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Na^+/K^+ Pump α_3 Isoform is a Universal Membrane Sensor for Weak Environmental Signals

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Abstract

In present review are presented data on functional role of α_3-Na^+/K^+ pump isoform-dependent Ca exchange in weak signal transduction in cells. According to the classic "Membrane theory" signals can modulate membrane functional activity by changing its conductive function: activation or inactivation of ionic channels leading to cell membrane depolarization or hyper-polarization. However, at present it is well established that there are a number of non-conductive mechanisms in membrane through which extra-weak signals having intensity even far from the threshold of channel activation could modulate the cell membrane function. The previous study on the sensitivity of α_3 receptors having higher ouabain affinity to different weak physical and chemical signals showed that they are universal sensors also for different environmental factors. Such sensitivity is determined by α_3- Na^+/K^+ pump isoform-dependent Na^+/Ca^{2+} exchange system, having crucial role in controlling cell hydration and intracellular Ca homeostasis. It is known that the latter regulates the number of functionally active protein molecules (enzymes, chemoreceptors and ionic channels) in membrane by its surface changing and the activity of intracellular macromolecules by protein-folding mechanism. On the basis of these data it is suggested that these carrier driven transporting mechanisms can be considered as candidates for extra-sensitive and universal sensors in neuromembrane through which the biological effects of extra-weak signals are realized.The overall aim of this chapter is to present a brief review on the physiological role of α_3 isoform Na^+/K^+ pump-dependent Na^+/Ca^{2+} exchange as a universal and extra-sensitive pathway through which the biological effects of weak signals on cells are realized.

Keywords: Na^+/K^+ pump; Chemoreceptors; Sensors

At present, when the technological progress leads to increase in environmental pollutions by different chemical and physical factors, the detection of the safety of environmental medium from the point of public health is one of the fundamental problems of modern Life Sciences. The lack of information on the mechanisms of weak signals transduction in cell is the main barrier for choosing the cellular marker, which can be used for detection of the hazardous effect of environmental pollution on organism. Numerous hypotheses have been proposed, but none of them provided a satisfactory and reproducible explanation of the variety of experimental results. The main difficulty for the adequate determination of harmful effects of environmental pollutions by low concentration of chemical substances and weak intensity physical factors on organism is due to their biological effects a) have nonlinear dose-dependent character; b) depends on environmental composition and c) depends on initial state of organism.

According to classical membrane theory the signal transduction in cells is realized as an activation of ionic channels in membrane leading to generation of transient ionic currents through the cell surface membrane and Na/K pump has only housekeeping role in controlling of intracellular ionic homeostasis. However from the point of this membrane conductive theory it is difficult to explain the biological effect of weak physical and chemical signals having intensity far from the threshold of channel activation, but having a strong modulatory effect on cell membrane function [1,2]. These data indicate that there are extra sensitive sensors in membrane the activation of which can modulate membrane functional activity trough potential-independent pathways. As cells are functioning in multicomponents and are continuously changing cell bathing medium it is supposed that these sensors could have a protective function, switching on metabolic cascade proceding generation of specific cell responses. It is clear that such sensors must have extra-sensitive and universal characters.

As the vectoral character of Na^+/K^+ pump having fundamental role in regulation of the close talking between cell metabolism and ionic channels-transporting mechanisms in membrane is a well established fact it can be considered as a one of potential candidates for universal sensor, through which the biological effects of weak signals are realized. This suggestion has served as a working hypothesis for our research group during three decades the result of which will be briefly presented in this review.

After proving the electrogenic character of Na^+/K^+ pump in different types of cells [3] it becomes clear that cell membrane potential (MP) in normal living state having a metabolic-dependent component, which is more sensitive to environmental factors than its diffusion component [4-6]. It was shown that the MP metabolic part variation could have crucial role in regulation excitability of pacemaker activity of neurons [7,8], smooth and cardiac muscles and squid giant axon [9,10].

Since the Na^+/K^+ pump controlling Na gradient on membrane serves as an energy source for different ionic and metbolits transporting exchangers [11] it has a crucial role also in regulation of cell volume [12-18].

Our previous study performed on snail neurons showed that the Na^+/K^+ pump-dependency of cell volume has great physiological

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significance in metabolic regulation of cell membrane function [16]. The pump-induced dynamic changes of the cell membrane active surface serve as a pathway through which the autoregulation of Na^+/K^+ pump and regulation of membrane conductive function are realized [19]. The number of functionally active protein molecules in neuronal membrane, having receptors [20], enzymatic [21] and ionic channels forming [22] properties, are in functionally active and inactive (reserve) states, depending on active membrane surface (cell hydration). The cell swelling leads to increase in the number of active protein molecules in membrane while its shrinkage has opposite effect on it [21]. This correlation can be clearly seen in table 1, where the dependence of the number of functionally active ^{3}H-ouabain receptors (Na-K ATP-ase molecules) on external solution tonicity is presented.

These data show that the number of ouabain binding sides in hypoosmotic solutions, where cells are in their swelling state, is much higher than in isotonic and hypertonic mediums where they are in less hydrated state. To elucidate the question of whether the number of functioning pump units is changing under the normal conditions in response to the increase of passive membrane permeability, the binding of ^{3}H–ouabain to membrane was studied in the presence of synaptic transmitters in external medium.

Table 2 shows that at presence of Ach and GABA the ^{3}H-ouabain binding with membrane significantly increases compared to the case of transmitters-free medium. More detailed investigation on this phenomenon showed that the transmitter-induced increase in the number of pump units in membrane is due to cell swelling in response to the increase in transmitter-induced membrane permeability [21]. These data allow us to consider cell hydration as the second messenger through which the functional activity of membrane is regulated [19]. At present it is well established that cell hydration-dependent caveolaes exist in membrane, having different metabolic properties and signalling function in cells [23].

As the dynamic changes of Na^+/K^+ pump activity lead to corresponding changes of transmembrane water fluxes, the latter could serve also as a gate of intracellular signaling system. Till the beginning of 1980s the water flow through the membrane was not properly considered in the investigations of nerve excitation and membrane currents. It was shown that transmembrane water flow can modulate the amplitudes and kinetics of neuromembrane action potentials and the ionic currents [22,24-27]. By our study performed on intracellular perfused squid axon and snail neurons we showed that inward water flow through the membrane caused the facilitating effect on the inward ionic currents and inactivation effect on the outward ones, while the outward water flow had opposite effect on these ionic currents. It is worth to note that the mentioned water fluxes effects did not depend on the type of the charge carrying ions [22].

Ouabain content (Mol)	Incubation medium		
	Hypotonic	Isotonic	Hypertonic
$1x10^{-10}$	4.59 ± 0.32	3.23 ± .24	2.03 ± 0.16
$3x10^{-10}$	18.3 ± 1.4	11.7 ± .87	6.29 ± 0.41
$6x10^{-10}$	28.9 ± 2.0	17.9 ± 1.2	10.0 ± 0.67
$1x10^{-9}$	32.0 ± 2.2	21.1 ± 1.4	12.2 ± 0.9
$3x10^{-9}$	144 ± 29.4	90.5 ± 5.7	53.8 ± 3.1
$6x10^{-9}$	431 ± 29.4	266 ± 15.8	147 ± 9.7
$1x10^{-8}$	793 ± 45.6	508 ± 30.1	283 ± 19.4

Table 1: Binding of [^{3}H] Ouabain to *Helix pomatia* Cell Membrane as a Function of Concentration of Glycoside in Solutions with Different Tonicity ($\times10^8$ molecules/mg dry weight).

Oubain content in the medium (mol)	Normal Ringer	Normal Ringer containing 10^{-4}M ACh	Normal Ringer containing 10^{-4}M GABA
$1x10^{-10}$	3.16 ± 0.48	5.13 ± 0.62	4.24 ± 0.21
$1x10^{-9}$	20.56 ± 0.55	30.54 ± 1.55	27.63 ± 3.17
$5x10^{-9}$	109.40 ± 10.47	170.29 ± 13.36	139.62 ± 11.43
$1x10^{-8}$	143.54 ± 8.91	270.93 ± 28.53	174.48 ± 13.54
$1x10^{-7}$	3254.47 ± 74.20	3944.33 ± 07.23	-
$1x10^{-6}$	23938.20 ± 852.44	28686.96 ± 963.47	-

Table 2: The Effects of ACh and GABA on [^{3}H] Ouabain Binding to Neuronal Membrane.

