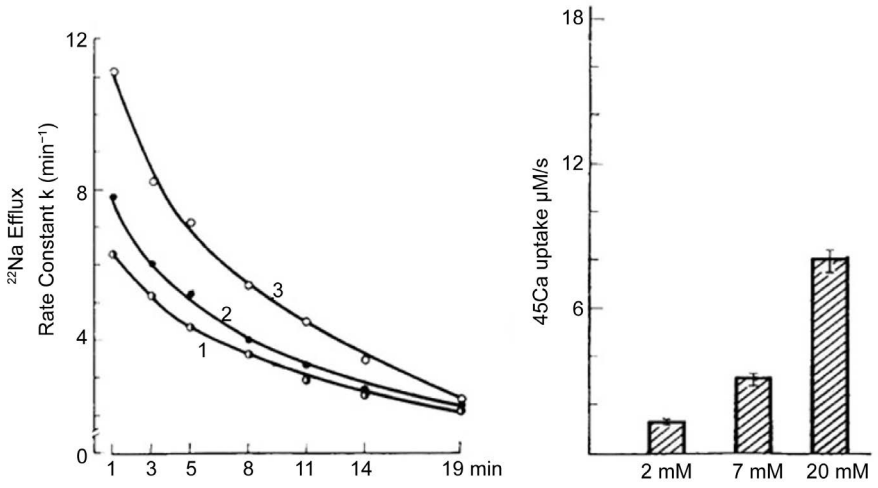

Figure 13. (a) The time-dependent ^{22}Na efflux from the neurons in presence of 2 mM (1); 7 mM (2) and 20 mM (3) Ca; (b) ^{45}Ca-uptake by neurons at 2 mM; 7 mM and 20 mM Ca [43].

It has been shown that activation of ^{22}Na efflux by low ouabain in intact neurons, like ouabain-insensitive ^{22}Na efflux in perfused axon [40], is due to activation of R Na/Ca exchange (**Figure 13(a)** and **Figure 13(b)**). The ^{22}Na effluxfrom neurons in K-free solution is activated by the increase of Ca content in cell bathing medium (**Figure 13(a)**), which is accompanied by ^{45}Ca uptake increase (**Figure 13(b)**).

In order to evaluate how the dose-dependent effect of ouabain on ^{22}Na efflux is related with ouabain binding with membrane receptors we have studied the dose-dependent [^{3}H]-ouabain binding with cell membrane of snail neurons.

It has been shown that two saturated components of ouabain binding may be distinguished on the dose-dependent curve of ouabain binding with cell membrane: one—at concentrations from 10^{-10} to 10^{-9} M and the other—at ouabain

concentrations between 10^{-9} and 10^{-7} M. Moreover, the kinetics of dose-dependent ouabain binding curve does not change by variation of osmolality of cell bathing medium (**Figure 14**) [18].

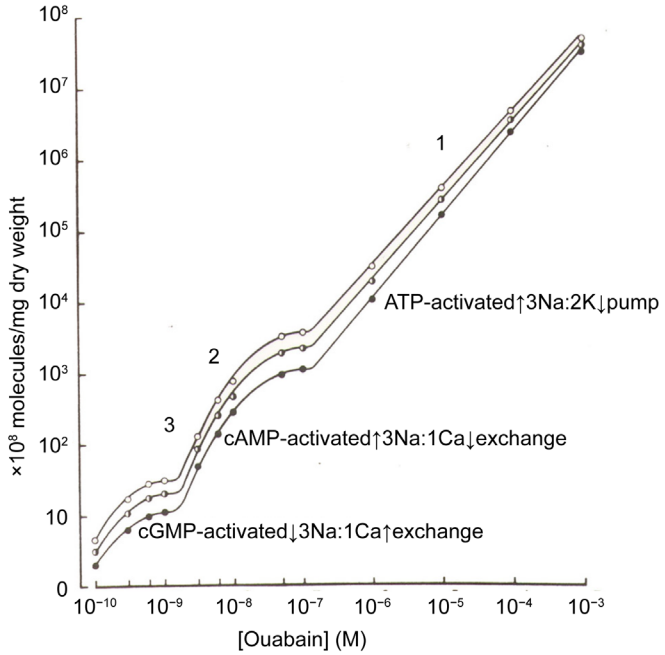

Figure 14. Dose-dependent [^{3}H]-ouabain binding with cell membrane in isotonic, hypertonic and hypotonic mediums [18].

