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Age-Dependent Impairment of Heart Muscle Contractility as a Primary Mechanism for Overexpression of Na^+/Ca^{2+} Exchanger in Brain Cortex Tissues

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Abstract: The cognitive function of brain and contractility of heart muscle are accompanied with age-dependent dehydration of tissues of these two organs. The aim of the present study is to reveal which of the abovementioned two organs primarily fail as a result of dysfunction of age-sensitive metabolic mechanism. For this purpose, the age-dependent sensitivity of cell hydration in brain cortex and heart muscle tissues are studied through depressing metabolic activity by cooling and its activation by supplying animals with distilled water, by inactivation of Na^+/K^+ pump and activation of Na^+/Ca^{2+} exchange in the reverse mode. The obtained data bring us to the conclusion that the metabolic regulation of brain cortex and heart muscle tissues has different nature. The age-dependent dysfunction of Na^+/K^+ pump–induced activation of RNa^+/Ca^{2+} exchange leads to dysfunction of heart muscle contractility because of activation of Ca-calmoduline-NO-cGMP production, which brings to FNa^+/Ca^{2+} exchange induced muscle relaxation and it could serve as a primary mechanism for dysfunction of brain tissues' metabolic control of cell hydration, which leads to overexpression of Na^+/Ca^{2+} exchanger in the membrane.

Keywords: Hydration, Brain Cortex, Heart Muscle, [^{3}H]-Ouabain, Na^+/K^+ Pump

1. Introduction

The dysfunction of metabolic controlling of cell hydration is a common consequence of any cell pathology, including aging. It is known that aging-induced impairments of cognitive function of brain and contractility of heart muscle are accompanied with dehydration of tissues of these two organs. However, which of the abovementioned two organs primarily fail as a result of dysfunction of age-sensitive metabolic mechanism is not clear yet.

It is known that cell hydration being cell dynamic parameter has a crucial role in metabolic regulation of cell function, which is realized by cell's surface-dependent changes of quantity of functional active proteins in membrane (enzymes, ionic channels and receptors) [1-4] as well as by hydration-dependent regulation of intracellular micromolecules' activity, through folding/unfolding mechanisms [5]. Moreover, the metabolic driving of water efflux from the cell, having inhibitory effect on inward ionic currents such as INa and ICa [3], serves as a key metabolic mechanism controlling low permeability of cell membrane (membrane excitability) for these ions [6].

It is also shown that because of negative feedback between the Na^+/K^+-pump activity and cell volume, the Na^+/K^+ pump–induced cell hydration serves as a powerful metabolic mechanism through which the autoregulation of Na^+/K^+-pump activity, as well as regulation of membrane excitability and chemosensitivity are realized [7-9]. It is known that the dysfunction of Na^+/K^+ pump leads to activation of Na^+/Ca^{2+} exchange in the reverse mode (RNa^+/Ca^{2+}) as a result of the intracellular Na^+ ($[Na^+]i$) increase [10, 11].

At present it is known that in neuronal and muscle membrane three types of ouabain receptors are expressed having different affinity: high (α3), middle (α2) and low (α1). Our earlier study showed that the function of α1 receptors, agonist of which <10-7M ouabain responsible for Na^+/K^+

pump, while the >10-7M ouabain has been activated through G protein (s) of the cAMP-dependent RNa^+/Ca^{2+} exchange [1, 12]. These receptors are universal and extrasensitive to different weak chemical and physical factors [13-15] and have age-dependent depressing characters [16].

Despite that RNa^+/Ca^{2+} working in stoichiometry $3Na^+:1Ca^{2+}$ [10] as a result of activation leads to age-dependent weakening metabolic-dependent cell hydration [16]. On the basis of these data it has been suggested that the impairment of RNa^+/Ca^{2+} exchange-induced hydration in brain is responsible for its age-dependent dehydration [17], while in heart muscle the age-dependent tissue dehydration is a result of activation of RNa^+/Ca^{2+} exchange-induced increase of the intracellular Ca^{2+} ($[Ca^{2+}]i$) -dependent contractility of myocites [18]. As the volume of myocites consists of more than 50% myosines, contractility of which depends on $[Ca^{2+}]i$, we suggest that the age-dependent heart muscle dehydration (contraction) could precede to brain tissue dehydration. The aim of the present work is to check this hypothesis. For this purpose, the age-dependency of brain and heart muscle tissue hydration-dependence on Na^+/K^+ pump and RNa^+/Ca^{2+} exchange has been activated as well as there have been studies on their affinity to [^{3}H] -ouabain in different experimental conditions.

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 117 young (6 weeks old) and 117 old (18 months old) albino 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±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) was used, with the following composition (in mM): 137 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.05 $MgCl_2$, 5 $C_6H_{12}O_6$, 11.9 $NaHCO_3$, 0.42 NaH_2PO_4, and adjusted to pH=7.4. A radiometer PHM-22r (Radiometer, Copenhagen, Denmark) was used for pH measurements. All chemicals were obtained from "Medisar" Industrial Chemical Importation Company (Yerevan, Armenia). The distilled water (DW) was received in laboratory by means of corresponding apparatus.

The [^{3}H]-ouabain (specific activity 25.34Ci/mM), non-radioactive ouabain and $^{45}Ca^{2+}$ (specific activity 40mCi/ml) were obtained from Perkin Elmer (Waltham, MA, USA). The [^{3}H] -ouabain was prepared on the basis of the physiological solution and used for intraperitoneal injection (*in vivo*) of animals. The volume of all injected solutions was adjusted according to the body mass of the animals (0.02 ml/g body mass). The cold (non-radioactive) ouabain was used for brain cortex and heart muscle tissue incubation *(in vitro*). In order to receive PS with 50% of NaCl (68.5mM), the 68.5mM of NaCl was replaced with 2M mannitol dissolved in PS for maintaining the osmolarity of the solution and these types of PS in Figure 4, Figure 5 and Figure 6 named as 50% NaPS.

2.3. Tissue Preparation

In present experiments, to avoid an anesthetic effect on initial functional state [19, 20] we preferred to use sharp freezing method [21]. Animals were immobilized by dipping their heads into liquid nitrogen (for 3-4s) and then they were decapitated. After decapitation their brain cortex and heart muscle tissues were immediately placed in the tube with PS, and then five pieces were taken from each tested brain cortex and heart muscle tissues with 50-60 mg wet mass (w. m.) per piece.

In *in vivo experiments*, 30 min before decapitation the animals were intraperitoneally injected by investigated solutions. After this they were immobilized and decapitated.

In *in vitro experiments* animals were firstly immobilized and decapitated. Then the brain cortex and heart muscle tissues were dissected in the same manner and then incubated in investigated solutions.

To remove surface-adherent and extracellular tracer, the samples were washed three times in PS. After that the wet mass (w.m.) of samples was determined.

2.4. Definition of the Water Content in Brain Cortex and Heart Muscle Tissues

Determination of the water content (hydration) of brain cortex and heart muscle tissues was performed by traditional "tissue drying" method [22]. After determination of wet mass, the samples were dried in a thermostat (Factory of Medical Equipment, Odessa, Ukraine) during 24 h at 105°C in order to estimate water content in samples. The quantity of water in 1 g of dry mass (d. m.) of tissue was derived by the following equation: (w.m.-d. m.)/d. m.

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

Radioactive [^{3}H]-ouabain is usually used to estimate the number of Na^+/K^+-pump units in the membrane. It is assumed that each binding site in the membrane binds one molecule of ouabain [23]. After 30 min of [^{3}H]-ouabain injection (*in vivo* experiments) the rats were decapitated and the brain cortex and heart muscle samples were washed three times (10min-5min-5min) with PS to remove surface-adherent and extracellular tracer. In *in vitro* experiments after decapitation of rats the brain cortex and heart muscle samples were incubated in cold ouabain (non-radioactive) for 30min and then washed three times (10min-5min-5min) with PS. After determination of water content by the method described previously, dried tissue samples were replaced into special tubs and homogenized in 50µl 68% HNO_3 solution. Finally, 2 ml of Bray's scintillation fluid was added and the

radioactivity of samples was calculated as counted per minute (cpm)/mg dry mass by Wallac 1450 liquid scintillation and luminescence counter (WallacOy, Turku, Finland).

2.6. Definition of $^{45}Ca^{2+}$ Uptake in Brain Cortex and Heart Muscle Tissues

In *in vitro* experiments brain cortex and heart muscle tissue samples were incubated in 20 ml PS containing 1.8µl $^{45}Ca^{2+}$ (as a control) or in 50%NaPS containing 1.8µl $^{45}Ca^{2+}$ (as a experiment) for 30 min. In experiments with ouabain in PS and 50%NaPS were added cold ouabain (10^{-4}M or 10^{-9}M) and all samples were also incubated in these solutions for 30 min. Then all the samples were dried in thermostat for 24 h at 105°C. After determination of dry mass, all samples homogenized in 50µl of 68% HNO_3 solution, and the radioactivity of the samples was measured as (cpm)/mg dry mass.

2.7. Statistical Analysis

The mean and standard error of the heart muscle hydration index, [^{3}H]-ouabain binding and $^{45}Ca^{2+}$ changes in different samples was calculated and the statistical probability was determined by Student's paired t-test by means of computer program Sigma Plot (Version 8.02A, San Jose, CA, USA).

The statistical probability was reflected in Figures by asterisks (*). For all statistical tests the P value was taken as $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

3. Results

In order to evaluate the difference between mechanisms of metabolic controlling and their age-dependent dysfunction on tissue hydration in the first series of experiments, the age-dependent changes of brain and heart muscle slices' hydration in bathing cold (7°C) PS (when all the metabolic processes in tissues were suppressed) were studied.