The theoretical analysis of the experimental data on the effect of the outward and inward water flows through the neuromembrane on the outward potassium currents showed that the outward water flow increases both the potassium conductance at a given potential (g_k) and the maximum potassium conductance (g_k^{max}), 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 [28].

Taking together these data on water fluxes activation and inactivation effect on ionic channels in membrane and data obtained by Terakawa and co-workers that membrane depolarization leads to cell swelling and hyperpolarization to cell shrinking [29,30] it can be concluded that the water fluxes through membrane serve as a gate of activation and inactivation mechanism for potential-dependent ionic channels in membrane. Therefore, from this point of view the Na^+/K^+ pump-induced potential independent inhibition of pacemaker activity of mollusc neurons observed in our work could be explained [31].

It is known that the cell hydration regulates the activity of intracellular molecules also, including gene expression [32]. At present it is well established that cell swelling triggers cell proliferation, while cell shrinkage promotes the apoptotic patterns [18,33].

Thus, based on above data it can be concluded that Na^+/K^+-pump-dependent changes of cell hydration serve as a powerful signal transduction system in cell through which the metabolic regulation of its functional activity is realized.

It is known that the cyclic nucleotides-dependent phosphorylation and dephosphorylation reactions represent one of the major regulatory networks controlling and integrating the multitude of basic cellular functions, including cell hydration, membrane transporting, chemoreceptive and excitable functions [34-36]. Therefore, the question of possible correlation between Na^+/K^+ pump activity and intracellular cyclic nucleotides-dependent membrane phosphorylation was the subject of our investigation. For this purpose the cyclic nucleotide content and level of membrane phosphorylation in molluscan neurons at the pump active (in normal Ringer's solution) and inactive states (K-free or 10^{-4} M ouabain containing solutions) were studied [34].

As shown in table 3, the K-free saline or ouabain-induced Na^+/K^+ pump inhibition leads to increase in the intracellular cAMP level in neurons by 164% and 55%, respectively. Addition of the ouabain to the K-free solution led to a 138% increase in intracellular cAMP contents as compared to the control one. The different affects of both factors on the level of intracellular cAMP concentration could be explained either by their different effects on membrane potential (K-free saline has hyperpolarization while the ouabain has depolarization effects on cell membrane) or by the fact that ouabain could inactivate adenylate

cyclase or activate phosphodiesterase activities. Still, more detailed investigations are needed to clarify the reason of different affectivities of the K-free saline and ouabain on intracellular content of cAMP. The incubation of nerve ganglia in intracellular content of cGMP was decreased by 30% compared to the control of both the K-free saline as well as in the presence of ouabain.

As was predicted the study of correlation between Na^+/K^+ pump activity and cell membrane phosphorylation level indicate that pump inhibition causes the increase in the membrane phosphorylation also [34]. Therefore, it can be suggested that under normal conditions of the cell the variation in Na^+/K^+-pump activity is accompanied by a corresponding change in the degree of phosphorylation of membrane proteins leading to change of the protein activity in membrane.

By study of the functional role of pump-dependent elevation of intracellular cAMP and phosphorylation membrane proteins in regulation of membrane chemosensitivity it was shown that ouabain- and K-free solution (but not cold) -induced pump inhibition leads to depression of synaptic transmitters binding with neuronal membrane and depressing transmitters-induced currents in neuromembrane [35]. More detail investigation of the mechanism of pump inhibition-induced depression of membrane chemosensitivity was shown that it is due to the elevation of cAMP-dependent phosphorylation receptors (Figure 1).

Figure 1 shows that intracellular application of cAMP has time-dependent depressing effect on Ach-induced current. More detailed investigation on the kinetics of the dose-dependent depressing effect of intracellular cAMP on the Ach-induced current (receptor affinity to their ligands) brought us to the conclusion that intracellular cAMP has depressing effect on the receptors' affinity [36,37].

It is known that the increase in the membrane phosphorylation causes increase in intracellular concentration of another second messenger, the Ca ions ($[Ca^{2+}]_i$). Ca^{2+} enters the cell via the potential-dependent ion channels and via the reverse (R) Na^+/Ca^{2+} exchange

Conditions	AMP level (pmole/mg protein)	Per ccent of increase	cGMP level (pmole/mg protein)	Per cent of decrease
Normal physiol. sol.	7.5 +0.82	-	3.80+0.42	-
K-free sol.	19.77+2.66	164	2.62+0.14	29
Ouabai 0.1 mM	11.61+0.89	55	2.71+0.25	30

Table 3: The effect of Na^+/K^+-pump inhibition (5 min) on the intracellular contents of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) [34].

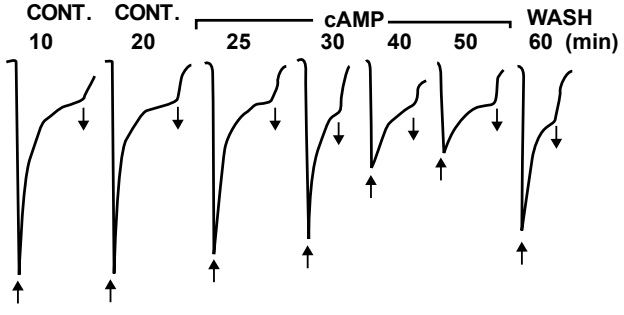

Figure 1: The dependency of Ach-induced current in internally dialyzed neuron on the internal concentration of cAMP in normal medium [36].

Cyclic nucleotide	Intracellular level, impulse/min for 1mg protein		
	Control	10^{-13} M GABA	10^{-11} M Ach
cAMP	7.5 ± 0.82	20.42 ± 1.53	15.71 ± 1.43
cGMP	3.8 ± 0.42	4.41 ± 0.63	3.4 ± 0.50

Table 4: The Effect of 10^{-13} M GABA and 10^{-13} M Ach on cAMP and cGMP Concentrations in Snail Neuron [34].

mechanism. It was established that both transport mechanisms are stimulated enhancing of the intracellular cAMP concentration [Doroshenko et al. 1982]. As the R- Na^+/Ca^{2+} exchange has more essential effect on $[Ca^{2+}]_i$ than potential-dependent Ca^{2+} channels [38] the correlation between Na^+/K^+ pump-dependent changes of cyclic nucleotides concentration and Na^+/Ca^{2+} exchange was the subject for our investigation.

It was shown that the direction of Na^+/Ca^{2+} exchange strongly depends on the ratio of cGMP/cAMP. The factors, which cause the elevation of intracellular cGMP lead to Na^+/Ca^{2+} exchange activation in forward (F) mode (Na influx and Ca efflux) [39], while the elevation of cAMP activates the R- Na^+/Ca^{2+} exchange (Na efflux and Ca influx) [40]. As the pump inhibition-induced small enhancement of intracellular Ca^+ concentration $[Ca^{2+}]_i$ could lead to dramatic changes in the activity of the number of enzyme systems, the cyclic nucleotides-dependent Na^+/Ca^{2+} exchange can be considered as a powerful signaling pathway through which the Na^+/K^+ pump could modulate the cell functional activity. It is known that the $[Ca^{2+}]_i$ is a strong modulator of lipids turnover in cells which is realized by changes in the activity such as phospholipases family, calmodulin-dependent activation of NO-syntetase and others [Case, 1980]. As the $[Ca^{2+}]_i$-induced activation of lipase leads to increase in membrane fluidity, having strong modulatory role in membrane functional activity, this pathway serves next through which the Na^+/K^+ pump could regulate cell functional activity. By our earlier works it was shown that the increase in membrane fluidity by involvement of short-chain unsaturated fatty acids such as 2-decenoid acid in its structure a strong blocking effect on Na current in neuronal membrane [41,42], agonist-activated K channels [43] as well as on the Na^+/K^+ pump and Na^+/Ca^{2+} exchange [44]. As both Na^+/K^+ pump and Na^+/Ca^{2+} exchange are highly lipid-dependent their correlation disappeared by changes of lipid-protein interaction in membrane by extracellular applying these acids [44] or by phospholipase A_2 treatments [34]. As can be seen in figure 2 the pump inhibition-induced activation of $^{45}Ca^{2+}$ uptake (Na^+/Ca^{2+} exchange) disappears after 5-minute treatment of neurons by 20 microgram/ml A_2-containing physiological solution (Figure 2).