Later, these three types of ouabain receptors were characterized as α_1, α_2 and α_3 isoforms of Na/K-ATPase [41]. The involvement of these receptors in R Na/Ca exchange was explained by ouabain-induced inactivation of Na/K pump leading to local increase of $[Na]_i$ [42].

However, our study on mollusk neurons, rat brain and heart muscle tissues has shown that the function of low ouabain-induced activation of Na/Ca exchange and the function of α_2 and α_3 receptors has no direct relation with Na/K pump activity [43]. It has been shown that the activation of Na efflux from the cells by both $[cGMP]_i$ and $[cAMP]_i$ are due to Na/Ca exchange in forward mode (F) and R Na/Ca exchange, respectively. The activation of α_3 and α_2 receptors stimulates the cGMP-dependent F Na/Ca exchange, and cAMP-dependent R Na/Ca exchange, respectively [43] [44].

It has also been shown that α_3/α_2 receptors are non-specific for ouabain molecules and serve as common membrane targets for the impact of various weak chemical and physical signals unable to activate ionic channels and Na/K pump activity such as low concentrations of transmitters ($<10^{-12}$ M) [45] [46], magnetized solution, microwave having intensity even less than the thermal thresholds [47] [48].

Figure 15 shows that low concentration of Ach, which is unable to activate ionic channels and Na/K pump activity in neurons, has potential-independent

modulation of GABA-induced ionic currents in neuron, while in oocytes injected with mRNA for different receptors this modulation is absent (Figure 15). These data clearly indicate that low Ach effect on GABA-induced current is not realized through Ach receptor or Na/K pump, and is due to activation of cAMP-dependent activation of R Na/Ca exchange [46].

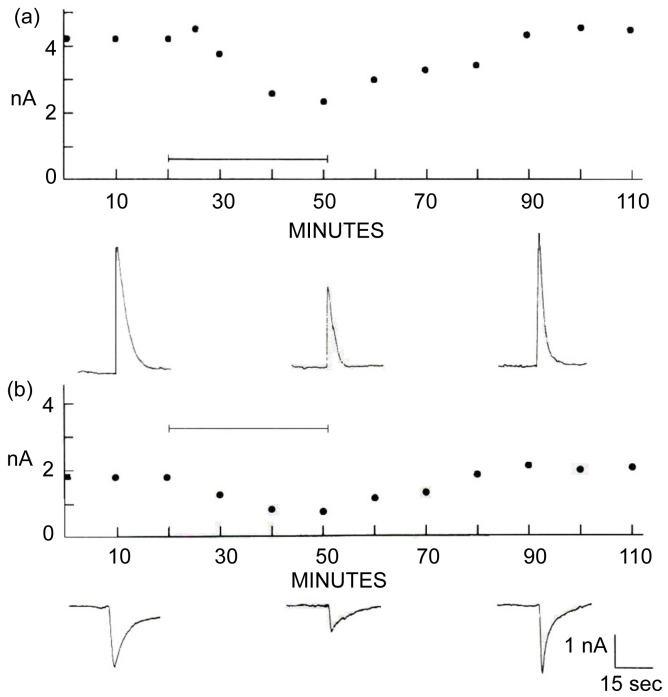

Figure 15. The effect of 10^{-13} M Ach (applied during the bars from 20 to 50 min) on GABA responses of a medial pleural neuron. Part A shows a plot of peak GABA response amplitude with time when the neuron was held at −50 mV. Three records of the currents are shown below the plot to illustrate the control, depressed, and recovered GABA currents. After microperfusion of Ach the peak amplitude declined to about 40% of control. Part B shows results of a repeat of this experiment in the same neuron, but with the holding potential at −65 mV [46].

The extra-sensitivity of α_3/α_2 receptors in membrane to various factors is in harmony with the groundbreaking discoveries of Robert Lefkowitz and Brian Kobilka regarding the extremely sensitive G-proteins-coupled receptors in cell membrane for which they have been awarded the Nobel Prize in Chemistry [49]. Thus, these data indicate that the high concentration of ouabain (>10^{-6} M) can only be considered as a specific inhibitor for Na/K-ATPase [3], while the low concentrations of ouabain have only intra-signaling function, which is thoroughly described in the review by Xie and Askari [50] [51].

It is worth noting that activation of both cGMP-dependent F Na/Ca exchange and cAMP-dependent R Na/Ca exchange generates water efflux from the cell, decreases membrane permeability for Na ions and controls Na gradient on the membrane: the cGMP-activated F Na/Ca exchange stimulates Na/K pump by

removing $[Ca]_i$, generating water efflux from the cells and decreasing membrane permeability for Na ions, while cAMP-activated R Na/Ca exchange, stimulates endogenous water formation by $[Ca]_i$-induced activation of mitochondrial function, which also generates water efflux from the cells and decreases membrane permeability for Na and controls Na gradient on the membrane. Thus, this explains the metabolic regulation of membrane semipermeability [44].