As can be seen in Figure 1 (A) there is an age-dependent increase of dehydration in brain tissue slices incubated in cold (7°C) PS compared with slices incubated in PS with room temperature (20°C). In heart muscle slices the cooling has an age-dependent reversed effects on hydration: dehydration effect in young animals and hydration-in slices of old once.

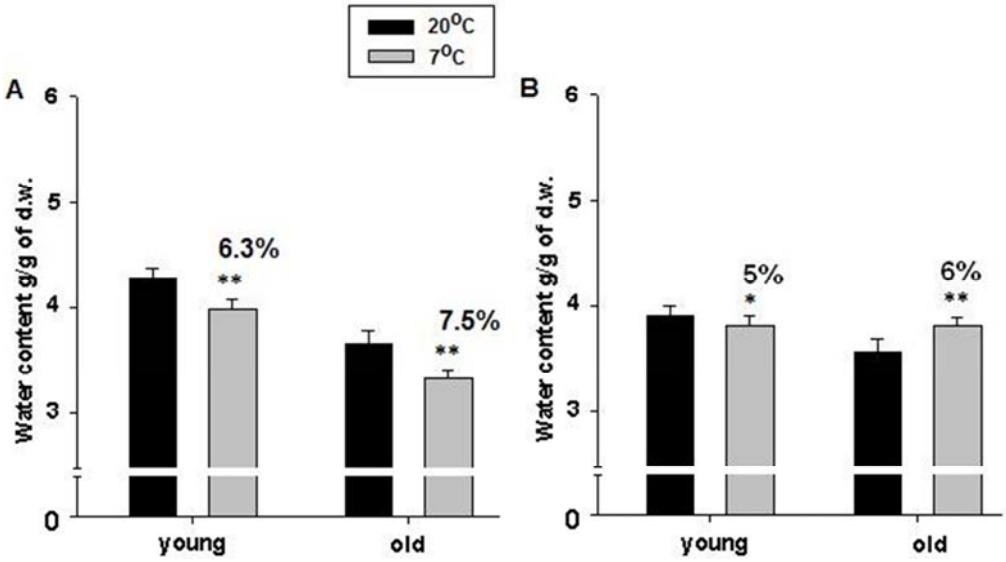


Figure 1. *The hydration effect of cold (7°C) PS on brain and heart muscle slices of young and old animals. The control samples of brain cortex and heart muscle tissues were incubated in PS at 20°C (black bars). The experimental samples of brain cortex and heart muscle tissues were incubated in PS at 7°C (grey bars). Each bar represents the mean±SEM (n=45). The symbols (*), (**) indicate p< 0.05 and p<0.01, respectively.*

It is known that Na^+/K^+ pump has a major role in metabolic regulation of cell volume in neurons and myocites. To evaluate the role of $Na^{+/}K^+$ pump hydration in age-dependent changes of brain and heart muscle tissues in the next series of experiments the effect of 10^{-4}M [^{3}H]-ouabain (inhibitor for Na^+/K^+ pump) on age-dependent changes of tissue hydration and on the number of [^{3}H]-ouabain bound molecules with cell membrane of brain and heart muscle tissues were studied.

As can be seen in Figure 2 (A) the injection with 10^{-4}M [^{3}H]-ouabain leads to age-dependent increase of hydration in brain cortex tissues, which is accompanied with age-dependent decrease of the number of [^{3}H]-ouabain bound molecules with cell membrane (Figure 2C, E). While in heart muscle tissues the 10^{-4}M [^{3}H]-ouabain injection has age-dependent reversion effect on tissue hydration: dehydration in young animals and hydration in the old ones (Figure 2B), which is accompanied with age-dependent decrease of the number of [^{3}H]-ouabain bound molecules with cell membrane (Figure 2D, F). The value of the coefficient (Figure 2E, F) of old animals in brain cortex and heart muscle tissues is lower than in the young ones.

As was noted above the 10^{-9}M ouabain activates cAMP-dependent RNa^+/Ca^{2+} exchange without changes of Na^+/K^+ pump activity [12].

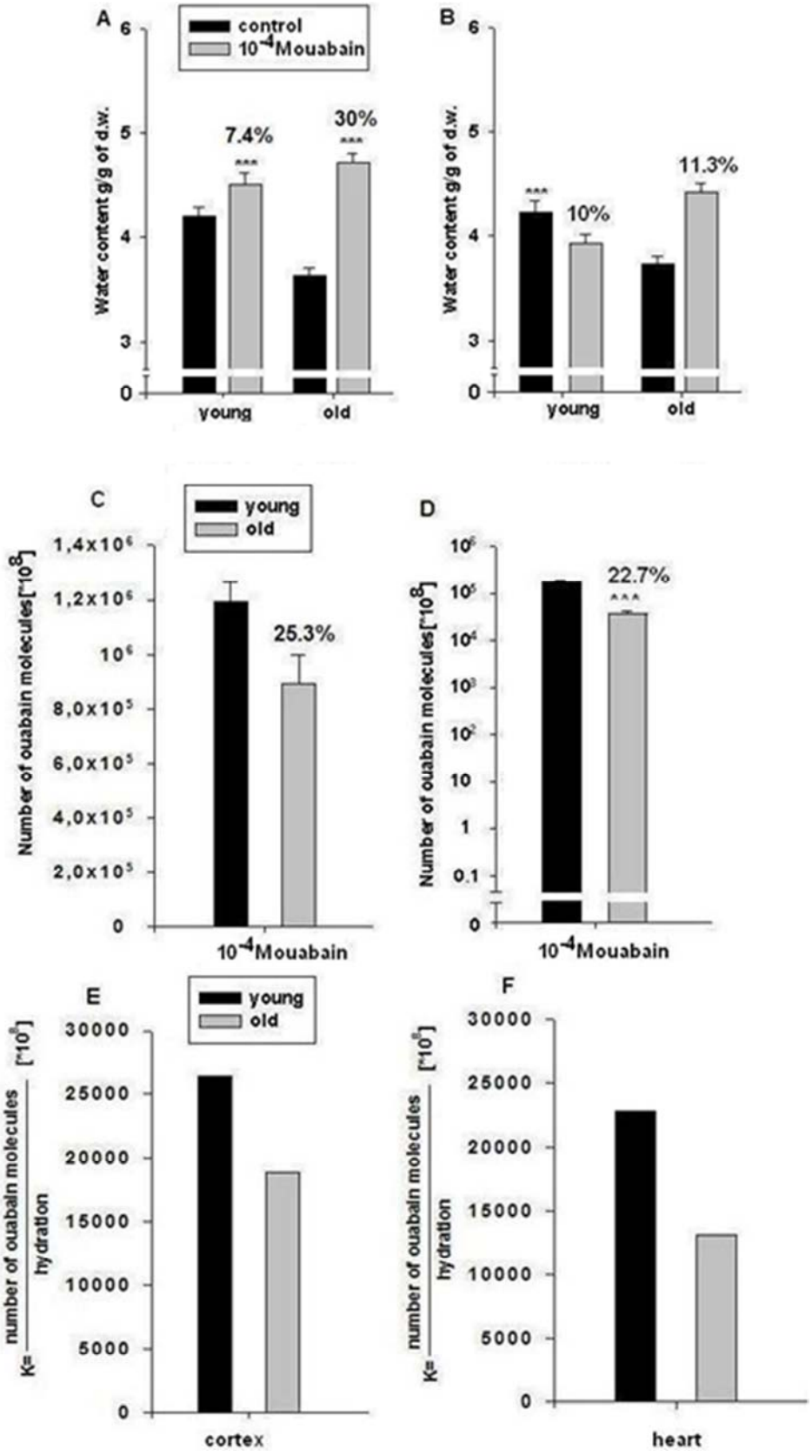


Figure 2. *The age-dependent changing of cell hydration and the number of 10^{-4}M [^{3}H] -ouabain bound molecules with cell membrane in brain cortex tissues (A, C), heart muscle tissues (B, D), and the value of the coefficient (E, F) calculated as the ratio of the number of bound molecules of ouabain to the level of tissues hydration. The control groups of animals being i/p injected by PS (0.02 ml/g body mass) (black bars). The experimental groups of animals being i/p injected by 10^{-4}M [^{3}H]-ouabain (0.02 ml/g body mass) (grey bars). Each bar represents the mean ±SEM (n=45). The symbol (***) indicates $p<0.005$.*

