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The Quantum-Mechanical Sensitivity of Cell Hydration in Mammals

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Abstract

The elucidation of the mechanism on the biological effects of weak chemical and physical factors on cells and organism is one of the modern problems in life sciences. According to the Receptor Theory of Prof. Bernard Katz the impact of the biological substances on cells is realized through the activation of ligand-gated ion channels in the membrane. However, this theory doesn't provide a satisfactory explanation on the similar biological effects of extremely low concentrations of different chemical substances, which are unable to activate the ionic channels in the membrane and have non-linear dose-dependent effect on cells. Previously we have suggested that the metabolic control of cell hydration serves as a universal quantum-mechanical sensor for different weak physical and chemical signals. For supporting this hypothesis, in this article the comparative study of the effects of low concentrations of both cold (non-radioactive) and [^{3}H]-ouabain (specific inhibitor for Na^+/K^+-ATPase) on the hydration in different tissues of rats has been performed. The obtained data have shown that cold and [^{3}H]-ouabain have different effects on cell hydration and such a difference depends on the initial metabolic state of tissues. On the basis of our previous and present results it is suggested that such a quantum-mechanical sensitivity of cell hydration is realized through the cyclic-nucleotides-dependent Na^+/Ca^{2+} exchange, having a crucial role in the metabolic regulation of cell hydration.

Keywords

Rat, Hydration, Ouabain

1. Introduction

The elucidation of the mechanism on the biological effects of weak chemical and physical factors on cells and organism is one of the modern problems in life

sciences. At present, our knowledge on signal transduction in cells is based on the Membrane Theory which explains this transduction by the changes of cell membrane permeability for inorganic ions leading to generation of transient ionic currents through cell membrane [1]. However, this theory based on classical thermodynamic approaches, cannot explain the biological effects of extremely low concentrations of chemical substances [2] [3] and weak physical signals [4] [5], which are unable to activate ionic channels in the membrane and have non-linear dose-dependent effect.

The main omission of the Membrane Theory is that it does not consider the direct role of cell metabolism in generation of cell membrane potential. Although the existence of the metabolic component of membrane potential in the living cells has been revealed in a number of studies [6] [7], there isn't any reliable theory explaining the sensitivity of cells to weak physical and chemical signals.

Based on our previous research data we have developed a new approach on quantum-mechanical sensitivity of living cells to different weak factors, which are realized through the metabolic control of cell hydration [8] [9] [10]. According to this hypothesis a water molecule, having valence angle with quantum-mechanical sensitivity to different factors, serves as a primary messenger for signal transduction in cells. The metabolically generated water efflux from the cell balances the osmotic water uptake and has inhibitory effect on inward going ionic currents [11] [12] serving as a gate by which the weak signals modulate cell metabolic activity. Therefore, the metabolic control of cell hydration has been suggested as a universal quantum-mechanical sensor through which the biological effects of extremely weak chemical and physical signals on cells and organisms are realized [9]

It is known that the Na^+/K^+ pump is a key mechanism through which the metabolic control of cell hydration is realized. In excitable membranes three isoforms (α_1, α_2, α_3) of α catalytic subunit of Na^+/K^+-ATPase (working molecule for Na^+/K^+ pump) are identified [13]. They are characterized by different affinities to cardiac glycoside ouabain (specific inhibitor for Na^+/K^+-ATPase) as well as functional roles: α_1 (with low affinity) and α_2 (with middle affinity) isoforms are involved in transportation of Na^+ and K^+, while α_3 (with high affinity) is not directly involved in transporting Na^+ and K^+ and has only signaling function [14] [15]. Our previous studies have shown that these isoforms are extremely sensitive not only to ouabain but also to extremely low concentrations of other biologically active substances [2] [3] [16] as well as to weak intensity of electromagnetic fields [17] [18]. From these data it is followed that the same chemical substances with different quantum-mechanical structures (e.g. non-radioactive ouabain and [^{3}H]-ouabain) can have individual effects on cell hydration. It has been shown that Na^+/K^+ pump is a key mechanism in regulation of cell hydration and its age-dependent dysfunction brings to tissue dehydration, which is accompanied by the decrease of α_3 isoform's affinity to ouabain [19]. Therefore,

in the presented article the age-dependent comparative study of the effects of pM (agonist for α_3) and nM (agonist for α_2) concentrations of cold (non-radioactive) ouabain and [^{3}H]-ouabain on the hydration of different tissues was performed.