This suggestion is supported by the data on weak intensity static pulsing and magnetic field effects on cGMP-activation of F Na/Ca exchange in normal PS and activation of cAMP-dependent R Na/Ca exchange in K-free medium (**Figure 16**) [52] [53].

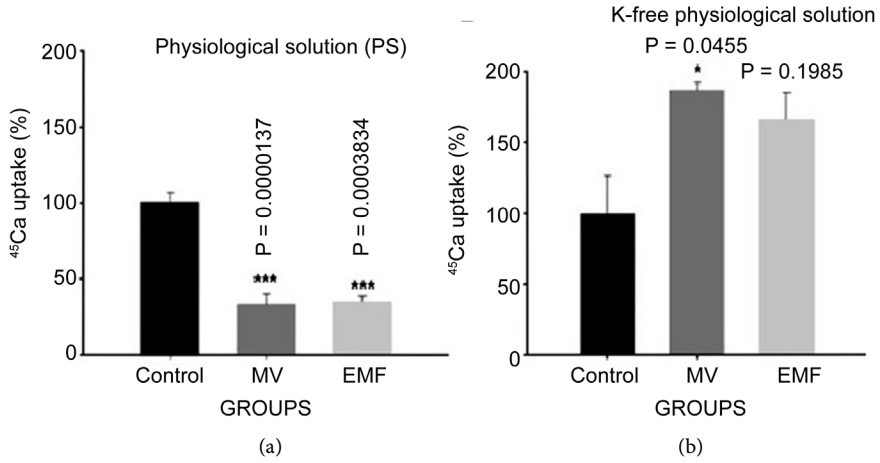

Figure 16. The effect of 4Hz mechanical vibration (MV) and ELF EMF on $^{45}Ca^{2+}$ uptake by neuronal ganglia in (a) normal PS and (b) K-free solution [53].

At present, the discovery of the role of intracellular cGMP/cAMP in regulation of Na/Ca exchange between cells and external medium as well as between different organoids and cytoplasm [54], allows to consider the extra-sensitivity of cyclic nucleotides-dependent changes of $[Ca]_i$ as a primary mechanism for modulation of Na/K pump activity in response to the impact of weak factors on cells and organism [55].

The study of synaptic transmitters sensitivity of cGMP-activated F Na/Ca exchange and cAMP-activated R Na/Ca exchange has shown that cGMP system is more sensitive to transmitters effects ($<10^{-15}$ M) than cAMP system (10^{-12} - 10^{-13} M) [56] [57].

On one hand the highest sensitivity of cGMP-dependent F Na/Ca exchange and on the other hand the high permeability of cell membrane for water allow us to suggest that the factor-induced cell hydration serves as a primary mechanism for the activation of soluble guanylate cyclase-induced cGMP synthesis. To check this suggestion, we have studied the effect of cell hydration on intracellular contents of cGMP and cAMP [56] [57]. It is known that membrane water permeability is elevated as a result of membrane fluidity increase. By our previous study we have shown that short chain fatty acids activate the membrane

fluidity, through which the strong modulation of membrane functions takes place [58] [59] [60].

Figure 17 shows that when K-free saline is added in cell bathing medium, non-metabolized 2-decenoic fatty acid (DA) leads to cell swelling, which is accompanied by the increase of cGMP without significant changes of cAMP contents. These data allow us to suggest that the increase of cell hydration could be a primary mechanism for activation of cGMP-dependent F Na/Ca exchange.

Experimental conditions	cAMP	cGMP
Control	100 ± 5	100 ± 5
DA	100 ± 9	124 ± 8

Figure 17. The 2-decenoid fatty acid-induced cell swelling in K-free solution. The first arrow indicates the moment when the K-free solution was replaced with K-free DA containing solution, the second arrow shows the moment of the effect of DA-induced water uptake on intracellular cyclic nucleotides (in %) [60].

Thus, the aforementioned data allow us to suggest that the dysfunction of cGMP/cAMP-dependent Na/Ca exchange-induced increase of $[Ca]_i$, which inhibits Na/K pump, is considered as a common consequence of cell pathology including cancer [61] [62] [63] [64], nerve disorders [65] [66] and cardio-vascular diseases [67] [68].