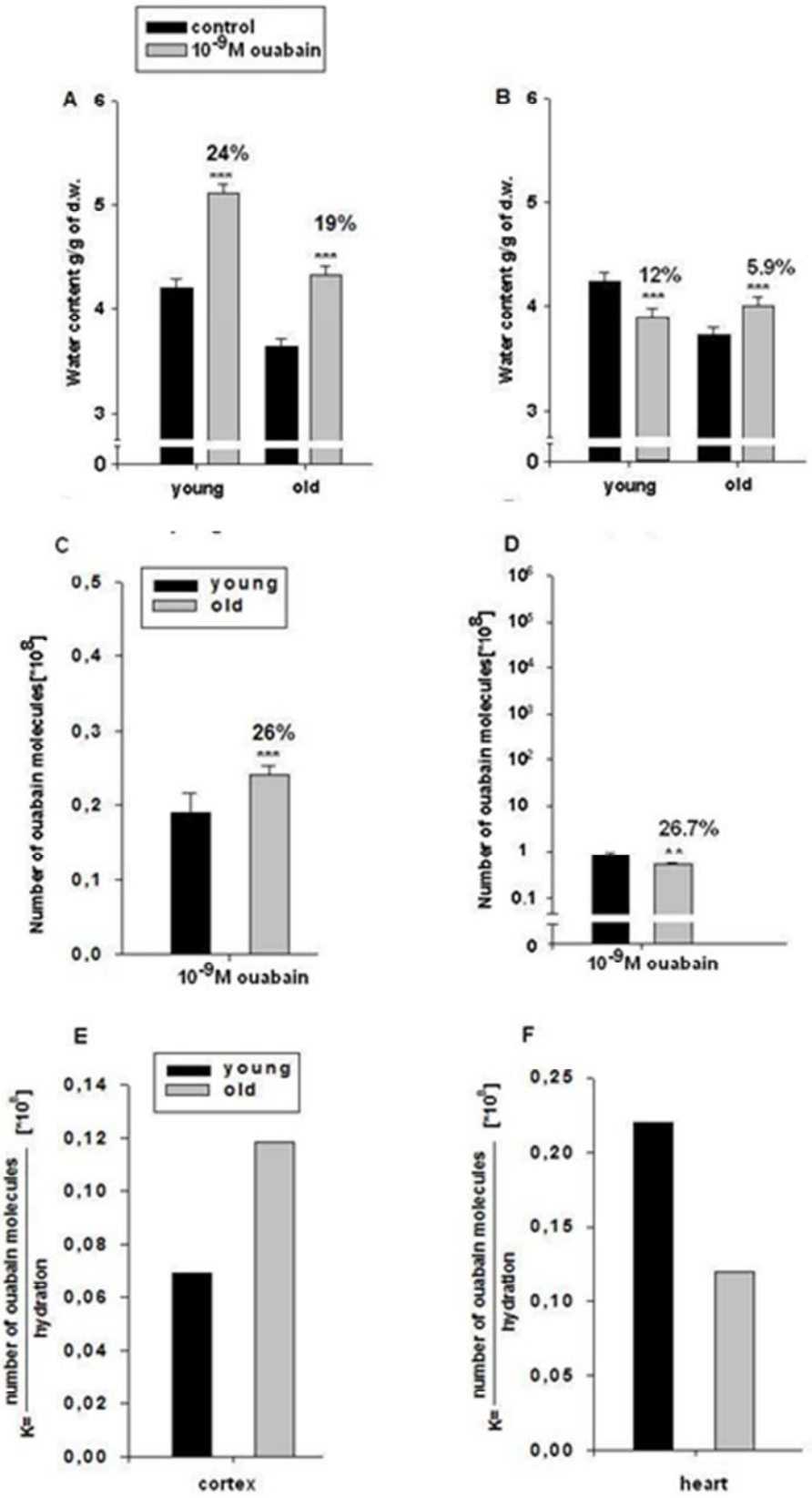


Figure 3. *The age-dependent changing of cell hydration and the number of 10^{-9}M [^{3}H] -ouabain bound molecules with cell membrane in brain cortex tissues (A, C), heart muscle tissues (B, D), and the value of the coefficient (E, F) calculated as the ratio of the number of bound molecules of ouabain to the level of the tissue hydration. The control groups of animals being i/p injected by PS (0.02 ml/g body mass) (black bars). The experimental groups of animals being i/p injected by 10^{-9}M [^{3}H] -ouabain (0.02 ml/g body mass) (grey bars). Each bar represents the mean ±SEM (n=45). The symbols (**) and (***) indicate $p<0.01$ and $p<0.005$, respectively.*

The result of 10^{-9}M [^{3}H]-ouabain injection on brain and heart muscle has shown that in brain cortex tissue it has an age-dependent weakening hydration effect which is accompanied with age-dependent increase of [^{3}H]-ouabain binding with the membrane (Figure 3A, C), while in heart muscle tissue it has dehydration effect on young and hydration on old animals (Figure 3B), but age-dependent reverse effects on hydration have age-dependent decrease of the number of [^{3}H]-ouabain bound molecules with the membrane (Figure 3D). In brain cortex tissues the value of the coefficient (Figure 3E) of young animals is lower than that in the old ones. In heart muscle tissues the value of the coefficient (Figure 3F) of young animals is higher than that in the old ones.

It is known that the source of energy for the Na^+/Ca^{2+} exchanger is a Na^+ gradient on the membrane, decrease of which also activates the RNa^+/Ca^{2+} exchange in cells [10]. To evaluate the sole role of Na^+ gradient decrease, which activates the RNa^+/Ca^{2+} exchanger, (without involvement of cAMP system) in the next series of experiments (*in vitro*) we study the effect of low Na^+ (50%) contents PS on age-dependent hydration and $^{45}Ca^{2+}$ uptake in brain cortex and heart muscle tissues.

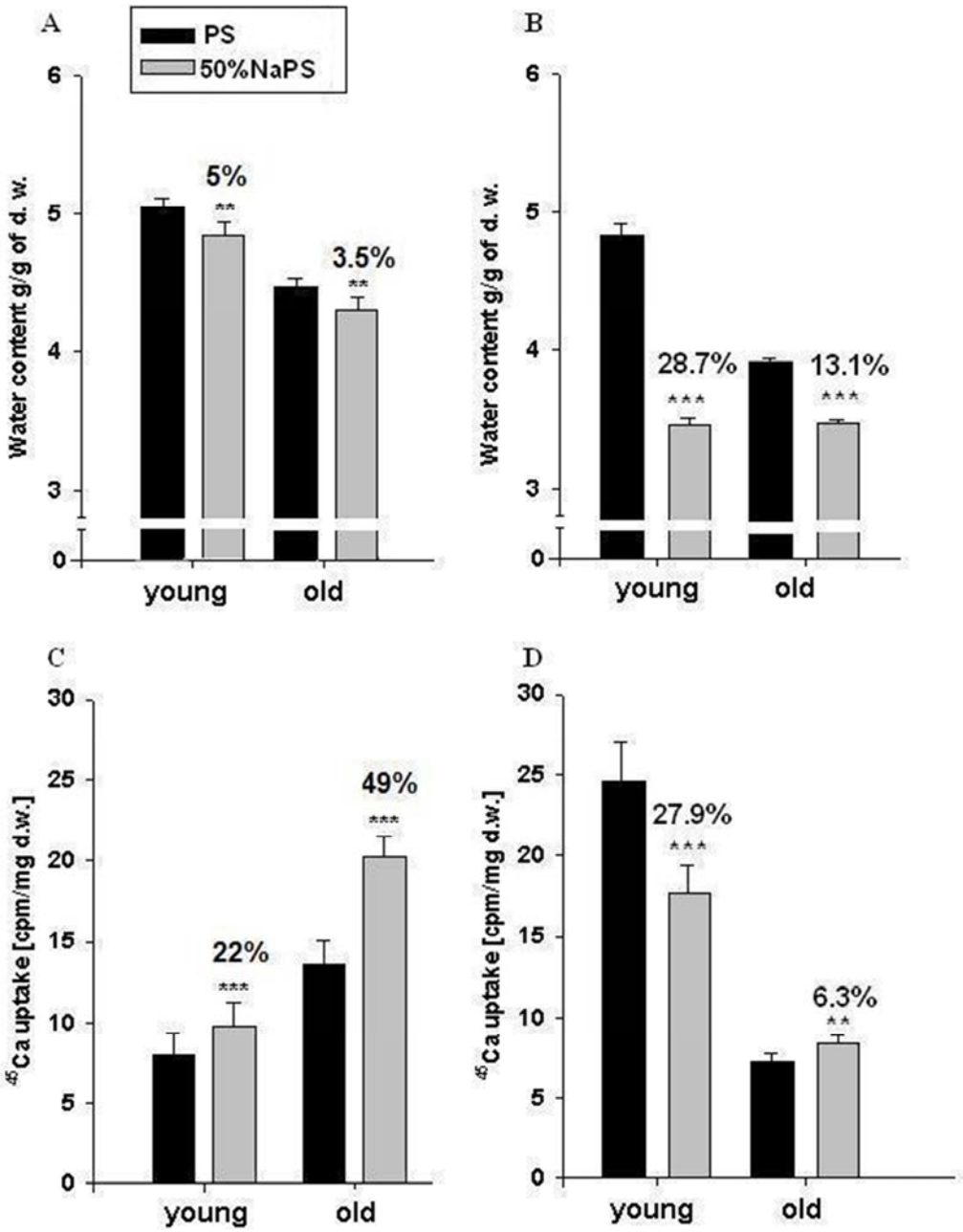


Figure 4. *The age-dependent changing of cell hydration and the $^{45}Ca^{2+}$ uptake in brain cortex (A, C) and heart muscle tissues (B, D) after 30min incubation in 10ml PS (black bars) or in 50%Na containing PS (grey bars). Each bar represents the mean ±SEM (n=45). The symbols (**) and (***) indicate p<0.01 and p<0.005, respectively.*

As can be seen in Figure 4 (A, B) incubation in 50%Na PS leads to age-dependent weakening tissue dehydration in brain cortex and heart muscle tissues. However, in brain cortex tissues the incubation in 50%Na PS brings to age-dependent

increase of $^{45}Ca^{2+}$ uptake (Figure 4C), while in heart muscle tissues it has an age-dependent suppressing effect on $^{45}Ca^{2+}$ uptake (Figure 4D).

To estimate the role of Na^+/K^+ pump on brain cortex and heart muscle tissue hydration and on $^{45}Ca^{2+}$ uptake, the protocols of the previous experiments are repeated by adding the 10-4M ouabain in both types of PS.