2. Materials and Methods

2.1. Animals

All procedures performed on animals were carried out following the protocols approved by Animal Care and Use Committee of Life Sciences International Postgraduate Educational Centre (LSIPEC, Yerevan, Armenia).

The experiments were performed on young (6 weeks old) and old (18 months old) Wistar rats. They were regularly examined, kept under control of the veterinarians in LSIPEC and reserved in a specific pathogen-free animal room under optimum conditions of 12 h light/dark cycles, at temperature of 22˚C ± 2˚C, with a relative humidity of 50% and were fed *ad libitum* on a standard lab chow and water.

2.2. Chemicals

Tyrode's Physiological solution (PS) containing (in mM) 137 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.05 $MgCl_2$, 5 $C_6H_{12}O_6$, 11.9 $NaHCO_3$, and 0.42 NaH_2PO_4 and adjusted to pH 7.4 with NaOH was used. All chemicals were obtained from "Medisar" Industrial Chemical Importation Company (Yerevan, Armenia). The [^{3}H]-ouabain with specific activity (25.34 Ci/mM) (PerkinElmer, Massachusetts, USA) and cold (non-radioactive) ouabain at pM (10^{-11} M) and nM (10^{-9} M) concentrations dissolved in PS were used for tissue injection and incubation. All ouabain solutions were also adjusted to pH 7.4.

2.3. Experimental Design

It is well known that the anesthetics with different chemical and pharmacological profiles significantly affect metabolic processes, which play an important role in regulation of cell volume [20] [21]. Therefore, in the present experiments animals were sharply immobilized by freezing method (dipping their noses into liquid nitrogen for 3 - 5 sec) and decapitated [22]. After such a procedure the full absence of somatic reflexes on extra stimuli was recorded.

For *in vivo* experiments 15 young and 15 old animals were taken. Each animal group was divided into five subgroups (n = 3). The animals of the control group were injected with PS (according to the animal weight) and the animals of the next four subgroups were injected with 10^{-11} M and 10^{-9} M concentrations of cold and 10^{-11} M and 10^{-9} M concentrations of [^{3}H]-ouabain. After the 15th min the animals were decapitated. Brain cortex, heart muscle and liver tissues were isolated. From each animal 5 samples of each tissue were taken, washed in PS three times and then weighed (15 samples from each tissue). The protocol of experiments was the same for both young and old animals. All data were received from three independent experiments.

For investigation of the water content variations and ouabain effect in *in vitro* conditions 15 young and 15 old animals were taken. After their decapitation 5 samples of the above mentioned tissues were taken from each animal (similar to *in vivo* experiments). These samples (n = 15) were incubated in PS (as sham), cold ouabain solution (10^{-11} M, 10^{-9} M) and [^{3}H]-ouabain solution (10^{-11} M, 10^{-9} M) for 15 min, then washed in PS three times and weighed.

2.4. Definition of Water Content of Brain Tissues

The water content of brain cortex, heart muscle and liver tissues was determined by traditional "tissue drying" method [23]. After measuring the wet weight (w.w.) of tissue samples they were dried in oven (Factory of Medical Equipment, Odessa, Ukraine) for 24 h at 105˚C for determination of dry weight (d.w.). The quantity of water in 1 g of d.w. tissue was counted by the following equation: (w.w. – d.w.)/d.w.

2.5. Counting of [^{3}H]-Ouabain Receptors in the Membrane

The tissue samples from *in vivo* experiments, which were subjected to [^{3}H]-ouabain, were homogenized in 50 µl of 68% HNO_3 solution after determination of wet and dry weights. Then 2 ml of Bray's scintillation fluid was added and chemoluminescence of samples was quantified with 1450-MicroBeta liquid scintillation counter (Wallac, Turku, Finland). The number of [^{3}H]-ouabain molecules' binding with cell membranes was defined per mg of dry weight of samples.

The same procedure (the definition of the number of [^{3}H]-ouabain molecules) was performed on the tissue samples from *in vitro* experiments after removing them from the oven and determining wet and dry weights.