It is known that the Na^+/Ca^{2+} exchanger is functioning in electrogenic regime in stoichiometry 3Na:1Ca [45]. Therefore, it has a strong modulatory effect on cell hydration: F- Na^+/Ca^{2+} exchange has hydration while the R- Na^+/Ca^{2+} exchange has dehydration effect on cells [46,47].

Thus, above presented data indicate that the electrogenic Na^+/K^+ pump besides housekeeping ionic homeostasis role serves also as an important gate for intracellular signaling systems controlling such fundamental cell parameters as cell hydration and $[Ca^{2+}]_i$ homoeostasis, which could have multisided effects on cell metabolism. Now question rises whether Na^+/K^+ pump-induced changes of cell hydration and $[Ca^{2+}]_i$ serve as common pathways through which the biological effects of weak physical and chemical signals on cells are realized.

It is known that extremely weak physical and chemical signals, intensity of which is rather far from the threshold of ionic channels activation could have biological effect on cells and organisms [1,2]. As

can be seen in figure 3 the neurons in snail isolated neuronal ganglia treated by 4 Hz 30 dB MV for 15 min in normal physiological solution (PS) got dehydrated while in K-free PS (Na^+/K^+ pump inactivated state) they were hydrated.

Similar to MV effects on cell hydration, the same takes place also in case of ganglia exposed to 4 Hz 0.2 mT EMF in normal and in K-free PS [48] figure 4.

Figure 5 indicates that the both 4 Hz MV-and EMF have depressing effect on Ca uptake in normal K containing PS (Figure 5A) and reverse effect in K-free solution when pump is in inactive state (Figure 5B). From these data it can be concluded that MV and ELF EMF have effect on cell hydration, which can be modulated by Na^+/K^+ pump activity.

By study of Na^+/K^+ pump dependency of biological effect of electromagnetic fields (EMF) and mechanical vibration (MV) on different types of cells was shown that pump-dependent cell hydration and Ca uptake are sensitive even to EMF- and MV –induced structural changes of cell bathing aqua solution [48]. Therefore, the questions which of known three Na^+/K^+ pump isoforms determines the higher sensitivity of cell hydration and is responsible for $[Ca]_i$ homoeostasis were the subject of our study.

At present it is established that in membrane of mammalian muscle and neuronal tissues three isoforms of the Na^+/K^+-ATPase are functioning having different catalytic α subunits with different affinities to ouabain: alpha α_1-low affinity, α_2-middle affinity, and α_3-high affinity [49-54].

The existence of three populations of ouabain receptors with different affinity in neuronal membranes was also demonstrated earlier by our work performed on snails isolated nerve ganglia [21]. By study of dose-dependent [3H]-ouabain biding with membrane it was shown, that its curve consists of two saturated (10^{-11}–10^{-9} M and 10^{-9}–10^{-7} M) and one linear component (10^{-7}–10^{-4} M) in iso-; hyper- and hypotonic medium (Figure 5). These data indicate that the existence of these three populations of ouabain receptors cannot be explained by ouabain-induced cell volume changes.

The fact that the α_1 and α_2 ouabain receptors are responsible for Na^+/K^+ pump activity is well documented, however, the real function of α_3 receptors remains controversial [49,54]. But there are a great number of literature data that α_2 and α_3 (Figure 6) receptors have a crucial role in the regulation of Na^+/Ca^{2+}exchange in cell [38,54,55].

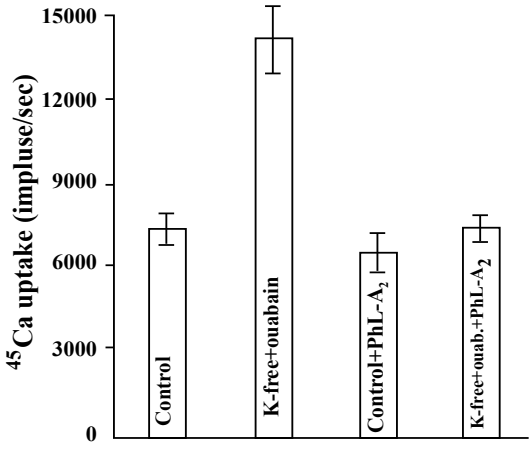

Figure 2: The effect of phospholipase-A_2 on pump inhibition (K-free + ouabain)-induced activation of ^{45}Ca uptake [34].

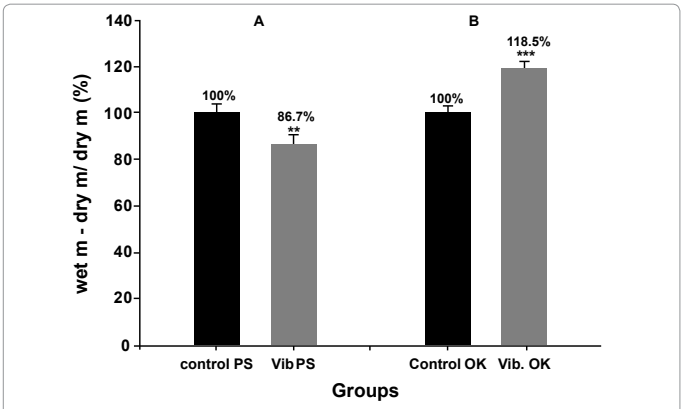

Figure 3: The effect of 4Hz 30DB MV 15 min. treatment on snail nerve ganglia's hydration in normal and K-free PS [48].

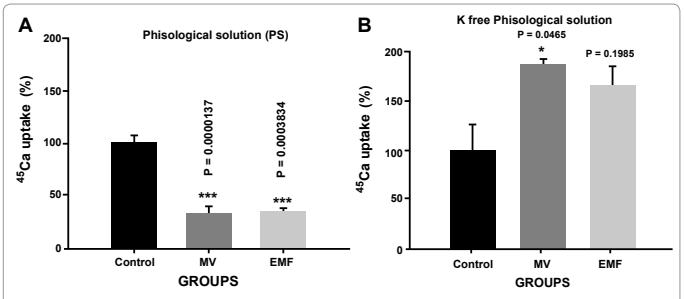

Figure 4: The effect of 4Hz MV and ELF EMF on $^{45}Ca^{2+}$ uptake by neuronal ganglia in (A) normal PS and (B) K-free solution.

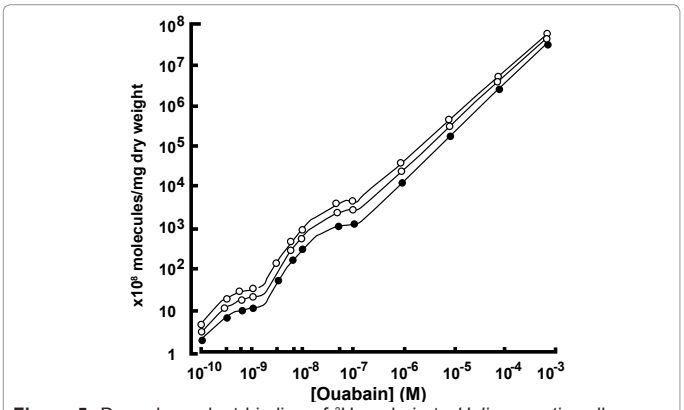

Figure 5: Dose-dependent binding of ^3H-ouabain to *Helix pomatia* cell membrane in PS with different tonicity. Binding was measured after 2 h of exposure to 3H-ouabain-containing PS. Cell exposed to hypotonic (o) (0 mM sucrose, p = 0,5); in isotonic (filled diamond) (63 mM sucrose; p=1); and hypertonic (filled circle) (189 mM sucrose; p = 2) PS. The curves were drawn by eye. Both axes are logarithmic [21].

Baker and co-authors [45] described the details of Na^+/Ca^{2+} exchange properties and their correlation with Na^+/K^+ pump on internally perfused squid axon. They observed two components of $^{22}Na^+$ efflux from the axon; ouabain sensitive (Na^+/K^+ pump) and ouabain insensitive (Na^+/Ca^{2+}exchange). However, in our study performed on snail neurons it was shown that ouabain at concentrations >10^{-7} M had an inhibitory while at low concentrations it had an activation effect on $^{22}Na^+$ efflux from neurons (Figure 7) [21].