Although the Na/Ca exchange functions in stoichiometry of 3Na:1Ca [40], its activation in forward mode has hydration effect, while in reverse mode its activation has dehydration effect only on brain tissue of old animals, when $[Ca]_i$ is high. However, at low $[Ca]_i$ (in young animals), the activation of F Na/Ca exchange has dehydration effect on brain tissue as $[Ca]_i$ decrease leads to activation of Na/K pump, while the activation of RNa/Ca exchange leads to hydration of brain tissue by $[Ca]_i$-induced activation of mitochondrial production of H_2O [44]. It is worth noting that in young animals the activation of both F Na/Ca and R Na/Ca exchanges stimulates water efflux from the cells, leading to the decrease of membrane permeability for Na, and Na efflux from the cells leads to the increase of Na gradient on membrane serving as energy sources for different secondary exchange systems. These data indicate that the Na/Ca exchange in both directions is a key protective cell mechanism, the dysfunction of which brings to cell pathology, including aging [65] [68].

7. Conclusions

1) The electrogenic Na/K pump generates metabolic components of membrane potential through which the control of potassium electrode properties predicted by Nernst's law is realized.

2) Unlike ionic gradient-induced membrane currents, the Na/K pump current is increased by the elevation of membrane resistance.

3) As Na/K pump functions in stoichiometry of 3Na:2K, and is the highest

ATP utilizing machine; it increases endogenous water formation by activation of oxidative phosphorylation and generates water efflux from the cells. The latter, besides balancing osmotic water influx, has a pivotal role in controlling semi-permeable properties of cell membrane in resting state of neuron, *i.e.* low permeability for Na and high permeability for K ions.

4) The Na/K pump activation decreases the number of functionally active Na channels in membrane by both surface-dependent decrease of the number of functionally active channels and water efflux-induced inactivation of these ionic channels. Thus, there is a negative feedback between the number of functionally active Na ionic channel in membrane and Na/K pump activity.

5) There is also a negative feedback between cell volume and Na/K pump activity, which is realized through surface-dependent changes in the number of Na/K pump units (ouabain receptors), through which the autoregulation of Na/K pump activity is realized.

6) In intact neurons the curve of dose-dependent ouabain binding with cell membrane consists of two saturated and one linear part, out of which low affinity receptors with linear dose-dependent character are identified by Na/K pump functions, while the high and middle affinity receptors have cGMP-dependent F Na/Ca exchange and cAMP-dependent R Na/Ca exchange functions, respectively.

7) The intact neurons, unlike in case of neurons in internally perfused axon, have low ouabain-activated Na efflux, which is due to cAMP-dependent R Na/Ca exchange. The activation of the latter also takes place upon the impact of various weak chemical and physical signals, which are unable to activate ionic channels and modulate Na/K pump activity. Thus, it is suggested that G-proteins in membrane serve as such common sensors which activate cGMP and cAMP contents in the cell.

8) The activation of both cGMP-dependent FNa/Ca exchange and cAMP-dependent R Na/Ca exchange generates water efflux from the cell, decreases membrane permeability for Na ions and controls Na gradient on the membrane. The cGMP-activated F Na/Ca exchange stimulates Na/K pump by removing $[Ca]_i$, generating water efflux from the cells and decreasing membrane permeability for Na ions, while cAMP-activated R Na/Ca exchange, stimulates endogenous water formation by activation of mitochondrial function, which also generates water efflux from the cells and decreases membrane permeability for Na and controls Na gradient on the membrane. Thus, this explains the metabolic regulation of membrane semi permeability.

9) The dysfunction of G-proteins-regulated GMP/cAMP-dependent Na/Ca exchange, which controls low level of $[Ca]_i$ and inhibits Na/K pump, is a common consequence of cell pathology.

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

References

[1] Hodgkin, A.L. (1964) The Consecution of the Nervous Impulse. University Press, Liverpool.

[2] Katz, B. (1966) Nerve, Muscle and Synapse. Raven Press, New York.