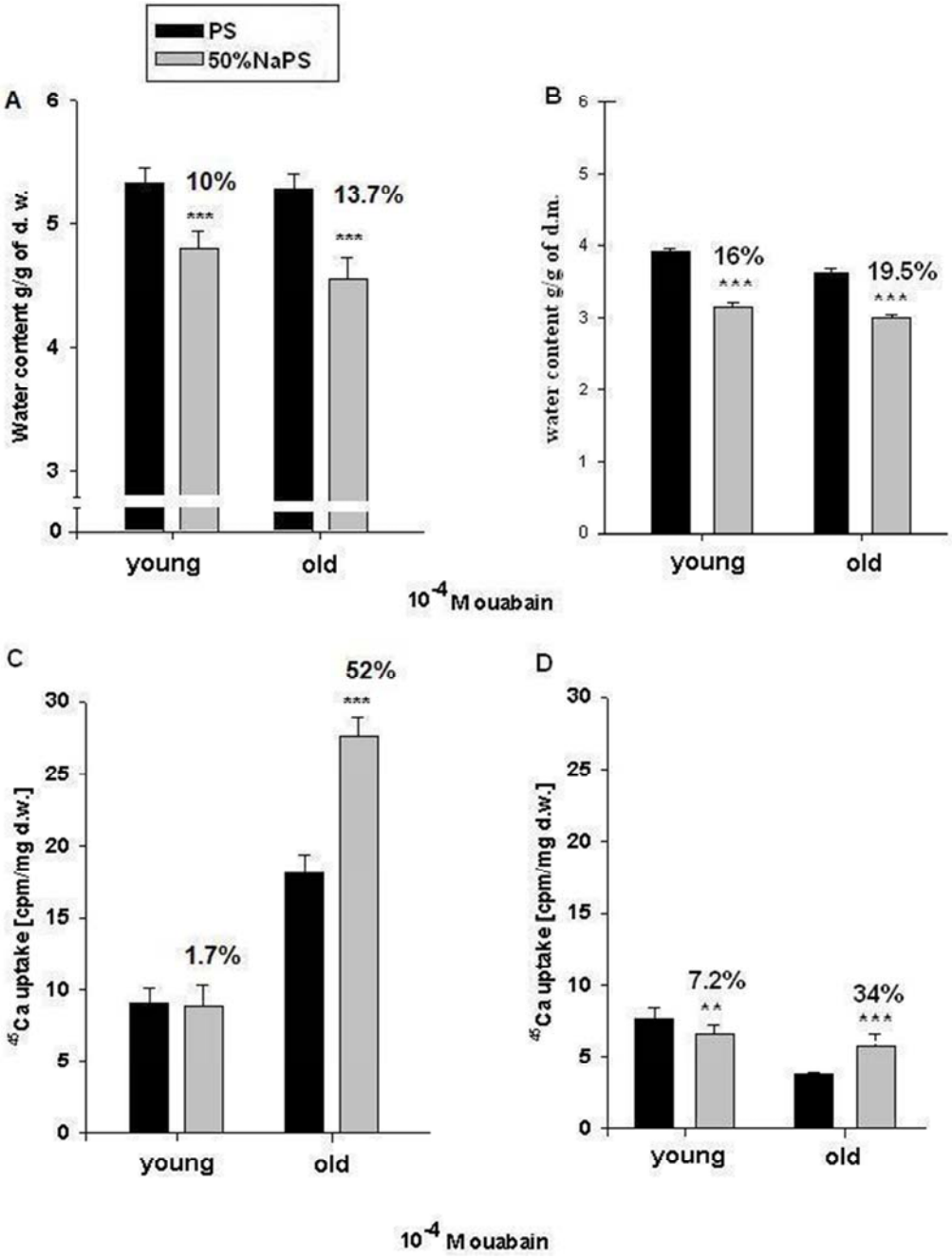


Figure 5. *The age-dependent changing of cell hydration and the $^{45}Ca^{2+}$ uptake in brain cortex (A, C) and heart muscle tissues (B, D) after 30min incubation in 10ml PS (black bars) or in 50%Na containing PS (grey bars) in which were added the 10^{-4}M ouabain. Each bar represents the mean ±SEM (n=45). The symbols (**), (***) indicate p<0.01 and p<0.005, respectively.*

The data presented in Figure 5 (A, C), indicate that the 10-4M ouabain has age-dependent dehydration effect on brain cortex which is accompanied with age-dependent increase of $^{45}Ca^{2+}$ uptake. In heart muscle tissues the incubation in 50%NaPS leads to dehydration in both age groups of rats, which is accompanied with decrease of $^{45}Ca^{2+}$ uptake in young animals and increase in the old ones (Figure 5 B, D).

To find out the differences between activation of RNa^+/Ca^{2+} exchange by decreasing Na^+ gradient on membrane and by cAMP-induced decreasing [Ca]i., in the next experiments the effect of 50% NaPS on age-dependent brain cortex and heart muscle tissue hydration and $^{45}Ca^{2+}$ uptake with 10^{-9}M ouabain are studied.

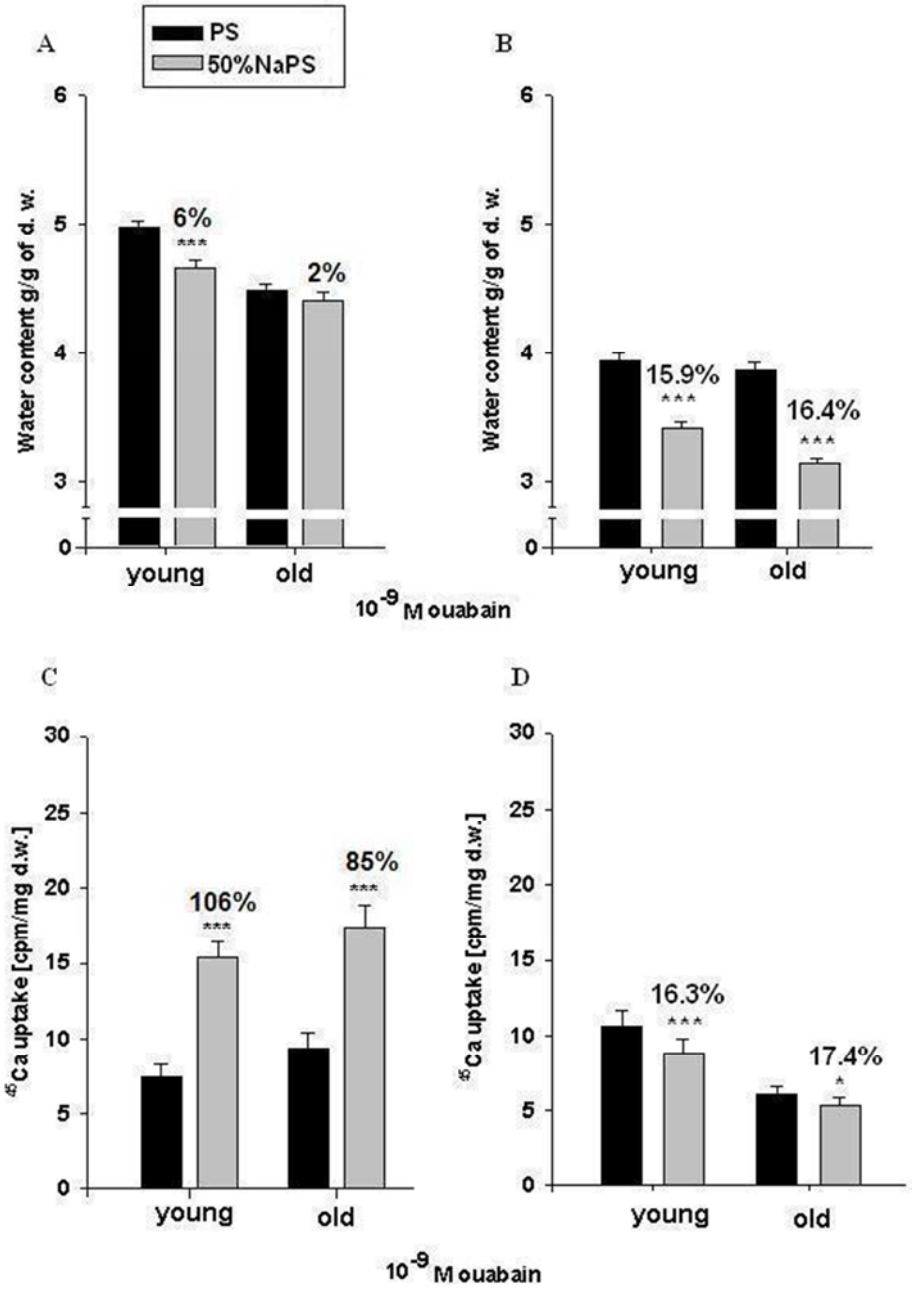


Figure 6. *The age-dependent changing of cell hydration and the $^{45}Ca^{2+}$ uptake in brain cortex (A, C) and heart muscle tissues (B, D) after 30min incubation in 10ml PS (black bars) or in 50%Na containing PS (grey bars) in which were added the 10^{-9}M ouabain. Each bar represents the mean ±SEM (n=45). The symbols (*), (***) indicate $p<0.05$ and $p<0.005$, respectively.*

As can be seen in Figure 6 (A, C) the 10^{-9}M ouabain brings in brain tissue to age-dependent significant dehydration effect in PS groups of animals, which is accompanied with age-dependent increase of $^{45}Ca^{2+}$ uptake, which is enhaced in 50%NaPS group of animals by age-dependent weakening maner, which is accompanied by age-dependent increasing of $^{45}Ca^{2+}$ uptake in all tissues in both groups of animals. However, in heart muscle tissues (Figure 6B, D) the 10^{-9}M ouabain has no significant effect on age-dependent hydration in PS groups of rats but has age-dependent suppression effect on $^{45}Ca^{2+}$ uptake. While in 50% NaPS groups of animals the 10^{-9}M ouabain has age-dependent dehydration effect on heart muscle tissues, which is accompanied with age-dependent suppression of $^{45}Ca^{2+}$ uptake (Figure 6D).