2.6. Statistical Analysis

Microsoft Excel and Sigma-Plot (Version 8.02A, NY, USA) were used for data analyses. The statistical significance in comparison with the sham group was calculated with Student's t-test with the following symbols (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

3. Results

3.1. Investigation of Brain Cortex Tissue Hydration

The comparative study of the hydration sensitivity of excitable (brain cortex, heart muscle) and non-excitable (liver) tissues to pM and nM concentrations of cold and [^{3}H]-ouabain in *in vivo* and in *in vitro* experimental conditions was performed. In i*n vivo* experiments the animals were preliminarily i/p injected with ouabain-free PS and 10^{-11} M and 10^{-9} M concentrations of cold and [^{3}H]-ouabain, while in *in vitro* experiments the tissue samples were incubated in the same solutions for 15 min.

As can be seen from **Figure 1(a)**, after i/p injection with 10^{-11} M cold ouabain

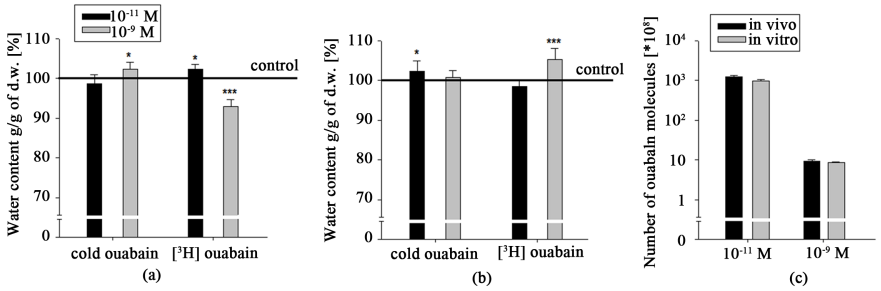

Figure 1. The water contents in cortex tissue of young rats in *in vivo* (a) and in *in vitro* (b) experiments upon the effect of pM and nM concentrations of cold and [^{3}H]-ouabain. The black and gray bars on (a) and (b) indicate the mean value of water contents in the tissues upon the effect of pM and nM ouabain, respectively. The continuous line shows the control value of tissue hydration after PS injection (a) and PS incubation (b). The black and gray bars on (c) show the number of ouabain molecules binding with cell membrane in *in vivo* and in *in vitro* conditions, respectively. Each bar represents the ±SEM (n = 45). The symbols (*) and (***) indicate $p < 0.05$ and $p < 0.001$, respectively. All data were obtained from three independent experiments.

insignificant dehydration in brain cortex tissues of young animals was observed compared with the control (injected with ouabain-free PS), while 10^{-9} M cold ouabain brought to significant hydration. The data on [^{3}H]-ouabain i/p injection (**Figure 1(a)**) revealed the opposite effect on brain tissue hydration, *i.e.* significant hydration at 10^{-11} M and expressed dehydration at 10^{-9} M [^{3}H]-ouabain.

In *in vitro experiments* (**Figure 1(b)**), where the metabolic activity of tissues was impaired, brain tissue hydration sensitivity to ouabain had a reverse character compared with those *in vivo* studies (**Figure 1(a)**): pM cold ouabain had hydration effect, while pM [^{3}H]-ouabain had dehydration effect. In case of nM cold ouabain there was no effect but nM [^{3}H]-ouabain had hydration effect on the tissues. **Figure 1(c)** illustrated that at each concentration of [^{3}H]-ouabain the number of ouabain molecules was higher in *in vivo* experiment compared with *in vitro* one.

According to the fact that aging leads to the depression of the metabolic activity, in the next series of experiments we repeated the above-mentioned protocol on old animals. As can be seen on **Figure 2(a)**, the hydration level in brain cortex tissue of old animals in *in vivo* experiments upon the impact of 10^{-11} M cold ouabain was significantly higher compared with the control, while the same concentration of [^{3}H]-ouabain sharply dehydrated the tissues. In case of 10^{-9} M cold ouabain the dehydration effect was observed, while the same concentration of [^{3}H]-ouabain had slight hydration effect on the tissues compared with the control.