[3] Skou, J.C. (1957) The Influence of Some Cations on an Adenosine Triphosphatase from Peripheral Nerves. *Biochimica et Biophysica Acta*, **23**, 394-401. https://doi.org/10.1016/0006-3002(57)90343-8

[4] Thomas, R.C. (1972) Electrogenic Sodium Pump in Nerve and Muscle Cells. *Physiological Reviews*, **52**, 563-594. https://doi.org/10.1152/physrev.1972.52.3.563

[5] Kerkut, G.A. and Thomas, R.C. (1965) An Electrogenic Sodium Pump in Snail Nerve Cells. *Comparative Biochemistry and Physiology*, **14**, 167-183. https://doi.org/10.1016/0010-406X(65)90017-4

[6] Thomas, R.C. (1969) Membrane Current and Intracellular Sodium Changes in a Snail Neuron during Extrusion of Injected Sodium. *The Journal of Physiology*, **201**, 495-514. https://doi.org/10.1113/jphysiol.1969.sp008769

[7] Grundfest, H., Kao, C.Y. and Mirano, M.A. (1954) Bioelectric Effect of Ions Microinjected into the Giant Axon of Loligo. *Journal of General Physiology*, **38**, 245-282. https://doi.org/10.1085/jgp.38.2.245

[8] Ayrapetyan, S.N. (1969) Metabolically Dependent Fraction of Membrane Potential and Electrode Properties of the Membrane of Giant Neurons in Mollusks. *Biofizika*, **14**, 1027-1031.

[9] Gorman, A.L. and Marmor, M.F. (1970) Contribution of Sodium Pump and Ionic Gradients to the Membrane Potential of Molluscan Neuron. *Physiology*, **210**, 897-917. https://doi.org/10.1113/jphysiol.1970.sp009248

[10] Ayrapetyan, S.N., Nasarenko, A.R. and Sorokina, Z.A. (1970) Dependency of Active Ion Transport in Snail Neurons of Ionic Composition of Extracellular Medium. *Biofizika*, **16**, 1037-1042.

[11] Ayrapetyan, G., Ayrapetyan, S. and Carpenter, D. (1991) The Electrogenic Sodium Pump Activity in Aplysia Neuron Is Not Potential-Dependent. *Acta Biological Hungarica*, **50**, 27-34.

[12] Carpenter, D.O. and Alving, B.O. (1968) A Contribution of an Electrogenic Na+ Pump to Membrane Potential in Aplysia Neurons. *Journal of General Physiology*, **52**, 1-21. https://doi.org/10.1085/jgp.52.1.1

[13] Carpenter, D.O. (1970) Membrane Potential Produced Directly by the Na Pump in Aplysia Neurons. *Comparative Biochemistry and Physiology*, **35**, 371-385. https://doi.org/10.1016/0010-406X(70)90602-X

[14] Ayrapetyan, S.N. (1969) Effect of Temperature on Membrane Potential of Giant Neurons in Snails. *Biofizika*, **14**, 663-668. https://doi.org/10.1007/BF01124277

[15] Ayrapetyan, S.N. (1969) Mechanism of Regulation of Spontaneous Activity of Snail Giant Neurons. *Biofizika*, **14**, 866-872. (In Russian)

[16] Kay, A.R. and Blaustein, M.P. (2019) Evolution of Our Understanding of Cell Volume Regulation by the Pump-Leak Mechanism. *Journal of General Physiology*, **151**, 407-416. https://doi.org/10.1085/jgp.201812274

[17] Hoffmann, E.K., Lambert, I.H. and Pedersen, S.F. (2009) Physiology of Cell Volume Regulation in Vertebrates. *Physiological Reviews*, **89**, 193-277. https://doi.org/10.1152/physrev.00037.2007

[18] Ayrapetyan, S.N., Suleymanyan, M.A., Sagian, A.A. and Dadalyan, S.S. (1984) Au-

toregulation of Electrogenic Sodium Pump. *Cellular and Molecular Neurobiology*, **4**, 367-384. https://doi.org/10.1007/BF00733598

[19] Carpenter, D.O., Fejtl, M., Ayrapetyan, S.N., Szarowski, D.H. and Turner, J.N. (1992) Dynamic Changes in Neuronal Volume Resulting from Osmotic and Sodium Transport Manipulations. *Acta Biologica Hungarica*, **43**, 39-48.

[20] Palade, G.E. (1953) Fine Structure of Blood Capillaries. *Journal of Applied Physics*, **24**, 1424-1432.

[21] Parton, R.G. and Simons, K. (2007) The Multiple Faces of Caveolae. *Nature Reviews*, **8**, 185-194. https://doi.org/10.1038/nrm2122

[22] Cooke, K.R. (1981) Ouabain and Regulation of Cellular Volume in Slices of Mammalian Renal Cortex. *The Journal of Physiology*, **320**, 319-332. https://doi.org/10.1113/jphysiol.1981.sp013952

[23] Cooke, K.R. (1978) Ouabain and Regulation of Cellular Volume in Freshly Prepared Slices of Rabbit Renal Cortex. *The Journal of Physiology*, **279**, 361-374. https://doi.org/10.1113/jphysiol.1978.sp012349