It was shown that low ouabain produces the elevation of intracellular cAMP level, which, in its turn, causes the activation of Na^+/Ca^{2+} exchange in its reverse mode [46]. Nevertheless, low ouabain-induced activation of $^{22}Na^+$ efflux from neuron was coupled by $^{45}Ca^{2+}$ uptake, without inactivation of Na^+/K^+ pump [46]. Such differences between results on effects of low concentration ouabain on $^{22}Na^+$ efflux in intracellular perfused axon and in whole cells allow us to suggest on existence of a mechanism in cytoplasm responsible for activation of $^{22}Na^+$ efflux from cells which was absent in intracellular perfused squid axon [45].

Since the difference between Na^+ and Ca^{2+} ions (E_{Na}-E_{Ca}) electrochemical gradients on membrane serves as energy sources for Na^+/Ca^{2+} exchange, the increase in $[Na^+]_i$ and decrease in $[Ca^{2+}]_i$ could bring in activation of the R-Na^+/Ca^{2+} exchange [45]. The fact that at ouabain concentration less than 10^{-7} M ouabain has no inactivation effect on Na^+/K^+ pump, the activation in R-Na^+/Ca^{2+} exchange cannot be explained by decrease in E_{Na}. Therefore, the low ouabain-induced activation of R-Na^+/Ca^{2+} exchange can be due to only by decrease in $[Ca^{2+}]_i$ leading to increase in E_{Ca}. It is obvious that the latter could occur in case of $[Ca^{2+}]_i$ absorption by intracellular structure. It is known that such absorption can be realized by cAMP-activated Ca^{2+}-pump localized in SR membrane, transporting Ca^{2+} from cytoplasm into the SR [56]. Recently we have shown that the nanomolar concentration of ouabain stimulates cAMP-dependent R-Na/Ca exchange in different zones of brain tissue and heart muscle of rats [57].

The data that the nanomolar ouabain could elevate the intracellular cAMP have been demonstrated in different tissues including dog renal cortex, goldfish intestinal mucosa, mouse pancreatic islets, murine epithelioid and fibroblastic cell lines, rat brain, rat renal collecting tubule cells in culture and astrocytes [58]. Earlier we also showed that low ouabain activation of R-Na^+/Ca^{2+} exchange was accompanied by increase in intracellular cAMP content in snail neurones [40]. Therefore, the low ouabain-induced activation of α_3 receptors leading to increase in R-Na^+/Ca^{2+} exchange can be considered as a consequence of cAMP-activated protein kinase activity leading to activation of Ca^{2+} pump in SR membrane. This conclusion is in agreement with Xie and Askari's [59] suggestion that in cell membrane two Na^+/K^+-ATPase subpopulations are in existence with different functions. The role of one subpopulation is connected with maintaining ionic gradients, and inhibition of these molecules leads to an increase in intracellular Ca^{2+} concentration. The other subpopulation of the enzyme mainly performs the signaling function [59].

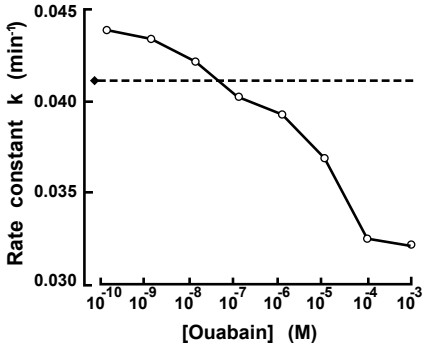

Figure 6: The dependence of the rate constant of ^{22}Na efflux from cell on the concentration of ouabain from 10^{-10} to 10^{-3} M. The dashed line indicates the rate constant in normal Ringer's solution [21].

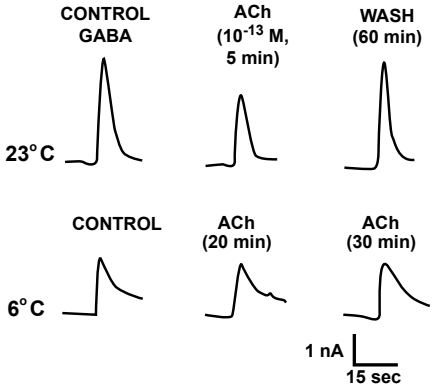

Figure 7: The effect of 10^{-13}M Ach on GABA induced current in *Aplysia* neurons in room and cold temperature [2].

In order to find out whether the observed α_3-dependent signalling system is a specific pathway for realization of low ouabain effect or its common sensor for extremely low concentration of other biological active substances and weak physical factors too, the low concentration of synaptic transmitters and their synthetic derivatives as well as magnetic and electromagnetic field effects on α_3 receptors affinity and their-dependent membrane conductive properties, intracellular cAMP and cGMP contents, Na^+/Ca^{2+} exchange in molluscan and mammal neurons and heart muscles were studied.

In mollusk neurons very low concentrations of synaptic transmitters (less than 10^{-10} M), unable to change the membrane electrical conductivity, modulate membrane chemosensitivity, excitability and Na^+/K^+ pump activity. These effects are relatively slow and temperature-dependent. They have clear threshold concentrations [2,38,60]. Figure 7 shows that the surprisingly low concentration of Ach (10^{-13} M) has depressing effect on 10^{-4} M GABA-induced responses of *Aplysia* neuron, which disappears in cold medium.

This modulation was accompanied by activation of cAMP–dependent R-Na^+/Ca^{2+} exchange [2,60,61].

The study of the effect of low concentration of Ach and GABA on intracellular cAMP content showed that even at the lowest concentration of the transmitters (10^{-13} M) they have an elevation effect on intracellular cAMP content (Table 4). Upon the 10^{-13} M GABA effect the cAMP concentration increased in snail neurones almost three-fold [61,62], while the same concentration of Ach led to its double increase [34].

It is interesting to note that in case of the study of low concentration transmitters' effect on cAMP level we have the dose-dependent regressive effects [2] (Figure 8). It is more pronounced at the lowest concentration of Ach (10^{-10} M) than at the highest one (10^{-4} M). The explanation can be that more than one mechanism of different directions are involved in high concentration transmitters induced effect on cAMP, while by lowering the concentration the involvement of a single mechanism is reached.

In order to elucidate the effect of transmitters on Na^+/Ca^{2+} exchange, experiments were performed in K-free and 10^{-4}M ouabain containing saline, where Na^+/K^+ pump was blocked. It is shown in figure 9 that the low concentrations of Ach and GABA in pump inactive state, had activating effect on ^{22}Na efflux reached its maximum at 10^{-11} and 10^{-10} M concentrations of Ach and GABA, respectively. Ach increased the ^{22}Na efflux by 25%, while GABA did it by 38% [60].

These observations indicate that transmitters have at least two actions on neuronal membrane: a) modulation of their and other transmitters-induced responses at very low concentrations and b) direct activation of ionic responses at higher concentrations [2]. More detailed investigation of the metabotropic effect of low doses of transmitters (LDT) on membrane functional activity proved the suggestion that cyclic nucleotides-dependent Na^+/Ca^{2+} exchange could serve as a gating mechanism for the intracellular metabolic cascade through which different interrelated messenger systems realize the metabotropic effect of LDT [61].

However, to find out whether the binding sites for LDT are part of channel binding ionic receptors in high concentrations of transmitters or whether their effects are realized by same sensors at low concentration of ouabain, the comparative study of the Na^+/K^+ pump current and transmitters-induced membrane current in neurons and different mRNA-injected *Xenopus* oocytes were studied. It is known that in the membrane of *Xenopus* oocytes the synaptic receptors are almost absent. Thus it can produce a synthesis of different individual receptors by means of preliminary mRNA injection from rat brain. Both in mollusc neurons and in oocyte membrane Na^+/K^+ pump is working in electrogenic regime and is generating quite large metabolic-dependent components of membrane potential. In neuron 10^{-11} M ouabain and rather low concentrations (less than 10^{-13} M) of transmitters have partially depressing effect on the pump current while in oocytes' membrane it is insensitive to low concentration of ouabain and even to high concentrations (10^{-4}–10^{-5} M) of transmitters [63]. Figure 10 shows the 10^{-11} M ouabain effect on pump current in neuron.