In *in vitro* experiments (**Figure 2(b)**) the hydration levels of brain cortex samples at all concentrations of ouabain were nearly the same. Compared with the control data at both concentrations of cold and [^{3}H]-ouabain the dehydration effect was observed. As indicated on the data presented on **Figure 2(c)** the number of ouabain molecules at 10^{-11} M concentration of [^{3}H]-ouabain in *in vivo* as well as in *in vitro* experiments was the same, while the number of ouabain

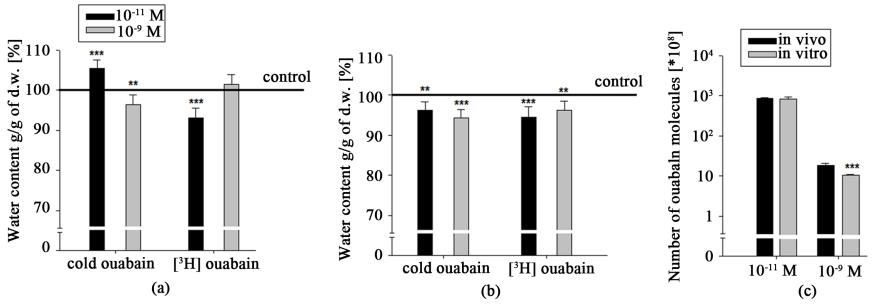

Figure 2. The water contents in cortex tissue of old rats in *in vivo* (a) and in *in vitro* (b) experiments upon the effect of pM and nM concentrations of cold and [^{3}H]-ouabain. The black and gray bars on (a) and (b) indicate the mean value of water contents in tissues upon the effect of pM and nM ouabain, respectively. The continuous line shows the control value of tissue hydration after PS injection (a) and PS incubation (b). The black and gray bars on (c) show the number of ouabain molecules binding with cell membrane in *in vivo* and in *in vitro* conditions, respectively. Each bar represents the ±SEM (n = 45). The symbols (**) and (***) indicate $p < 0.01$ and $p < 0.001$, respectively. All data were obtained from three independent experiments.

molecules at 10^{-9} M concentration of [^{3}H]-ouabain was much higher in *in vivo* experiment than in *in vitro* one.

3.2. Investigation of Heart Muscle Tissue

Considering the fact that unlike brain tissue hydration heart muscle hydration significantly depends on myosin contraction, in the next series of experiments the above mentioned protocol was performed on heart muscle tissues. The data of *in vivo* experiments presented on **Figure 3(a)** showed that the pM cold ouabain had significant dehydration effect on heart muscle tissue of young rats, while the same concentration of [^{3}H]-ouabain had hydration effect.

The effects of both nM cold ouabain and [^{3}H]-ouabain were similar to the effects of pM ouabain, but nM ouabain effect was less pronounced. In *in vitro* experiments the pM cold ouabain had slight hydration effect, while the same concentration of [^{3}H]-ouabain had expressed hydration effect on heart muscle tissue. The nM cold ouabain had more pronounced hydration effect than pM ouabain, while nM [^{3}H]-ouabain had less expressed hydration effect than pM [^{3}H]-ouabain (**Figure 3(b)**). As can be seen from **Figure 3(c)**, the number of ouabain molecules binding with cell membrane in *in vivo* and in *in vitro* experiments was the same.

The investigation of heart muscle tissues in old rats in *in vivo* condition (**Figure 4(a)**) showed significant over hydration at both concentrations (10^{-11} M and 10^{-9} M) and types of ouabain. However at 10^{-9} M [^{3}H]-ouabain the hydration level was higher.

As can be seen from **Figure 4(b)**, 10^{-11} M cold ouabain in *in vitro* experiments led to significant dehydration in old animals, while 10^{-11} M [^{3}H]-ouabain to hydration. As for the effects of 10^{-9} M ouabain the hydration reached to the level recorded at control, but 10^{-9} M [^{3}H]-ouabain brought to significant dehydration.