[24] Ayrapetyan, S.N. and Suleymanian, M.A. (1979) On the Pump-Induced Cell Volume Changes. *Comparative Biochemistry and Physiology*, **64A**, 571-575. https://doi.org/10.1016/0300-9629(79)90585-1

[25] Bloedel, J., Gage, P.W., Llinás, R. and Quastel, D.M. (1966) Transmitter Release at the Squid Giant Synapse in the Presence of Tetrodotoxin. *Nature*, **212**, 49-50. https://doi.org/10.1038/212049a0

[26] Iwasa, K., Tasaki, I. and Gibbons, R.C. (1980) Swelling of Nerve Fibers Associated with Action Potentials. *Science*, **210**, 338-339. https://doi.org/10.1126/science.7423196

[27] Terakawa, S. (1990) Intracellular Pressure and the Excitable Membrane. In: Ayrapetyan, S.N., Ed., *Metabolic Regulation of Membrane Function*, Academy of Sciences of Armenian SSR Publishing, Yerevan, 140-148.

[28] Kojima, M., Ayrapetyan, S. and Koketsu K. (1984) On the Membrane Potential Independent Mechanism of Sodium Pump-Induced Inhibition of Spontaneous Electrical Activity of Japanese Land Snail Neurons. *Comparative Biochemistry and Physiology*, **77**, 577-583. https://doi.org/10.1016/0300-9629(84)90232-9

[29] Ayrapetyan, S.N., Rychkov, G.Y. and Suleymanyan, M.A. (1988) Effects of Water Flow on Transmembrane Ionic Currents in Neurons of Helix Pomatia and in Squid Giant Axon. *Comparative Biochemistry and Physiology*, **89**, 179-186. https://doi.org/10.1016/0300-9629(88)91076-6

[30] Rychkov, G.Y., Suleymanian, M.A. and Ayrapetyan, S.N. (1989) The Dependence of Water Flow Effect on the Ionic Currents of Dialyzed Neuron on Fluidity of Somatic Membrane. *Biological Membranes*, **6**, 733-740. (In Russian)

[31] Suleymanian, M.A., Ayrapetyan, S.N., Arakelyan, V.B. and Ayrapetyan, V.Y. (1993) The Effect of Osmotic Gradient on the Outward Potassium Current in Dialyzed Neurons of Helix Pomatia. *Cellular and Molecular Neurobiology*, **13**, 183-190. https://doi.org/10.1007/BF00735374

[32] Baker, P.F., Hodgkin, A.L. and Shaw, T.I. (1962) The Effects of Changes in Internal Ionic Concentrations on the Electrical Properties of Perfused Giant Axons. *The Journal of Physiology*, **164**, 355-374. https://doi.org/10.1113/jphysiol.1962.sp007026

[33] Ayrapetyan, S.N. (1985) Activation and Inactivation Effect of the Transmembrane Water Flows on the Transmembrane Currents in Squid Giant Axon. *Biological Journal of Armenia*, **38**, 245-250.

[34] Keynes, R.D. (1965) Energy Transformations in the Generation of Bioelectricity. In: Chance, B., Estabrook, R.W. and Williamson, J.R., Eds., *Control of Energy Metabolism*, Academic Press, New York, 375-381. https://doi.org/10.1016/B978-1-4832-3161-7.50046-X

[35] Mullins, L.J. and Awad, M.Z. (1965) The Control of the Membrane Potential of Muscle Fibers by the Sodium Pump. *Journal of General Physiology*, **48**, 761-775. https://doi.org/10.1085/jgp.48.5.761

[36] Venosa, R.A. (1978) Stimulation of the Na^+-Pump by Hypotonic Solutions in Skeletal Muscle. *Biochimica et Biophysica Acta*, **510**, 378-383. https://doi.org/10.1016/0005-2736(78)90038-X

[37] Ayrapetyan, S.N. (1976) Involvement of the Na Pump in Slow Oscillations Underlying the Bursting Patterns in Helix Neurons. In: Salanki, J., Ed., *Neurobiology of Invertebrates*, Academiai Kiado, Budapest, 353-370.