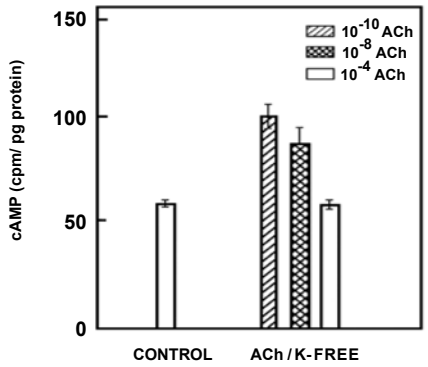

Figure 8: The dose-dependent effect of ach on intracellular level of cAMP in *Aplysia* neurones in K-free solution [34].

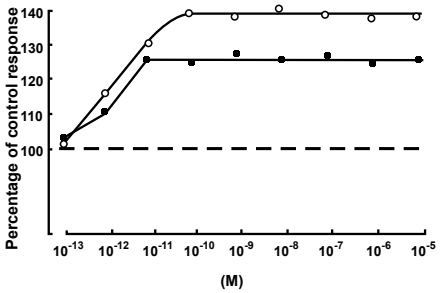

Figure 9: The effect of Ach (•) and GABA (o) on ^{22}Na efflux from cells in K-free, 10^{-4} M ouabain-containing solution. Abscissa – log concentration of transmitters; ordinate – Ach and GABA induced changes of ^{22}Na efflux from cells as compared with the efflux in K-free, 10^{-4} M ouabain-containing solution [60].

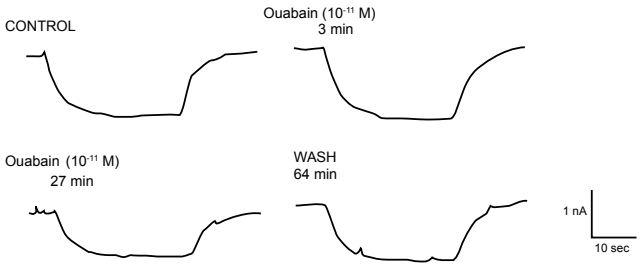

Figure 10: The effect of prolonged microperfusion of a solution containing 10^{-11} M ouabain on K-free solution-induced inward (pump inhibition) currents in *Aplysia* neuron. The neuron was perfused by normal sea water before 1 minute exposure in K-free solution. Similar effects of >10^{-10} M transmitters are not shown in figure.

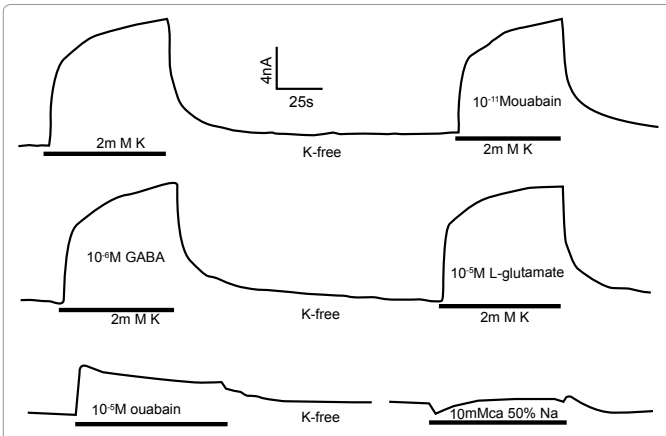

Figure 11: The intensity 2 mM-induced outward (pump) currents to transmitters. Fresh isolated oocytes, which were continuously perfused by K-free per 2 min., for 1 min. it was exposed 2 mM K containing medium with 10^{-11} M ouabain and different transmitters. In the bottom ouabain depressed that pump current while 10 mM Ca and 50% Na containing medium has no significant changing of study level of membrane current [63].

Similar inhibitory effects on pump current in neuron have LDT. In oocytes similar to pump current, the transmitters-induced current was also insensitive to low ouabain and LDT (Figures 10-12).

It is known that Na^+/Ca^{2+} exchange can be stimulated by the decrease of Na ions' or the increase of Ca ions' gradients on the membrane [45]. Such activation produces an outward (hyperpolarizing) current through the membrane [40]. Figure 11 demonstrates that the similar changes of Na and Ca ions' concentrations in oocytes' bathing medium does not generate any membrane current. These data allow us to suggest that in oocyte membrane the electrogenic Na^+/Ca^{2+} exchange is very weak or is absent [63].

As it was described above (Figure 6) the higher affinity ouabain binding sites (saturated component of [^3H]-ouabain binding) in neuronal membrane are connected with the function of Na^+/Ca^{2+} exchange [40]. To check the suggestion on the absence of Na^+/Ca^{2+} exchange in oocyte membrane, the dose-dependent ouabain binding was studied. Figure 13 shows that the higher affinity binding sites are absent in oocyte membrane, because there is no statistically significant ouabain binding to membrane. Only at higher than 10^{-7} M ouabain concentrations (threshold for ouabain-induced inhibition of Na^+/K^+ pump) the dose-dependent binding was observed.

This data could serve as an additional evidence for the absence of the family of higher affinity ouabain receptors in oocyte membrane that serves as sensor for LDT.

Based on the above data it can be concluded that nanomolar ouabain and LDT in excitable cell membrane have a common sensor having individual structure separate from the transporting Na^+/K^+-ATPase and channel binding receptors, function of which is connected with cAMP-dependent R-Na^+/Ca^{2+} exchange system [63].

It is known that besides plasma membrane mechanisms the intracellular Ca^{2+}-buffer system (sarcoplasmic reticulum (SR), mitochondria, calmoduline and other proteins) have a crucial role in regulation of $[Ca^{2+}]_i$ homeostasis [56].

To find out whether the above mentioned extra-sensitive chemosensors serve as a sensor for physical factors also, the effect of static magnetic fields (SMF) exposure on dose-dependent ^{3}H-ouabain binding with membrane, cell hydration, Na^+/Ca^{2+} exchange, intracellular cAMP and cGMP contents in rats heart muscle [64] and different zones of rats brain [57] were studied. Previous study performed on snail neurons and heart muscle have shown that both cell hydration and ^{45}Ca uptake by cells are sensitive to non-ionizing radiation and their effects can be reversed by Na^+/K^+ pump inactivation (Figures 3 and 4). However, in the available literature, the data on the individual roles of different types of catalytic α subunits of Na^+/K^+-ATPase in regulation of cell hydration and on the detailed mechanism on the role of α_3 receptors in regulation of Na^+/Ca^{2+}exchange as well as their sensitivity to MV and EMF are absent. Therefore, these questions were the subject for our recent investigations performed in rat's brain and heart tissues. For these purposes first of all the dose-dependent ^{3}H-ouabain binding and the effects of the latter on cell hydration and ^{45}Ca uptake in brain tissues and heart muscles were studied.

As can be seen in Figures 14b and 15b, in both tissues of adult rats there exist clearly distinguishable three populations of ^{3}H-ouabain receptors having different affinity and dose-dependent kinetics. The dose-dependent curves of ^{3}H-ouabain binding with cell membrane which was obtained by intraperitoneal injection of different concentration of ^{3}H-ouabain, consist of two saturated (10^{-11}–10^{-9} M and 10^{-9}–10^{-6} M) and one linear components (10^{-6}–10^{-4} M) corresponding

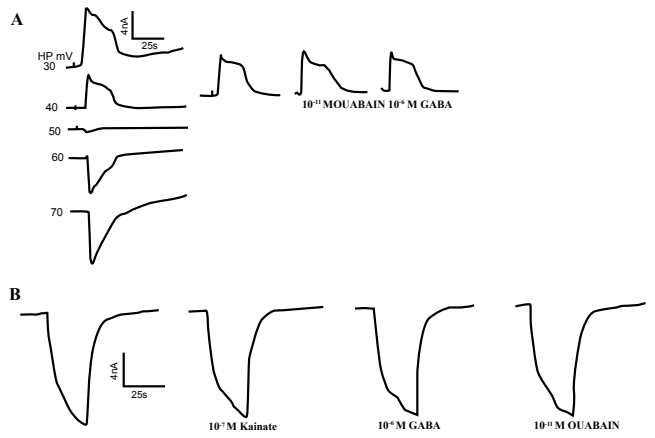

Figure 12: A- The insensitivity of nicotinic Ach responses of oocytes to 10^{-11} M and low concentration of Ach and GABA.B- The intensity of kinate (10^{-4} M) responses of oocytes to 10^{-7} M kinate, 10^{-6} GABA and 10^{-11}ouabain. Holding potential was 50 mV [63].