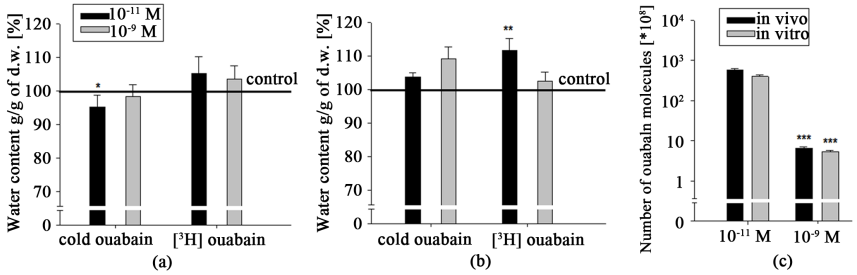

Figure 3. The water contents in heart muscle tissue of young rats in *in vivo* (a) and *in vitro* (b) experiments upon the effect of pM and nM concentrations of cold and [^{3}H]-ouabain. The black and gray bars on (a) and (b) indicate the mean value of water contents in tissues upon the effect of pM and nM ouabain, respectively. The continuous line shows the control value of tissue hydration after PS injection (a) and PS incubation (b). The black and gray bars on (c) show the number of ouabain molecules binding with cell membrane in *in vivo* and in *in vitro* conditions, respectively. Each bar represents the ±SEM (n = 45). The symbols (*) and (**) indicate $p < 0.05$ and $p < 0.01$, respectively. All data were obtained from three independent experiments.

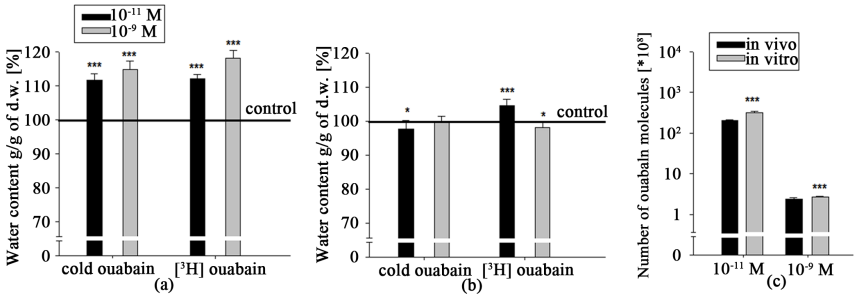

Figure 4. The water contents in heart muscle tissue of old rats in *in vivo* (a) and in *in vitro* (b) experiments upon the effect of pM and nM concentrations of cold and [^{3}H]-ouabain. The black and gray bars on (a) and (b) indicate the mean value of water contents in tissues upon the effect of pM and nM ouabain, respectively. The continuous line shows the control value of tissue hydration after PS injection (a) and PS incubation (b). The black and gray bars on (c) show the number of ouabain molecules binding with cell membrane in *in vivo* and in *in vitro* conditions, respectively. Each bar represents the ±SEM (n = 45). The symbols (*) and (***) indicate $p < 0.05$ and $p < 0.001$, respectively. All data were obtained from three independent experiments.

However, in spite of different effects on tissue hydration in *in vivo* and in *in vitro* conditions the number of ouabain molecules at [^{3}H]-ouabain was approximately the same at two different ouabain concentrations (**Figure 4(c)**).

3.3. Investigation of Liver Tissue

It is known that in soft tissues of healthy animals only a_1 isoform of Na^+/K^+-ATPase with low affinity to ouabain is expressed [24]. At the same time our previous study has shown that nM and pM ouabain concentrations activate cyclic nucleotides-dependent Na^+/Ca^{2+} exchange without any effect on Na^+/K^+ pump activity [25] [26]. Therefore, it was interesting to perform the same protocol used for the previous series of experiments on non-excitable tissues, such as liver.

Although in liver cell membrane a_2/a_3 isoforms of Na^+/K^+-ATPase were not expressed the data presented on Figure 5 indicated the modulation effect of pM and nM ouabain on its tissue hydration. Moreover, the modulation effects of low concentrations of cold and [^{3}H]-ouabain on liver tissue hydration were different compared with the effects on brain cortex and heart muscle tissues.