[38] Ayrapetyan, S.N., Arvanov, V.L., Maginyan, S.B. and Azatyan, K.V. (1985) Further Study of the Correlation between Na-pump Activity and Membrane Chemosensitivity. *Cellular and Molecular Neurobiology*, **5**, 231-243. https://doi.org/10.1007/BF00711009

[39] Pivavarov, A.S., Calahorro, F. and Walker, R.J. (2019) Na/K Pump and Neurotrasmitters Membrane Receptor. *Invertebrate Neuroscience*, **19**, 1-27. https://doi.org/10.1007/s10158-018-0221-7

[40] Baker, P.F., Blaustein, M.P., Hodgkin, A.L. and Steinhardt, S.A. (1969) The Influence of Ca on Na Efflux in Squid Axons. *The Journal of Physiology*, **200**, 431-458. https://doi.org/10.1113/jphysiol.1969.sp008702

[41] Juhaszova, M. and Blaustein, M. (1982) Na^+ Pump Low and High Ouabain Affinity Alpha Subunit Isoforms Are Differently Distributed in Cells. *Proceedings of The National Academy of Sciences of the United States of America*, **94**, 1800-1805. https://doi.org/10.1073/pnas.94.5.1800

[42] Blaustein, M.P. and Lederer, W.J. (1999) Na/Ca Exchange. Its Physiological Implications. *Physiological Reviews*, **79**, 763-854. https://doi.org/10.1152/physrev.1999.79.3.763

[43] Sagian, A.A., Ayrapetyan, S.N. and Carpenter, D.O. (1996) Low Dose of Ouabain Stimulates the Na:Ca Exchange in Helix Neurons. *Cellular and Molecular Neurobiology*, **16**, 180-192. https://doi.org/10.1007/BF02150229

[44] Ayrapetyan, S. (2012) Cell Hydration as a Universal Marker for Detection of Environmental Pollution. *Environmentalist*, **32**, 210-221. https://doi.org/10.1007/s10669-011-9380-3

[45] Dadalyan, S.S., Kiss, T., Azatyan, K.V., Ayrapetyan, S.N. and Salanki, J. (1988) The Effect of Low Concentration of GABA on the ACH Sensitivity of Snail Neurons. In: Salanki, J., Ed., *Neurobiology of Invertebrates*, AcademiaiKiado, Budapest, 643-653.

[46] Ayrapetyan, S. and Carpenter, D.O. (1991) On the Modulatory Role of Extra-Low Concentrations of Synaptic Transmitters for Membrane Functional Activity. *Journal of Evolutionary Biochemistry and Physiology*, **26**, 513-528.

[47] Ayrapetyan, S.N. (2006) Cell Aqua Medium as a Preliminary Target for the Effect of Electromagnetic Fields. In: Ayrapetyan, S.N. and Markov, M.S., Eds., *Bioelectromagnetics: Current Concepts. NATO Security through Science Series*, Springer Press, Netherlands, 31-64. https://doi.org/10.1007/1-4020-4278-7_3

[48] Ayrapetyan, S.N. (2015) The Role of Cell Hydration in Realization of Biological Effects of Non-Ionizing Radiation (NIR). *Electromagnetic Biology and Medicine*, **34**,

197-210. https://doi.org/10.3109/15368378.2015.1076443

[49] Clark, R.B. (2013) Profile of Kobilka BK, Lefkowitz RJ. Nobel Laureates in Chemistry. *Proceedings of The National Academy of Sciences of The United States of America*, **110**, 5274-5275. https://doi.org/10.1073/pnas.1221820110

[50] Xie, Z. and Askari, A. (2002) Na/K- ATPase as a Signal Transducer. *European Journal of Biochemistry*, **269**, 2434-2439. https://doi.org/10.1046/j.1432-1033.2002.02910.x

[51] Askari, A. (2019) The Sodium Pump and Digitalis Drugs: Dogmas and Fallacies. *Pharmacology Research & Perspectives*, **2019**, e00505. https://doi.org/10.1002/prp2.505

[52] Ayrapetyan, S.N., Avanesian, A.S., Avetisian TH and Majinian, S.B. (2017) Physiological Effects of Magnetic Fields May Be Mediated through Actions on the State of Calcium Ions in Solution. In: Carpenter, D. and Ayrapetyan, S., Eds., *Biological Effects of Electric and Magnetic Fields*, Volume 1, Academic Press, New York, 181-192. https://doi.org/10.1016/B978-0-12-160261-1.50012-2

[53] Ayrapetyan, S. (2013) Na^+/K^+ Pump $\alpha 3$ Isoform is a Universal Membrane Sensor for Weak Environmental Signals. *Journal of Bioequivalence & Bioavailability*, **5**, 31-40. https://doi.org/10.4172/jbb.1000131

[54] Brini, M. and Carifolly, E. (2009) Calcium Pumps in Health and Disease. *Physiological Reviews*, **9**, 1341-1378. https://doi.org/10.1152/physrev.00032.2008

[55] Ayrapetyan, S. (2001) Na-K Pump and Na:Ca Exchanger as Metabolic Regulators and Sensors for Extra-Weak Signals in Neuromembrane. In: Ayrapetyan, S.N. and North, A.C.T., Eds., *Modern Problems of Cellular and Molecular Biophysics*, Noyantapan, Yerevan, 31-57.