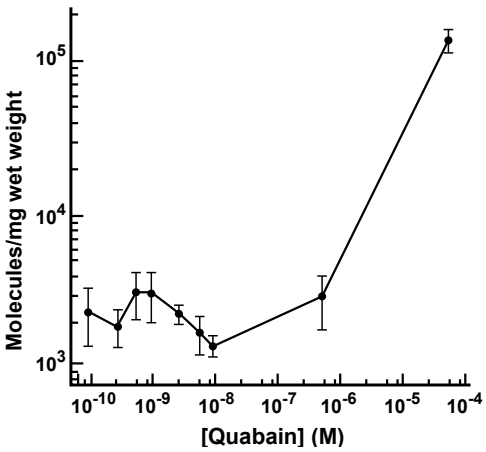

Figure 13: Binding of [^{3}H]-ouabain to the *Xenopus* oocytes membrane as a function of the glycoside concentration.

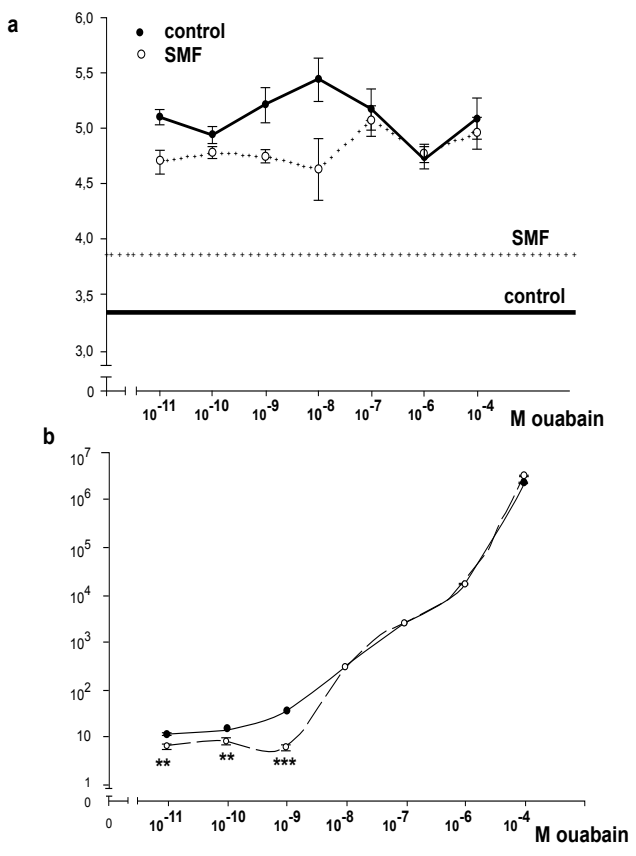

Figure 14: Dose-dependent ^{3}H-ouabain-effect on cell hydration (a) and binding (b) with cell membrane of adult rats brain cortex tissue [57].

to the so called α_3, α_2 and α_1 receptors described in literature [49]. The study on dose-dependent ouabain effect on brain cortex and heart muscle tissues' hydration has shown that even the intra-peritoneal injection of extremely low concentrations of ouabain (10^{-11} M) had a strong hydration effect on cells. On cortex cells, the ouabain-induced activation of all α_3, α_2 and α_1 receptors causes a cell hydration as compared to the control (ouabain non-injected animals) ones (Figure 14A), while in heart muscle the 10^{-7} and 10^{-6} M ouabain had

a dehydration effect on cells (Figure 15A). The fact that the ouabain-induced activation of all the three receptors on cortex and heart tissues lead to different modulation effects on cells hydration, indicate that each of these three types of receptors has their own specific metabolic pathways through which the modulation of cell hydration is realized. From the dose-dependent hydration curves on cortex and heart it can be clearly distinguished that all α_3 and α_2 receptors activations have different dose dependency and opposite effects on cell hydrations. It is worth to note that although the ouabain binding with high-affinity receptors of cortex and heart tissues has the same character their activations have different effects on these tissues' hydration, which can be explained by differences in metabolic pathways of cell volume regulation in neurons and muscles. We came to similar conclusion by studying the magneto-sensitivity. It can be seen in Figures 14B and 15B that in ouabain-free medium, the SMF-exposed animals lead to hydration of brain cortex and dehydration of heart muscle tissues (dash lines). In both tissues the SMF exposure had an opposite effect on ouabain-induced tissue hydration. At 10^{-11} M ouabain concentration, SMF exposure has hydration effect on both cortex and heart muscle tissue, while at 10^{-10}–10^{-8} M concentration of ouabain SMF had dehydration effect on cortex and hydration effect on heart muscle. However, although the SMF exposure has different effects on cortex and muscle tissues, it has depressing effect on ouabain binding with α_3 receptors. These data clearly indicate that α_3 receptors affinity in both tissues only is magneto-sensitive, while the ouabain bindings with, α_2 and α_1 receptors' affinity was non-magnetosensitive (Figures 14B and 15B). These data allow us to conclude that high-affinity ouabain receptors are a magneto-sensor through which the modulation effect of SMF on cell hydration is realized. However a question arises, which

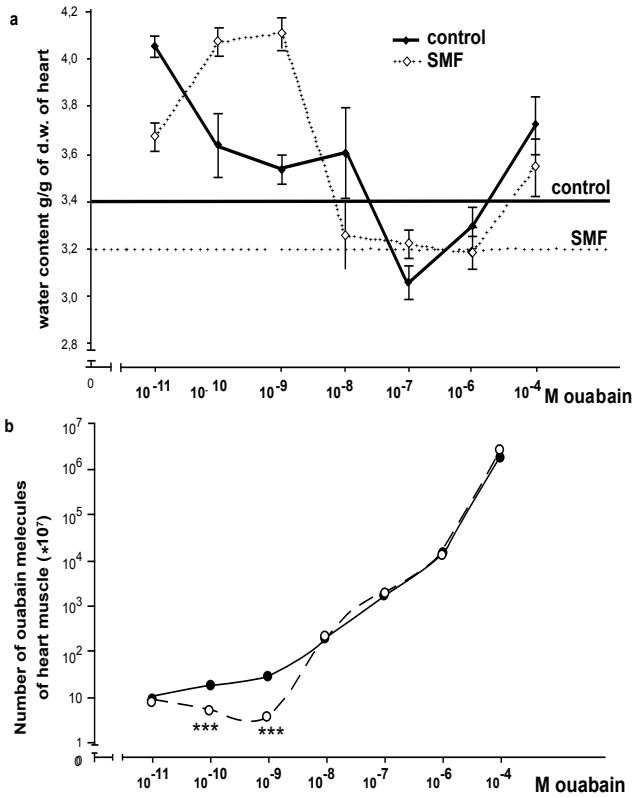

Figure 15: Dose-dependent ^{3}H-ouabain-effect on cell hydration (a) and binding (b) with cell membrane of adult rats heart muscle [57].