In i*n vivo* experiments (Figure 5(a)) cold ouabain led to dose-dependent hydration, while [^{3}H]-ouabain brought to dehydration. The significant hydration at 10^{-9} M cold ouabain turned to dehydration at 10^{-9} M [^{3}H]-ouabain. In *in vitro* experiments (Figure 5(b)) the incubation of liver samples at 10^{-11} M cold ouabain brought to more pronounced dehydration, while 10^{-11} M [^{3}H]-ouabain had weak dehydration effect on it. Cold ouabain at 10^{-9} M had no effect on tissue hydration, while [^{3}H]-ouabain at the same concentration had dehydration effect on it. It is worth to note that the ouabain binding with cell membrane at both concentrations was higher in *in vivo* state than in *in vitro* one. In old animals the effects of both cold and [^{3}H]-ouabain i/p injections on liver tissue hydration were more pronounced than in young animals (Figure 6(a)). It is interesting to note that there was no difference between cold and isotope ouabain effects on cell hydration, while 10^{-9} M cold ouabain-induced hydration was more expressed than 10^{-9} M [^{3}H]-ouabain-induced effect.

The incubation of the samples in cold ouabain solutions led to significant dehydration at 10^{-11} M, while at the same concentration of [^{3}H]-ouabain had hydration effect (Figure 6(b)). Both cold and [^{3}H]-ouabain at 10^{-9} M concentration had hydration effect on tissue but [^{3}H]-ouabain effect was less pronounced than the effect of the cold ouabain. It is interesting to note that the ouabain binding with cell membrane in *in vivo* experiment had dose-dependent increasing character, while in *in vitro* experiment it had dose-dependent weakening character (Figure 6(c)).

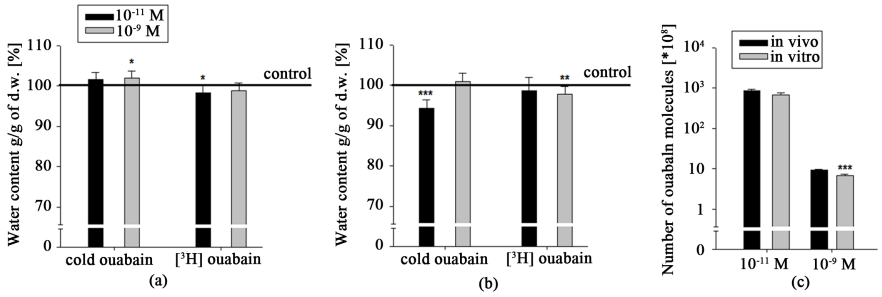

Figure 5. The water contents in liver tissue of young rats in *in vivo* (a) and in *in vitro* (b) experiments upon the effect of pM and nM concentrations of cold and [^{3}H]-ouabain. The black and gray bars on (a) and (b) indicate the mean value of water contents in tissues upon the effect of pM and nM ouabain, respectively. The continuous line shows the control value of tissue hydration after PS injection (a) and PS incubation (b). The black and gray bars on (c) show the number of ouabain molecules binding with cell membrane in *in vivo* and in *in vitro* conditions, respectively. Each bar represents the ±SEM (n = 45). The symbols (*), (**) and (***) indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. All data were obtained from three independent experiments.

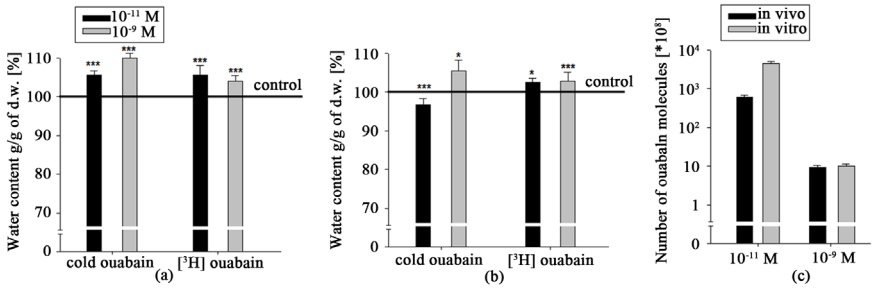

Figure 6. The water contents in liver tissue of old rats in *in vivo* (a) and in *in vitro* (b) experiments upon the effect of pM and nM concentrations of cold and [^{3}H]-ouabain. The black and gray bars on (a) and (b) indicate the mean value of water contents in tissues upon the effect of pM and nM ouabain, respectively. The continuous line shows the control value of tissue hydration after PS injection (a) and PS incubation (b). The black and gray bars on (c) show the number of ouabain molecules binding with cell membrane in *in vivo* and in *in vitro* conditions, respectively. Each bar represents the ±SEM (n = 45). The symbols (*) and (***) indicate $p < 0.05$ and $p < 0.001$, respectively. All data were obtained from three independent experiments.