As was noted above, the cell hydration leads to the activation of metabolic processes of cells [1, 5]. Therefore, it is suggested that when the animals drink distilled water (DW) instead of tap water, it will stimulate the metabolic activity of cells.

Thus, in the next experiments the experimental animals (young and old) drinking DW for 10 days and after this they are i/p injected with PS. The results of these experiments are compared with control groups of animals drinking the tap water and they also are injected with PS.

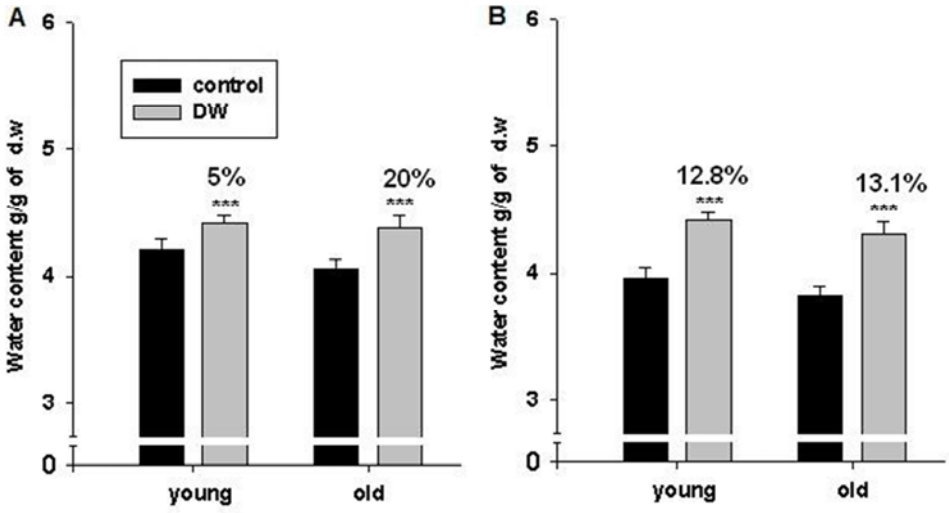


Figure 7. *The age-dependent changing of cell hydration in brain cortex (A) and heart muscle tissues (B) after drinking DW for 10 days. After 10 days all animals being i/p injected by PS (0.02 ml/g body mass). Control bars indicate the data of animals drinking the tap water. DW bars indicate the data of animals drinking the distilled water. Each bar represents the mean ±SEM (n=45). The symbol (***) indicates p<0.005.*

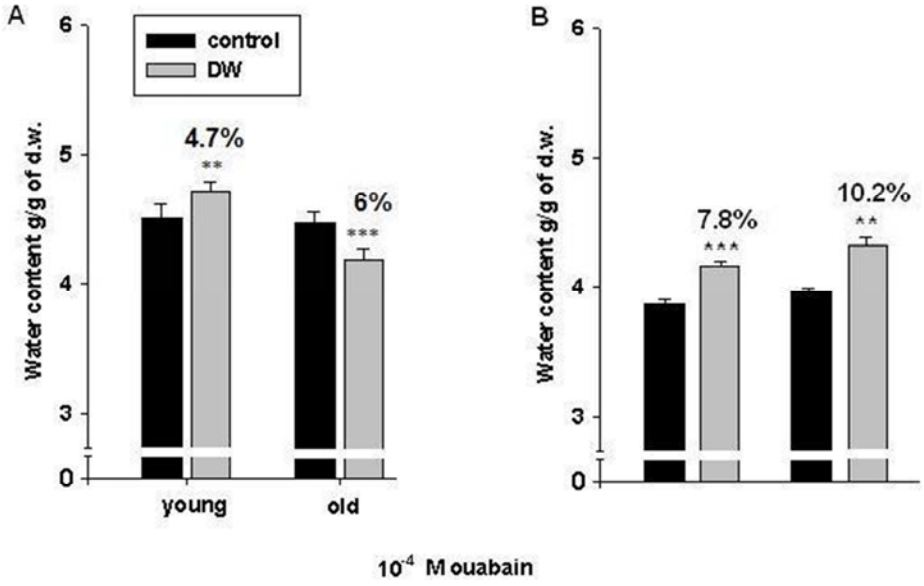
As can be seen in Figure 7 (A, B) in brain cortex and heart muscle tissues the overhydration is observed in two age groups of animals.

It is interesting to note that age-dependency of brain and heart muscle tissue dehydrations in control animals (black bars) becomes minimum in animals drinking DW (grey bars).

To evaluate the role of Na^+/K^+ pump in generation of DW-induced hydration in brain cortex and heart muscle tissues in the next experiments the control groups of animals and animals drinking DW are injected by 10^{-4}M [^{3}H]-ouabain.

As Figure 8 shows there can be noticed significant hydrations in brain cortex (A) and heart muscle tissues (B) in young animals, which are injected by 10^{-4}M [^{3}H]-ouabain. In brain cortex tissues of old rats 10^{-4}M [^{3}H]-ouabain injection leads to dehydration, while in heart muscle tissues it brings to hydration. As it can be seen in Figure 8 (A) in spite of the fact that in old animals (drinking DW and which are injected by 10^{-4}M [^{3}H]-ouabain) in brain cortex tissues there can be noticed more significant dehydration than that in the young ones and in heart muscle the opposite effect is observed (Figure 8B). The number of bound 10^{-4}M [^{3}H]-ouabain molecules with membrane in tissues of these two age groups of animals (Figure 8C, D) shows no difference between each other. In brain cortex tissues the value of the coefficient (Figure 8E) of young animals is lower than that in the old ones. In heart muscle tissues the value of the coefficient (Figure 8F) of young animals is higher than that in the old ones.

To estimate the role of cAMP-dependent RNa^+/Ca^{2+} exchange in age-dependent changes of hydration in brain cortex and heart muscle tissues the comparative study on age-dependency tissue hydrations after i/p injection with 10^{-9}M [^{3}H]-ouabain in control (drinking tap water) and animals drinking DW is performed.



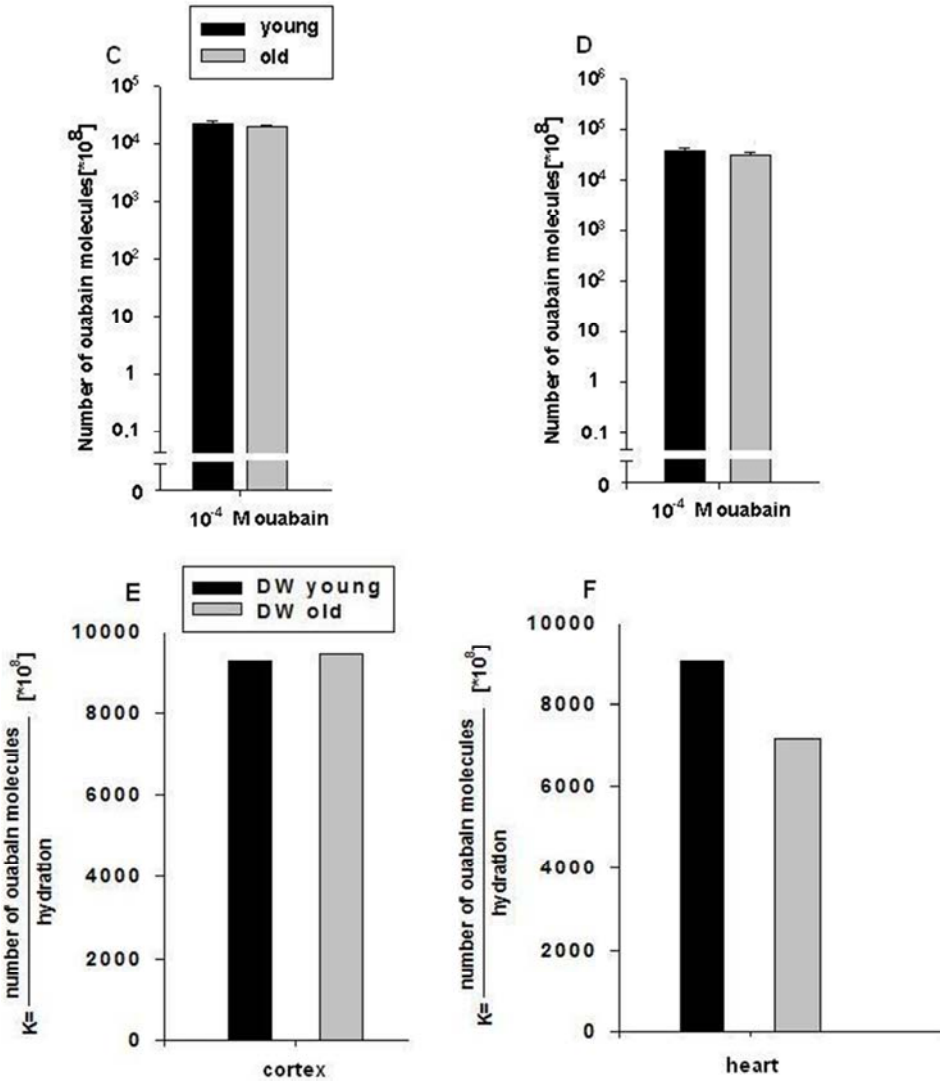
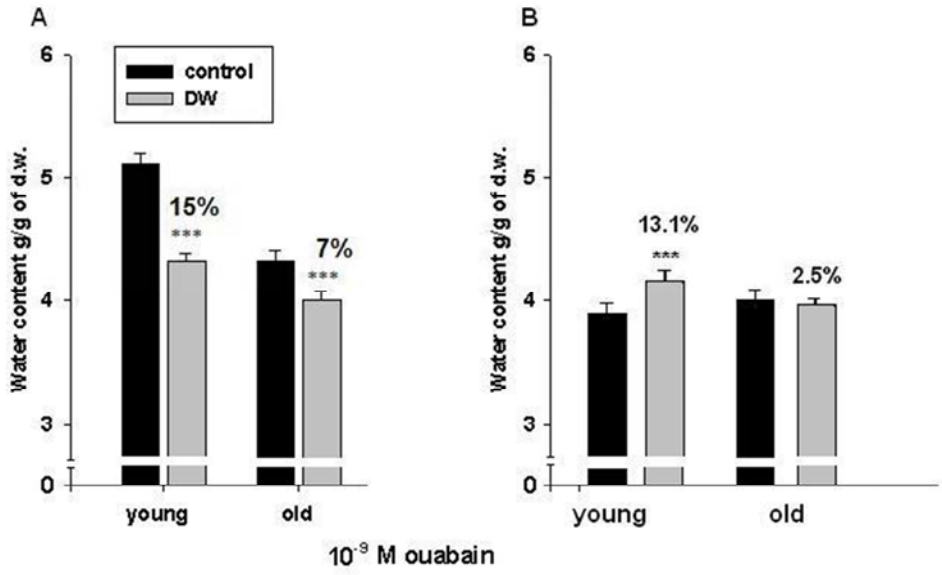