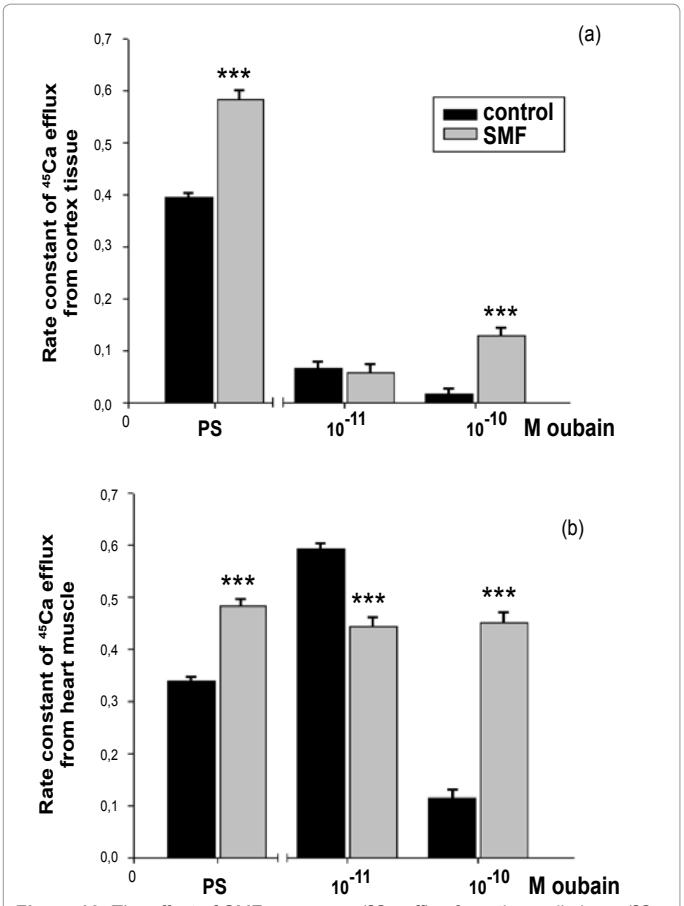

Figure 16: The effect of SMF expose on ^{45}Ca efflux from the preliminary ^{45}Ca enriched brain cortex (a) and heart muscle (b) cells in ouabain-free, 10^{-11} and 10^{-10} M ouabain containing PS [57].

mechanism is responsible for the magnetosensitivity of cell hydration and ouabain binding with α_3 receptors?

As α_3 receptors' functions closely correlate with the cAMP-dependent R- Na^+/Ca^{2+}exchange, having a key role in both intracellular Ca-dependent receptors' affinity and cell volume regulation, it can be considered as a mechanism responsible for determination of magnetosensitivity of α_3 receptors' ouabain binding properties and cell hydration dependent on it. The study of dose-dependent ouabain effect on $^{45}Ca^{2+}$ uptake and efflux as well their magnetosensitivity in brain cortex and heart muscle has revealed that the 10^{-11} M ouabain concentration has an inactivation effect on Ca efflux (Figure 16A) in brain cortex cells and activation effect on Ca efflux in heart muscle [57]. The SMF exposure of animals has an activation effect on Ca efflux in both tissues compared to its basal one in control. The SMF exposure on cortex at 10^{-11} M oubain concentration had no significant effect on Ca efflux in brain cortex cells, while in the case of heart muscle it has a strong activation effect on it (Figure 16B).

It is obvious that by decreasing ouabain concentration in cell bathing medium the number of mechanisms decreased through which ouabain could modulate the Na^+/Ca^{2+}exchange and cell volume. Therefore, it is suggested that the highest affinity- α_3 receptors could modulate the cell volume by activation of mechanism having highest sensitivity to ouabain.

Previously it has been shown that SMF exposure inhibits $^{45}Ca^{2+}$

uptake by neurons and heart muscle which was accompanied by elevation of intracellular cyclic guanosine monophosphate (cGMP) and decrease in cAMP [65]. It was shown that NO, which has effect on elevation of cGMP also had similar effect like that of SMF, i.e., the depression of Ca^{2+} uptake [39]. Therefore, as the α_3 receptors' functions closely correlate with the Na^+/Ca^{2+} exchange, the SMF- induced activation of ^{45}Ca efflux from cells, preliminary enriched by Ca, can be considered as a result of activation in cGMP-dependent F- Na^+/Ca^{2+} exchange only, as the Ca pump is inhibited by high $[Ca]_i$ [38,56].

Thus, the nanomolar ouabain- induced activation of R- Na^+/Ca^{2+}exchange can be explained by AMP-dependent activation of Ca pump in endoplasmatic membrane, the SMF-induced activation F-Na^+/Ca^{2+}exchange can be considered as a result of cGMP-dependent release of Ca from intracellular storage.

Based on the above data on common mechanisms such as cAMP/cGMP-dependent Na^+/Ca^{2+} exchange through which the nanomolar concentration ouabain, extremely low concentration synaptic transmitters and biological effects of SMF on neuronal and muscle membrane are realized, which is absent in oocyte membrane preinjected with specific mRNA for different synaptic transmitters, it can be suggested that the α_3 Na^+/K^+ pump isoform-dependent intracellular signaling system controlling [Ca] homeostasis is the universal and extra-sensitive pathways through which the biological effect of weak chemical and physical signals on cells are realized.

References

1. Adey WR (1981) Tissue interactions with nonionizing electromagnetic fields. Physiol Rev 61: 435-514.
2. Ayrapetyan S, Carpenter D (1991) Very low concentrations of acetylcholine and GABA modulate transmitter responses. Neuroreport 2: 563-565.
3. Thomas RC (1972) Electrogenic sodium pump in nerve and muscle cells. Physiol Rev 52: 563-594.
4. Airapetyan SN (1969) The effect of temperature on the membrane potential of giant neurons of snails (in Russian). Biofizika 14: 663-669.
5. AÄirapetian SN (1969) [Mechanism of regulation of the spontaneous activity of snail giant neurons]. Biofizika 14: 866-872.
6. Gorman AL, Marmor MF (1970) Temperature dependence of the sodium-potassium permeability ratio of a molluscan neurone. J Physiol 210: 919-931.
7. Ayrapetyan SN (1969) Metabolically-Dependent Part of Membrane Potential and Electrode Properties of Giant Neuron Membrane of Mollusk (in Russian). Biofizika 14: 1027-1031.
8. Carpenter DO (1971) Ionic Mechanisms and Models of Endogenous Discharge of Aplysia Neurones, Neurobiology of Invertebrates 35-58.
9. Casteels R, Droogmans G, Hendrickx H (1971) Membrane potential of smooth muscle cells in K-free solution. J Physiol 217: 281-295.
10. De Weer P, Geduldig D (1973) Electrogenic sodium pump in squid giant axon. Science 179: 1326-1328.
11. Evans DH (2008) Osmotic and ionic regulation. CRC Press.
12. USSING HH (1949) Transport of ions across cellular membranes. Physiol Rev 29: 127-155.
13. T.osteson DC (1964) Regulation od cell volume by Na and K transport. In "Cellular function of membrane transport" (Hoffman J. F.,ed.) Prentice-Hall.
14. Okamoto K, Quastel JH (1970) Water uptake and energy metabolism in brain slices from the rat. Biochem J 120: 25-36.
15. Cooke KR (1978) Ouabain and regulation of cellular volume in freshly prepared slices of rabbit renal cortex. J Physiol 279: 361-374.
16. Ayrapetyan SN, Suleymanyan MA (1979) On the pump-induced cell volume changes. Comp Biochem Physiol A Physiol 64: 571-575.
17. Lang F (2007) Mechanisms and significance of cell volume regulation. J Am Coll Nutr 26: 613S-623S.
18. Hoffmann EK, Lambert IH, Pedersen SF (2009) Physiology of cell volume regulation in vertebrates. Physiol Rev 89: 193-277.
19. Ayrapetyan SN (1980) On the Physiological Significance of Pump Induced Cell Volume Changes. Adv Physiol Sc 23: 67-82.
20. Ayrapetyan SN, Arvanov VL (1979) On The Mechanism of The Electrogenic Sodium Pump Dependence of Membrane Chemosensitivity. Comp Biochem Physiol 64: 601-604.
21. Ayrapetyan SN, Suleymanyan MA, Saghyan AA, Dadalyan SS (1984) Autoregulation of the electrogenic sodium pump. Cell Mol Neurobiol 4: 367-383.
22. Ayrapetyan SN, Rychkov GY, Suleymanyan MA (1988) Effects of water flow on transmembrane ionic currents in neurons of Helix pomatia and in squid giant axons. Comp Biochem Physiol A Comp Physiol 89: 179-186.
23. Parton RG, Simons K (2007) The multiple faces of caveolae. Nat Rev Mol Cell Biol 8: 185-194.
24. Kryshtal' OA, Osipchuk IuV, Pidoplichko VI (1981) [Kinetics of the incoming sodium current in perfused neurons governed by the transmembrane gradient of osmotic pressure]. Dokl Akad Nauk SSSR 259: 1253-1256.
25. Osipchuk YuV (1983) Modulation of gating mechanism of Na inward current by osmotic pressure gradient in membrane of perfused neuron. Neurophysiologia 15: 437-440.
26. Chizmakov IV, Sorokina ZA (1986) The dependence of the effect of osmotic pressure on the Na inward current on temperature in membrane of spinal ganglia neurons. Neurophysiologia 18: 518-525.
27. Chizmakov IV (1988) The investigation of action mechanism of osmotic gradient on the sodium inward current. Biol Membr 5: 517-527.
28. Suleymanian MA, Ayrapetyan SN, Arakelyan VB, Ayrapetyan VY (1993) The effect of osmotic gradients on the outward potassium current in dialyzed neurons of Helix pomatia. Cell Mol Neurobiol 13: 183-190.
29. Iwasa K, Tasaki I, Gibbons RC (1980) Swelling of nerve fibers associated with action potentials. Science 210: 338-339.
30. Terakawa S (1985) Potential-dependent variations of the intracellular pressure in the intracellularly perfused squid giant axon. J Physiol 369: 229-248.
31. Kojima M, Ayrapetyan SN, Koketsu K (1984) On The Membrane Potential Independent Mechanism of Sodium Pump-Induced Inhibition of Spontaneous Electrical Activity of Japanese Land Snail Neurons. Comp Biochem Physiol 77: 577-583.
32. Parsegian VA, Rand RP, Rau DC (2000) Osmotic stress, crowding, preferential hydration, and binding: A comparison of perspectives. Proc Natl Acad Sci U S A 97: 3987-3992.
33. Haussinger D (1996) The role of cellular hydration in the regulation of cell function. Biochem J 313 : 697-710.
34. Ayrapetyan S, Carpeter D, Sagian A, Dadalyan S, Mndalyan V (1992) Extralow neutotransmitter doses-induced triggering of neuronal intracellular messenger systems. Nauka, Moscow, 89-96 (in Russian).
35. Ayrapetyan SN, Arvanov VL (1988) The Metabolic Regulation of Membrane Chemosensitivity. In: Neurobiology of Invertebrates (Ed. Salanki) Budapest 36: 669-684.
36. Arvanov VL, Ovakimyan KS, Stepanian AS, Ayrapetyan SN (1992) Ouabain blocks some rapid concentration- induced clamp acetylcholine responses on Helix neurons. Cell Mol Neurobiol 12: 143-153.
37. Ayrapetyan SN, Arvanov VL, Maginyan SB, Azatyan KV (1985) Further study of the correlation between Na-pump activity and membrane chemosensitivity. Cell Mol Neurobiol 5: 231-243.
38. Blaustein MP, Lederer WJ (1999) Sodium/calcium exchange: its physiological implications. Physiol Rev 79: 763-854.
39. Azatian KV, White AR, Walker RJ, Ayrapetyan SN (1998) Cellular and molecular mechanisms of nitric oxide-induced heart muscle relaxation. Gen Pharmacol 30: 543-553.
40. Saghian AA, Ayrapetyan SN, Carpenter DO (1996) Low concentrations of ouabain stimulate Na/Ca exchange in neurons. Cell Mol Neurobiol 16: 489-498.