4. Discussion

Previously it was shown that in brain and heart tissues of rats the pM [^{3}H]-ouabain stimulated the cGMP-dependent Na^+/Ca^{2+} exchange in forward (F) mode [27] which was accompanied by cell dehydration in young and hydration in old animals [26]. The pM [^{3}H]-ouabain-induced cell dehydration in young animals was explained by the activation of Na^+/K^+ pump as a result of Na^+/Ca^{2+} exchange-induced decrease of intracellular contents of $[Ca^{2+}]_i$, while its hydration effect on the tissues of old animal was explained by the direct effect of F Na^+/Ca^{2+} exchange because of high $[Ca^{2+}]_i$ [10]. The nM [^{3}H]-ouabain activated the cAMP-dependent Na^+/Ca^{2+} exchange in reverse mode (R) [28], having age-dependent weakening character which was reversed in old animals [18]. It is worth to note that though [^{3}H]-ouabain has been used in biological experiments for 5 decades [29] [30] the comparative studies of cold and [^{3}H]-ouabain effects on cell metabolism have not been considered. As the metabolic control of cell hydration has quantum-mechanical sensitivity [10] it becomes possible to estimate the different effects of ouabain molecules with different quantum-mechanical structure, such as cold and [^{3}H]-ouabain, on cell hydration. The data presented in this article clearly indicate that the biological effects of cold and [^{3}H]-ouabain are not identical.

The data presented on **Figure 1(a)** showed that the 10^{-11} M and 10^{-9} M cold ouabain, as was noted above (**Figure 1(a)**), had dehydration and hydration effects on brain tissues in *in vivo* experiments, respectively, while their effects were reversed in *in vitro* experiments (**Figure 1(b)**), where the metabolic state of the slices was depressed. The pM and nM concentrations of [^{3}H]-ouabain injection had opposite effects on brain tissue hydration compared with cold ouabain injection. Moreover, the reverse of dose-dependent effect of [^{3}H]-ouabain on tissue hydration in *in vitro* experiments indicated that the modulation effect of the same doses of cold and [^{3}H]-ouabain had different metabolic nature.

Previously it has been shown that [^{3}H]-ouabain binding with cell membrane depends on both the number of ouabain receptors and their affinity: the number of receptors increases with cell swelling [30], while ouabain receptors affinity increases with the decrease of $[Ca^{2+}]_i$ [19]. The data obtained in *in vivo* experiment indicating that the number of ouabain molecules at pM concentration was higher than at nM ouabain (**Figure 1(c)**) can be explained by the pM ouabain-induced activation of F Na^+/Ca^{2+} exchange leading to the decrease of $[Ca^{2+}]_i$ and increase of receptors affinity to ouabain, while the decrease of ouabain binding with cell membrane in *in vitro* experiments can be a result of $[Ca^{2+}]_i$ increase.

The received data allow us to suggest that different effects of pM and nM ouabain as well as the difference in the effects of radioactive and cold ouabain is due to the initial level of $[Ca^{2+}]_i$. This suggestion is consistent with the data of the same protocol of experiments performed on old rats, when the initial level of $[Ca^{2+}]_i$ in brain tissues was higher than in young animals [31].

The data obtained in old animals showed that *in vivo* experiment the pM cold ouabain led to hydration, while [^{3}H]-ouabain had strong dehydration effect on brain cortex tissues (**Figure 2(a)**). In case of the nM ouabain i/p injection we recorded opposite data: cold and [^{3}H]-ouabain had dehydration and slight hydration effect on tissue, respectively. Moreover, in *in vitro* experiments, in which the tissue samples had high Ca^{2+} contents compared with that in *in vivo* experiments, both concentrations of cold and [^{3}H]-ouabain ouabain had dehydration effect on tissues. The differences between the effects of pM and nM as well as between cold and isotope ouabain were inconsistent (**Figure 2(b)**).

The data on dose-dependent binding with membrane in the cortex tissue of old animals indicated that there was dose-dependent increase of ouabain binding (**Figure 2(c)**). These age-dependent differences can be explained by the fact that in old animals $[Ca^{2+}]_i$ is higher. As a result of this, the activation of cGMP-dependent F Na^+/Ca^{2+} exchange had its direct hydration effect on cells [18].