Figure 8. *The age-dependent changing of cell hydration and the number of 10^{-4}M [^{3}H] -ouabain binding molecules with cell membrane in brain cortex (A, C) and heart muscle tissues (B, D). After 10 days all animals being i/p injected by 10^{-4}M [^{3}H] -ouabain (0.02 ml/g body mass). Control bars indicate the data of animals drinking the tap water. DW bars indicate the data of animals drinking distilled water. The value of the coefficient (E, F) calculated as the ratio of the number of bound molecules of ouabain to the level of tissues hydration. Each bar represents the mean ±SEM (n=45). The symbols (**) and (***) indicate p<0.01 and p<0.005, respectively.*



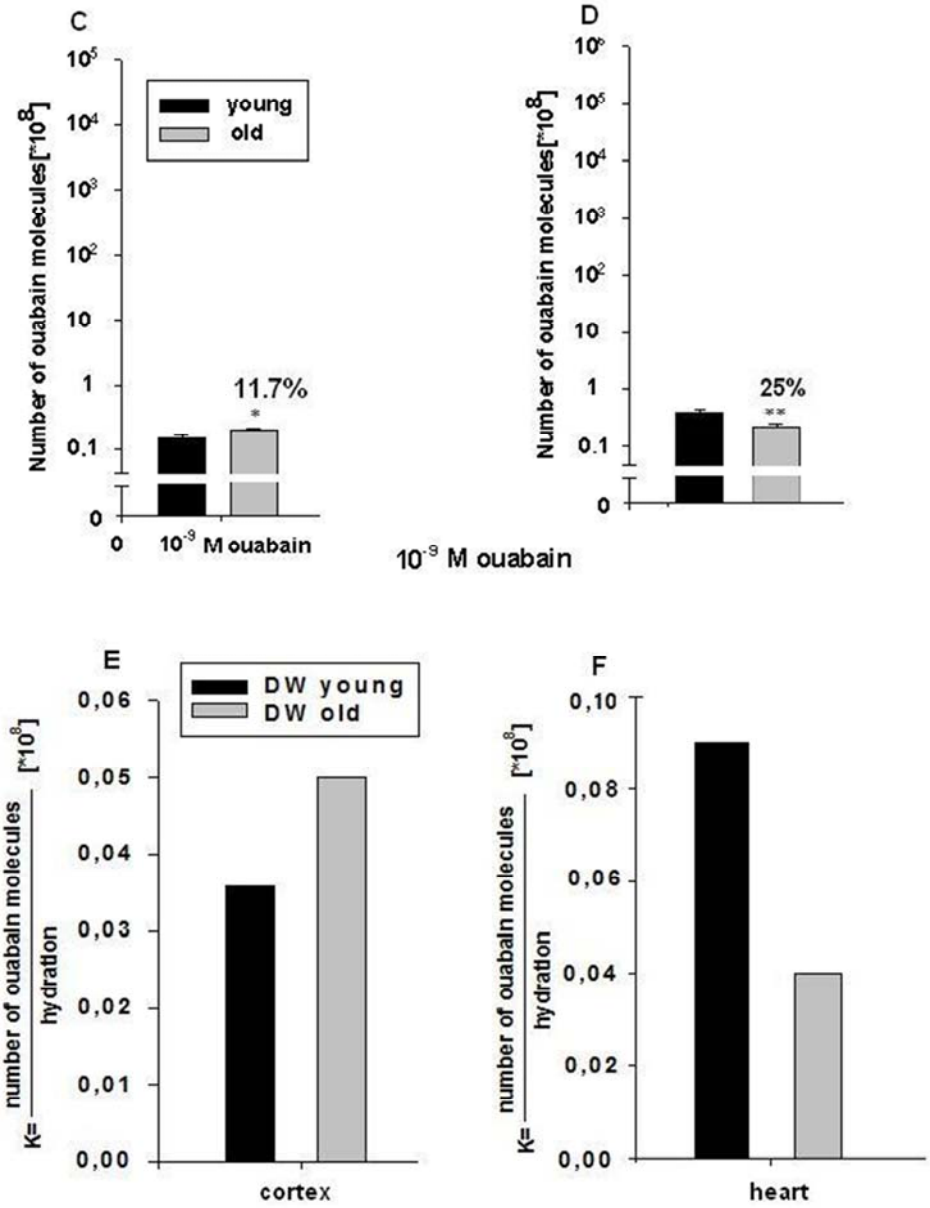


Figure 9. *The age-dependent changes of cell hydration and the number of 10^{-9}M [^{3}H] -ouabain binding molecules with cell membrane in brain cortex (A, C) and heart muscle tissues (B, D). After 10 days all animals being i/p injected with 10^{-9}M [^{3}H] -ouabain (0.02 ml/g body mass). Control bars indicate the data of animals drinking the tap water. DW bars show the data of animals drinking the distilled water. The value of the coefficient (E, F) calculated as the ratio of the number of bound molecules of ouabain to the level of tissue hydration. Each bar represents the mean ±SEM (n=45). The symbols (*), (**) and (***) indicate p< 0.05, p<0.01 and p<0.005, respectively.*

As it is shown in Figure 9 (A) there are significant dehydration effects in brain cortex tissues in two groups of animals after injection with 10^{-9}M [^{3}H]-ouabain. In heart muscle tissues of young animals after injection with 10^{-9}M [^{3}H]-ouabain the hydration level is increased compared to control, while in the old ones it's decreased.

In spite of the fact that in brain cortex tissues (Figure 9A) of old animals (DW group) more significant dehydration effect is observed than that in the young ones (DW group), the number of bound 10^{-9}M [^{3}H]-ouabain molecules with membrane in brain cortex tissues of old animals is higher than that in the young ones (Figure 9C). In heart muscle tissues of old (DW group) animals the level of hydration is less than that in the young (DW group) ones (Figure 9B) and such result is correlated with the number of bound molecules of 10^{-9}M [^{3}H]-ouabain (Figure 9D).

In brain cortex tissues the value of the coefficient (Figure 9E) of young animals is lower than that in the old ones. In heart muscle tissues the value of the coefficient (Figure 9F) of young animals is higher than that in the old ones.

As the above presented data indicate aging-induced tissue dehydration is accompanied with increased expression of nM ouabain receptors (Na^+/Ca^{2+} exchangers) in brain and decreased affinity of these receptors because of the increase of $[Ca^{2+}]_i$ in heart. To find out which of these two processes: the over expression of nM ouabain receptors in brain tissues

or decrease these receptors affinity in heart muscle, are more sensitive to aging the dose-dependent binding ouabain molecules with membrane in brain cortex and heart muscle tissues of young and old animals have been studied.