41. Takenaka T, Horie H, Kawasaki Y (1983) Effect of fatty acids on the membrane fluidity of cultured chick dorsal root ganglion measured by fluorescence photobleaching recovery. J Neurobiol 14: 457-461.

42. Suleymanyan MA, Salanki J, Ayrapetyan SN (1985) The dependence of Pump-Induced Hyper polarization on the Tonicity of the Surrounding Medium and Intracellular Sodium Concentration. Comp Biochem Physiol 78: 591- 595.

43. Arvanov VL, Suleymanyan MO, Majinian SB, Ayrapetyan SN (1984) Effect of Ouabain on Interaction Processes between ATP and External Neuronal Membrane. Proceedings of Arm. NAS 29: 41-45 (in Russian).

44. Saghyan AA, Dadalian SS, Takenaka T, Suleymanian MA, Ayrapetyan SN (1986) The effects of short-chain fatty acids on the neuronal membrane functions of Helix pomatia. III. 22Na efflux from the cells. Cell Mol Neurobiol 6: 397-405.

45. Baker PF, Blaustein MP, Hodgkin AL, Steinhardt RA (1969) The influence of calcium on sodium efflux in squid axons. J Physiol 200: 431-458.

46. Takeuchi A, Tatsumi S, Sarai N, Terashima K, Matsuoka S, et al. (2006) Ionic mechanisms of cardiac cell swelling induced by blocking Na+/K+ pump as revealed by experiments and simulation. J Gen Physiol 128: 495-507.

47. Ayrapetyan SN (2001) Na-K Pump and Na:Ca Exchanger as Metabolic Regulators and Sensors for Extraweak Signals in Neuromembrane. Modern Problems of Cellular and Molecular Biophysics.

48. Ayrapetyan SN (2006) Cell aqua medium as a preliminary target for the effect of electromagnetic fields. In: Bioelectromagnetics: Current Concepts, S. Ayrapetyan and M. Markov, eds., NATO Science Series, Springer Press, The Netherlands 31-64.

49. Juhaszova M, Blaustein MP (1997) Na+ pump low and high ouabain affinity alpha subunit isoforms are differently distributed in cells. Proc Natl Acad Sci U S A 94: 1800-1805.

50. Adams RJ, Schwartz A, Grupp G, Grupp I, Lee SW, et al. (1982) High-affinity ouabain binding site and low-dose positive inotropic effect in rat myocardium. Nature 296: 167-169.

51. Lucchesi PA, Sweadner KJ (1991) Postnatal changes in Na,K-ATPase isoform expression in rat cardiac ventricle. Conservation of biphasic ouabain affinity. J Biol Chem 266: 9327-9331.

52. Blanco G (2005) The NA/K-ATPase and its isozymes: what we have learned using the baculovirus expression system. Front Biosci 10: 2397-2411.

53. Wymore T, Deerfield DW 2nd, Hempel J (2007) Mechanistic implications of the cysteine-nicotinamide adduct in aldehyde dehydrogenase based on quantum mechanical/molecular mechanical simulations. Biochemistry 46: 9495-9506.

54. Blaustein MP, Zhang J, Chen L, Song H, Raina H, et al. (2009) The pump, the exchanger, and endogenous ouabain: signaling mechanisms that link salt retention to hypertension. Hypertension 53: 291-298.

55. DiPolo R, Beauge L (2006) Sodium/calcium exchanger: influence of metabolic regulation on ion carrier interactions. Physiol Rev 86: 155-203.

56. Brini M, Carafoli E (2009) Calcium pumps in health and disease. Physiol Rev 89: 1341-1378.

57. Heqimyan A, Narinyan L, Nikoghosyan A, Deghoyan A, Yeganyan A, et al. (2012a) Age-dependency of high affinity ouabain receptors and their magnetosensitivity. The Environmentalist 32: 228-235.

58. Siegel GJ (1999) Basic Neurochemistry. (6thedn), Molecular, Cellular and Medical Aspects, Lippincott-Raven, Philadelphia.

59. Xie Z, Askari A (2002) Na(+)/K(+)-ATPase as a signal transducer. Eur J Biochem 269: 2434-2439.

60. Dadalyan SS, Kiss T, Azatian KV, Ayrapetyan SN, Salanki J (1988) The Effect of Low Concentration of GABA on the ACh Sensitivity of Snail Neurons. In: J Salanki (ed).Neurobiology of Invertebrates, Budapest 643-653.

61. Azatian KV, Ayrapetyan SN, Carpenter DO (1997) Metabotropic GABA receptors regulate acetylcholine responses on snail neurons. Gen Pharmacol 29: 67-72.

62. Ayrapetyan G, Papanyan A, Hayrapetyan H, Ayrapetyan S (2005) Metabolic pathway of magnetized fluid-induced relaxation effects on heart muscle. Bioelectromagnetics 26: 624-630.

63. Ayrapetyan (1998) The Application of the Theory of Metabolic Regulation to Pain. Pain Mechanism and Management (Eds.S. N. Ayrapeyan and A. V. Apkarian), IOS Press, Amsterdam, Netherland 3-14.

64. Narinyan L, Ayrapetyan G, Ayrapetyan S (2012) Age-dependent magnetosensitivity of heart muscle hydration. Bioelectromagnetics 33: 452-458.

65. Ayrapetyan SN, Grigorian CV, Avanesian AS (1994) On a mechanism of action of magnetic field on the electrical conductivity of water solutions and some properties of Helix neurons. Bioelectromagnetics 15: 133-142.