Thus, the obtained data indicating that the same concentrations of cold and [^{3}H]-ouabain had different effects on cortex tissue hydration. The fact that the effect of pM [^{3}H]-ouabain was approximately the same as in case of cold nM ouabain clearly indicates that the existence of [^{3}H] in ouabain molecules increases its effect on cell hydration. This suggestion is supported by the obtained data of the similar study on heart muscle tissues.

It is known that approximately 50% of cardiomyocyte volume consists of myofibrils [32] and $[Ca^{2+}]_i$-dependent contractility of the latter has a determining role in muscle tissues hydration. The data presented on **Figure 3** and **Figure 4** indicate that as in case of cortex tissue (**Figure 1** and **Figure 2**) the cold and [^{3}H]-ouabain at the same concentrations had different effects on heart tissue hydration. The i/p injection of cold ouabain in young animals had dose-dependent dehydration effects on muscle compared with control, while [^{3}H]-ouabain had dose-dependent weakening effect on hydration in heart muscle tissue (**Figure 3(a)**). The data that in heart muscle tissues of old animals (containing higher $[Ca^{2+}]_i$ than in young animals) when the cold and [^{3}H]-ouabain injection led to

the same dose-dependent hydration effect on muscle (**Figure 4(a)**) indicate that their effects on cell hydration is realized by different mechanisms controlling $[Ca^{2+}]_i$. The data on the difference between cold and $[^3H]$-ouabain effects appeared in heart muscle samples incubated in *in vitro* experiments seem extremely interesting. The facts that in *in vitro* experiments where $[Ca^{2+}]_i$ is considered to be higher than in *in vivo* experiments and the increase of $[Ca^{2+}]_i$ activates cGMP-dependent F Na^+/Ca^{2+} exchange through the activation of Ca^{2+}-calmodulin-induced NO production, which in its turn stimulates cGMP formation, allow us to suggest that the modulation effect of $[^3H]$-ouabain on cell hydration is realized through this chain. However, to prove this suggestion more detailed investigation is needed.

It is known that all three isoforms of Na^+/K^+-ATPase are expressed in excitable tissues (nerve and muscle membrane), while in soft tissues of healthy animals only a_1 isoform is expressed [24]. At the same time by our previous experiments performed on snail neurons [28] [30], heart muscles and brain tissues of rats [18] it was shown that pM and nM ouabain-induced modulation of Na^+/Ca^{2+} exchange did not depend on Na^+/K^+ pump and it was realized through the changes of intracellular nucleotides [17] [18].

The data presented on **Figure 5(a)** indicate that after pM and nM cold ouabain injections of young animals the liver tissue hydration increased by dose-dependent manner, while the same concentrations of $[^3H]$-ouabain injections led to dehydration effect. These data support the above suggestion that cold and isotope had different biological effects on cells. It is worth to note that as in case of excitable tissues, the sensitivity of liver tissue hydration to low concentrations of both cold and $[^3H]$-ouabain was higher in old animals as well as in the samples incubated in *in vitro* experiment, when the metabolic activity of tissues was depressed. Therefore, the data that such low concentrations of non-radioactive and radioactive ouabain effects depend on $[Ca^{2+}]_i$ and the data that $[^3H]$-ouabain binding with cell membrane is depressed in *in vitro* experiments allow us to suggest that the modulation of tissue hydration at both concentrations of cold and $[^3H]$-ouabain takes place through the changes of $[Ca^{2+}]_i$.

Thus, the obtained data bring us to the following conclusions:

1) Cold and $[^3H]$-ouabain at pM and nM concentrations have different effects on cell hydration;

2) The different effects of cold and $[^3H]$-ouabain on cell hydration is due to their different activities on cGMP-dependent Na^+/Ca^{2+} exchange controlling intracellular Ca^{2+} concentration. Therefore, these data allow us to conclude that in biological experiments cold ouabain and radioactive ouabain effects cannot be considered as equivalent. These data indicate that Na^+/Ca^{2+} exchange is Na^+/K^+ pump-independent mechanism in the membrane having quantum-mechanical sensitivity.

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