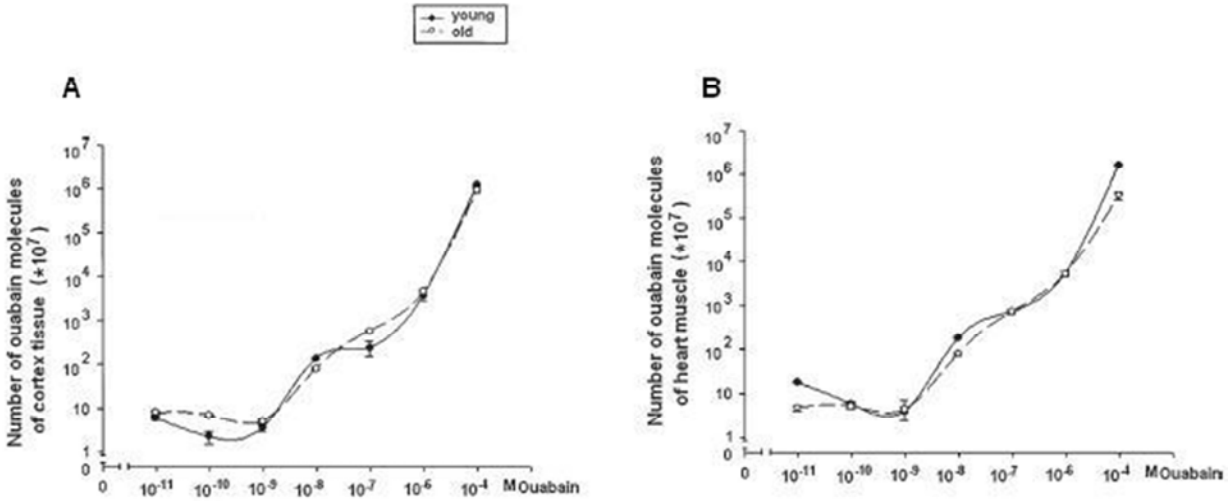


Figure 10. *Dose-dependent [^{3}H] -ouabain binding molecules with cell membrane in brain cortex (A) and heart muscle (B) tissues of young and old rats. Each point of curve represents the mean ±SEM (n=45). Error bars of each point are not detected because being blended with it.*

The results presented in Figure 10 indicate that in brain cortex tissues the age-dependent increase of [^{3}H]-ouabain receptors is more pronounced at 10^{-10}M [^{3}H]-ouabain, while decrease of the affinity of these receptors in heart muscle tissues has more pronounced effect at 10^{-11}M [^{3}H]-ouabain. These data indicate that the decrease of ouabain receptors' affinity precedes to the increased expression of ouabain receptors in brain cortex.

4. Discussion

Our previous study has shown that in living cells the metabolic driving of water efflux from the cells is a key regulating the cell membrane excitability, which is realized by direct inactivation of inward ionic currents, such as Na^+ and Ca^{2+} currents, through the membrane and surface-dependent decrease of functional active ionic channels in the membrane [1, 3]. The metabolic driving of water efflux from the cell is due to electrogenic ion transporting mechanisms in cell membrane such as: a) Na^+/K^+ pump and Na^+/Ca^{2+} exchange; b) the release of water molecules to cytoplasm during the intracellular oxidative process; c) contraction of myosin-like proteins in cytoskeleton and intracellular filaments in neurons, while in myocytes by contractility of myosin.

It is known that in brain cortex and heart muscle tissues the Na^+/K^+ pump has a key role in generation of water efflux from the cell, which is realized by its electrogenic properties, higher ATP-utilizing mechanism and activation of endogen water molecule formation in the process of oxidative phosphorylation. By these mechanism can be explained the age-dependent decrease of hydration in brain cortex tissues (Figure 1A, black colomn). The data on dehydration of brain cortex tissue at cooling can be explained by suppression of endogen water production as well as by activation of RNa^+/Ca^{2+} exchange in cold medium. The age-dependent increase of tissue dehydration can be explained by age-dependent decrease of mitochondrial function generating endogen water (Figure 1A). The hydration in heart muscle tissues depends on both activation of RNa^+/Ca^{2+} exchange and myosin contractility, so after incubation in cold PS the tissue dehydration in heart muscle of young animals is a result of increasing $[Ca^{2+}]_i$, due to the activation of RNa^+/Ca^{2+} exchange and myosin contractility. While in old rats, where both these processes are suppressed, because of initial high concentration of $[Ca^{2+}]_i$ and low concentration of intracellular cAMP in cells [24], the impairments of water efflux lead to increase of hydration (increase the osmotic uptake of water) (Figure 1B).

Our previous study has shown that the Na^+/K^+ pump inactivation is accompanied with the increase of intracellular cAMP contents [2], which by activation of Ca^{2+} pump in membrane of ER [*25*] stimulates the mitochondrial function and releases water molecules to cytoplasm [26]. Therefore, the hydration of cortex tissues, which has age-dependent increasing character after i/p injection with 10^{-4}M [^{3}H] -ouabain (Figure 2A) can be explained by the following facts: the direct inactivation of Na^+/K^+ pump brings to osmotic uptake of water, and from the other sides by the increase of $[Na^+]_i$ which leads to the activation of cAMP-dependent RNa^+/Ca^{2+} exchange having direct dehydration effect on brain tissues, and indirect - the activation of endogens water molecules formation [27]. As in old animals the RNa^+/Ca^{2+} exchange is in suppressing state because of high $[Ca^{2+}]_i$, its dehydration effect is also suppressed. Therefore, in brain cortex tissues of old animals the hydration effect is more pronounced than in the young ones. It is interesting to note that, in spite of the fact that 10^{-4}M [^{3}H] -ouabain in old animals' brain cortex leads to more pronounced hydration effect than that in the young ones, the number of ouabain binding molecules with cell membrane is decreased in the old animals (Figure 2C). In our earlier experiments have shown

that there is a positive correlation between membrane surface and the number of ouabain binding sides in the membrane [1], while the affinity of these receptors to ouabain decreases by the increased $[Ca^{2+}]_i$ [28]. Therefore, the decreasing of the number of 10^{-4}M [^{3}H] -ouabain binding molecules with membrane of cells in brain cortex tissues of old animals compared with that of young ones can be explained by initial high $[Ca^{2+}]_i$ in old animals' cells [29].

As is noted above, heart muscle tissue hydration depends on contractility of myosin, which is highly sensitive to $[Ca^{2+}]_i$. The Na^+/K^+ pump inactivation-induced heart muscle tissue dehydration in young animals is due to the activation of RNa^+/Ca^{2+} exchange leading to contraction by generation of osmotic gradient on membrane as well as by $[Ca^{2+}]_i$-induced contraction of myosin, while the muscle hydration in the old animals is due to the increase of $[Ca^{2+}]_i$ (Figure 2B), which by activation of Ca-Calmoduline-NO pathways brings to cGMP formations having activation effect on FNa^+/Ca^{2+} exchange [25, 30], which has hydration effect on muscle [31]. This suggestion is supporting the data on age-dependent decrease of the number of ouabain binding molecules with cell membrane (Figure 2D).

Since the energy source for Na^+/Ca^{2+} exchange is a different between ionic gradients of these ions on membrane, the reversion of Na^+/Ca^{2+} exchange can occur by the decrease of Na^+ (or the increase of Ca^{2+}) gradients on membrane [10]. Traditionally, the activation of RNa^+/Ca^{2+} exchange is the result of pump inhibition explained by the increase of $[Na^+]_i$ [11]. However, by our previous study we have shown that the RNa^+/Ca^{2+} exchange can be activated by extremely low concentration of biological substances, including nM ouabain, which have no effect on Na^+/K^+ pump activity and are unabale to activate the ionic channels in the membrane [12, 13]. The activation of nM ouabain induced cAMP-dependent RNa^+/Ca^{2+} exchange through activation of Ca^{2+} pump in membrane of ER stimulates metachondrial function and releases water molecules to cytoplasm, which brings to the increase of tissue hydration in brain cortex tissues (Figure 3A). The age-dependent weakening of nM ouabain-induced hydration can be considered as impairments of mitochondrial function in brain of old rats. The data on age-dependent increase of the number of 10^{-9}M [^{3}H] –ouabain binding molecules with membrane seems extremely interesting, it indicates that in response to age-dependent weakening of RNa^+/Ca^{2+} exchange in brain cortex tissues there is an increased expression of Na^+/Ca^{2+} exchanger in the membrane (Figure 3C, E).

The 10^{-9}M [^{3}H]-ouabain in heart muscle tissues has age-dependent reversal effect, which is accompanied with age-dependent decreae of the number of 10^{-9}M [^{3}H]-ouabain binding molecules with the membrane (Figure 3B, D). This can be explained by the activation of RNa^+/Ca^{2+} exchange, which leads to dehydration and $[Ca^{2+}]_i$-induced contraction of muscle in the young animals. While in heart muscle of the old animals because of initial high $[Ca^{2+}]_i$ the hydration is due to Ca-Calmodulin activation-induced increase of cGMP-dependent FNa^+/Ca^{2+} exchange. This suggestion supports the data of ouabain binding with cell membrane where it suppresses in old animals compared with the young ones (Figure 3D).

The data that the activation of RNa^+/Ca^{2+} by decrease of extracellular Na^+ ($[Na^+]_o$) has dehydration effect on brain (Figure 4A), while in case of nM ouabain–induced cAMP-dependent activation of RNa^+/Ca^{2+} exchange has hydration effect on brain (Figure 3A) clearly indicate that the brain cortex tissue hydration upon the effect of 10^{-9}M ouabain is due to formation of cAMP, which activates endogen formation of water molecules by the activation of Ca^{2+} pump in ER membrane, having stimulation effect on mitochondrial function. The age-dependent decrease of tissue dehydration in both PS and 50%NaPS groups of animals, which accompanied with age-dependent increase of $^{45}Ca^{2+}$ uptake can be explained by the increase of Na^+/Ca^{2+} exchanger's expression in brain cortex tissues of old animals (see Figure 3A, C). The same level of dehydration in heart muscle tissues of young and old animals at 50%Na PS indicates that muscles of both ages were in contracted by increase of $[Ca^{2+}]_i$ (Figure 4B, D). But the decrease of $^{45}Ca^{2+}$ uptake in heart muscle of young and the increase in the old ones is due to age-dependent weakening of Na^+/K^+ pump activity, which brings to more activation of RNa^+/Ca^{2+} exchange. These suggestions are supported by the data on age-dependent effects of 50%Na PS on brain and heart muscle tissues where Na^+/K^+ pump is inhibited by 10^{-4}M ouabain (Figure 5). The inhibition of Na^+/K^+ pump by 10^{-4}M ouabain at 50%NaPS (Figure 5A, B) leads to age-dependent increase of brain tissue dehydration, which is accompanied with slight decrease of $^{45}Ca^{2+}$ uptake in tissues of young animals and pronounced increase in the old ones. While in heart muscle the age-dependent same levels of muscle hydration, which is accompanied with decrease of $^{45}Ca^{2+}$ uptake in young and increase in the old animals is also due to age-dependent weakening of Na^+/K^+ pump (Figure 5B, D).