[56] Azatian, K.V., Karapetian, I.C. and Ayrapetyan, S.N. (1993) Effect of Low Dose Acetylcholine on Ca Ions Influx into Helix Pomatia Neurons. *Biological Membranes*, **10**, 317-320.

[57] Azatian, K.V., Ayrapetyan, S.N. and Carpenter, D.O. (1997) Metabotropic GABA Receptors Regulate Acetylcholine Responses on Snail Neurons. *General Pharmacology*, **29**, 67-72. https://doi.org/10.1016/S0306-3623(96)00568-X

[58] Saghian, A.A., Dadalian, S.S., Takenaka, T., Suleymanian, M. and Ayrapetyan, S.N. (1986) The Effect of Short-Chain Fatty Acids on the Neuronal Membrane Function. 3. The Na Efflux from the Cells. *Cellular and Molecular Neurobiology*, **6**, 397-405. https://doi.org/10.1007/BF00711408

[59] Arvanov, V.L., Takenaka, T., Dadalian, S.S. and Ayrapetyan, S.N. (1986) The Effect of Short-Chain Fatty Acids on the Neuronal Membrane Functions of Helix fPomatia. 2. Cholinoreceptive Properties. *Cellular and Molecular Neurobiology*, **6**, 165-175. https://doi.org/10.1007/BF00711068

[60] Suleymanian, M., Takenaka. T., Stamboltsyan, K. and Ayrapetyan, S. (1986) The Effect of Short-Chain Fatty Acids on the Neuronal Membrane Functions of Helix Pomatia. *Cellular and Molecular Neurobiology*, **6**, 151-163. https://doi.org/10.1007/BF00711067

[61] Ayrapetyan, S., Yeganyan, L., Bazikyan, G., Muradyan, R. and Arsenyan, F. (2012) Na^+/K^+ Pump α3 Isoform-Dependent Cell Hydration Controlling Signaling System Dysfunction as a Primary Mechanism for Carcinogenesis. *Journal of Bioequivalence & Bioavailability*, **4**, 112-120. https://doi.org/10.4172/jbb.1000123

[62] Dvoretsky, A.I., Ayrapetyan, S.N. and Shainskaya, A.M. (2012) High-Affinity Ouabain Receptors: Primary Membrane Sensors for Ionizing Radiation. *The Environ-*

mentalist, **32**, 242-248. https://doi.org/10.1007/s10669-012-9393-6

[63] Mikaelyan, Y. and Ayrapetyan, S. (2019) Over-Expression of Na/Ca Exchangers in Soft Tissues as a Novel Diagnostic Marker for Carcinogenesis. *Cancer Research and Molecular Mechanisms*, **5**.

[64] Mikaelyan, Y., Eloyan, N. and Ayrapetyan, S. (2019) The Na/Ca Exchange as a Target for Antitumor Effect of 4Hz Pulsing Magnetic Field. *Electromagnetic Biology and Medicine*, **219**, 1-9. https://doi.org/10.1080/15368378.2019.1685542

[65] Narinyan, L. and Ayrapetyan S. (2019) Age-Dependent Comparative Study of 4Hz and 8Hz EMF Exposure on Heart Muscle Tissue Hydration of Rats. *Open Journal of Biophysics*, **9**, 70-82. https://doi.org/10.4236/ojbiphy.2019.91005

[66] Narinyan, L., Ayrapetyan, G. and Ayrapetyan, S. (2013) Age-Dependent Magnetosensitivity of Heart Muscle Ouabain Receptors. *Bioelectromagnetics*, **34**, 312-322. https://doi.org/10.1002/bem.21769

[67] Narinyan, L. and Ayrapetyan, S. (2019) Age-Dependent Impairment of Heart Muscle Contractility as a Primary Mechanism for Overexpression of Na/Ca Exchanger in Brain Cortex Tissues. *European Journal of Biophysics*, **7**, 27-42.

[68] Nikoghosyan, A., Heqimyan, A. and Ayrapetyan, S. (2019) Aging Leads to Over-Expression of Na^+/K^+ Pump Units in Liver and Na^+/Ca^{2+} Exchangers in Brain Cortex. *Open Journal of Biophysics*, **9**, 218-237. https://doi.org/10.4236/ojbiphy.2019.93016