The result of 10^{-9}M ouabain, which activates the cAMP-dependent RNa^+/Ca^{2+} the 50%NaPS brings to age-dependent dehydration of brain tissue and is accompanied with age-dependent increase of RNa^+/Ca^{2+} exchange (Figure 6A, C). The data that 50%NaPS has age-dependent increase of heart muscle tissue dehydration which is accompanied with age-dependent decrease of $^{45}Ca^{2+}$ uptake (Figure 6B, D) indicate that because of high sensitivity of muscle contraction to $[Ca^{2+}]_i$, the activation of RNa^+/Ca^{2+} brings to contraction of muscle, which by negative feedback inhibits the RNa^+/Ca^{2+} exchange.

In experiments when the animals are supplied by DW instead of tap water (Figure 7), the age-dependent increase of hydration in brain cortex and heart muscle tissues probably can be explained by the increase of metabolic water efflux leading to the increase of osmotic water uptake, which has age-dependent increasing character because of age-dependent weakening metabolic activity of cells (Figure 7). This suggestion supports the data on age-dependent decrease of 10^{-4}M [^{3}H]-ouabain binding with membrane, which is due to the increase of $[Ca^{2+}]_i$ (Figure 8).

Our previous data have shown that cell hydration leads to increasing inhibitory effect on activity of Na^+/K^+ pump because of the increase of the pump units in the membrane [1]. From this point of view the hydration effect of DW on brain cortex tissues of young animals can be considered a result of increasing the inhibitory effect of 10^{-4}M [^{3}H]-ouabain on Na^+/K^+ pump, while in old animals where Na^+/K^+ pump is in inactive state the cell dehydration can be explained by the activation of cAMP-dependent RNa^+/Ca^{2+} exchange as a result of surface-dependent increase of functional active G proteins in the membrane [1], which are accompanied with age-dependent decrease of the number of ouabain binding molecules with membrane (Figure 8A, C). In heart muscle where hydration depends on muscle contractility the age-dependent increase of muscle hydration in DW group can be considered as a dilution of $[Ca^{2+}]_i$ (Figure 8B), which is accompanied with age-dependent decrease of the number of ouabain binding molecules with membrane (Figure 8D), and which brings to reactivation of Na^+/K^+ pump leading to stimulation of endogen water formation in cytoplasm of brain cortex tissue, while in heart muscle such dilution of $[Ca^{2+}]_i$ leads to relaxation of muscle, which in old rats is more pronounced because of its initial high $[Ca^{2+}]_i$.

The data that 10^{-9}M [^{3}H]-ouabain-induced age-dependent weakening of tissue hydration can be explained by cAMP activation-induced stimulation of mitochondrial function leading to endogen water formation, which is accompanied with age-dependent increase of the number of ouabain binding molecules, as it is mentioned above (Figure 3), is due to overexpression of RNa^+/Ca^{2+} exchanger in brain tissues of old animals. In DW group of animals there were age-dependent dehydration in brain tissues, which accompanied with suppression of expression of RNa^+/Ca^{2+} exchager indicates on inactivation of the metabolic effect of cAMP-dependent RNa^+/Ca^{2+}exchange, which is also by age-dependent overexpression of Na^+/Ca^{2+} exchanger the mechanism of which needs more detailed investigation. The result that 10^{-9}M [^{3}H]-ouabain, which has age-dependent hydration effect in heart muscle tissues accompanied with age-dependent decrease of the number of ouabain binding molecules with membrane in control animals indicates on age-dependent increase of $[Ca^{2+}]_i$ leading to the activation of FNa^+/Ca^{2+} exchange in old animals. The DW-induced hydration in heart muscle tissue of young animals, which is accompanied with decrease of ouabin binding molecules indicates on increase of $[Ca^{2+}]_{I,}$ which stimulates endogen water formation while in old animals because of impariement of mitochondrial function the water formation is suppressed (Figure 9B, D).

Our previous study has shown that age-dependent dehydration of brain cortex and heart muscle tissues is due to Na^+/K^+ pump impairments-induced activation of RNa^+/Ca^{2+} exchange [28]. The data on dose-dependent ouabain binding in brain and heart muscle tissues indicate that there is age-dependent decrease of high affinity ouabain receptors in heart muscle, while in brain cortex tissues the number of these receptors is increased (Figure 10). The fact that age-dependent decrease of ouabain binding molecules in heart muscle tissues appear at 10^{-11}M [^{3}H]-ouabain concentration, while in brain cortex tissues the increase of ouabain binding takes place at 10^{-10}M [^{3}H]-ouabain clearly indicates on higher sensitivity to aging-induced decrease of ouabin receptors' affinity in heart muscle than to overexpression of ouabain receptors in cell membrane of brain tissue. Considering the data that the high affinity ouabain receptors have RNa^+/Ca^{2+} exchanger's function, which increasing by age-dependent impairments of Na^+/K^+ pump allow us to suggest the age-dependent dysfunction of heart muscle contractility because of high sensitivity to $[Ca^{2+}]_i$ serves as a primary mechanism for brain tissue dehydration due to depression of blood circulation in brain.

Thus, the obtained data in the present work brings us to the following conclusions:

1. The data that the age-dependent suppression of metabolic water efflux from the cells brings to age-dependent increase of brain cortex tissue dehydration, while in heart muscle of young animals it has dehydration and in old animals has hydration effect indicate that the metabolic regulation of brain and heart muscle tissues has different nature.
2. The age-dependent dysfunction of Na^+/K^+ pump, which brings to the activation of RNa^+/Ca^{2+} exchange leads to age-dependent increase of brain tissue hydration, while in heart muscle of young animals it has dehydration and in the old ones hydration effects. These data indicate that the Na^+/K^+ pump has a key role in controlling hydration in brain tissues, while in heart muscle tissue the hydration solely depends on RNa^+/Ca^{2+} exchange regulating level of $[Ca^{2+}]$i, which in young animals leads to contractility of myocytes and in heart muscle of old rats because of high $[Ca^{2+}]$i leads to hydration through the activation of Ca-Calmoduline-NO-cGMP production, which brings to FNa^+/Ca^{2+} exchange.
3. The activation of RNa^+/Ca^{2+} exchange by 10^{-9}M [^{3}H] - ouabain has age-dependent weakening hydration effect on brain cortex tissues, which is accompanied with age-dependent overexpression of Na^+/Ca^{2+} in cell membrane, while in heart muscle of young animals by $[Ca^{2+}]$i–dependent contraction-induced dehydration and in the old ones due to the activation of Ca-calmoduline-NO-cGMP production, which brings to FNa^+/Ca^{2+} exchange-induced muscle hydration, which is accompanied with age-dependent decrease of affinity of nM receptors in the membrane.
4. The activation of RNa^+/Ca^{2+} exchange by 50%NaPS incubation in ouabain-free, 10-4M ouabain and 10-9M ouabain solutions leads to age-dependent weakening dehydration in brain tissue, which is accompanied with age-dependent elevation of $^{45}Ca^{2+}$ uptake while in heart muscle tissues of both age groups is noticed equal dehydration effect accompanied with the decrease of $^{45}Ca^{2+}$ uptake in heart muscle of young rats and by increase of $^{45}Ca^{2+}$ uptake in the old ones compared with normal PS.
5. In rats drinking DW the brain cortex and heart muscle tissues have age-dependent elevation of hydration compared

with rats drinking tap water. While in case of inactivation of Na^+/K^+ pump by 10^{-4}M [^{3}H]-ouabain, in brain tissues of young rats hydration effect is observed and in the old ones it brings to dehydration, which is accompanied with decrease of the number of ouabain binding molecules in the membrane. In heart muscle tissue the Na^+/K^+ pump inactivation leads to age-dependent elevation of hydration, which is accompanied with age-dependent decreasing affinity of ouabain receptors in the membrane.

6. In rats drinking DW the activation of cAMP-dependent RNa^+/Ca^{2+} exchange by nM ouabain leads to age-dependent dehydration in brain cortex tissue, which is accompanied with the increase of nM ouabain receptors' expression in the membrane, while in heart muscle the age-dependent dehydration is accompanied with age-dependent decrease of the number of nM ouabain receptors in the membrane.
7. The age-dependent impairment of Na^+/K^+ pump leads to the activation of RNa^+/Ca^{2+} exchange in brain cortex tissue, which brings to cells dehydration generating water efflux from the cells. In heart muscle tissues the RNa^+/Ca^{2+} exchange besides this function through $[Ca^{2+}]$i-dependent activation of myosin through positive feedback leads to further increase of $[Ca^{2+}]$i, which brings to hydration in heart of young animals (contraction) and in the old rats through the activation of Ca-calmoduline-NO-cGMP production, which brings to FNa^+/Ca^{2+} exchange-induced muscle hydration (relaxation).

Thus, it is suggested that age-dependent dysfunction of Na^+/K^+ pump–induced activation of RNa^+/Ca^{2+} exchange leads to heart muscle contractility because of activation of Ca-calmoduline-NO-cGMP production, which brings to FNa^+/Ca^{2+} exchange induced muscle relaxation and it could serve as a primary mechanism for dysfunction of brain tissues' metabolic control of cell hydration, which leads to overexpression of Na^+/Ca^{2+} exchanger in the membrane.

Conflict of Interest Statement

There are no conflict of interest between the author and suggested reviewers.

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