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Age-Dependent Comparative Study of 4 Hz and 8 Hz EMF Exposure on Heart Muscle Tissue Hydration of Rats

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Abstract

Previously we have shown that 4 Hz and 8 Hz EMF exposures have depressing effect on the thermodynamic activity of water, which decreases peroxide formation. It has also been shown that 4 Hz EMF-treated physiological solution modulates the growth and development of microbes and heart muscle contractility, but 8 Hz EMF has pronounced inhibitory effect on bacterial growth and development. Therefore, in order to elucidate the possible mechanism of 4 Hz and 8 Hz EMF effects on heart muscle function, in the present work the effects of 4 Hz and 8 Hz EMF exposures on heart muscle tissue hydration, the sensitivity of 4 Hz and 8 Hz EMF-induced tissue hydration to 10^{-4} M ouabain (Na^+/K^+ pump inhibition) and 10^{-9} M ouabain (activation of intracellular signaling system) as well as the effects of 4 Hz and 8 Hz EMF exposures on the number of Na^+/K^+ pump units in the membrane of both young and old rats have been studied. The obtained data allow us to suggest that 8 Hz EMF exposure has more pronounced age-dependent modulation effect on tissue hydration of heart muscle than 4 Hz EMF and this effect is sensitive to Na^+/K^+ pump activity and intracellular signaling system.

Keywords

EMF, Tissue Hydration, Heart, Na^+/K^+ Pump, Na^+/Ca^{2+} Exchange

1. Introduction

In literature there are a lot of contradictory data on the biological effect of electromagnetic fields (EMF) on heart function [1] [2] [3] [4]. Our weak knowledge about cellular and molecular mechanisms of EMF effect on heart muscle is the reason of the variability of these data.

Since cell hydration (water content) is a fundamental cellular parameter controlling metabolism, it is predictable that any factor able to change cell hydration can modulate cell metabolism and, conversely, cell metabolism changes will cause variation of cell hydration level [5] [6] [7] [8].

As physicochemical properties of water are sensitive to EMF [9] [10] [11] and cell membrane is highly permeable for water [12] [13], water molecules take the role of a primary messenger for EMF signal transduction from cell bathing medium into cell metabolism.

The Na^+/K^+ pump with a key role in cell volume regulation [14] [15] [16] [17] [18] is determining for the magnetic sensitivity of cell hydration. Our previous work has demonstrated that age-dependent decrease in magnetic sensitivity of heart muscle hydration is clearly expressed, when the Na^+/K^+ pump is in an inhibited state [19].

The Na^+/K^+-ATPase (working molecules of Na^+/K^+ pump) has three catalytic isoforms (α_1, α_2, α_3) in neuronal and muscle membranes [14]. These isoforms have different affinities to cardiac glycoside-ouabain and functional activities: α_1 and α_2 isoforms are involved in transportation of Na^+ and K^+ through membrane, while α_3 has only intracellular signaling function [14] [15]. Previously it has been shown that among these three families of receptors, α_3 isoform is a target for EMF effect [19] [20].

By our previous experiment, performed on snail hearts, it has been shown that 4 Hz EMF-treated physiological solution (PS) modulates the growth and development of microbes and heart muscle contractility [3] [21].

Our previous study on the effect of extremely low frequencies of EMF (ELF EMF) (<10 Hz) on physicochemical properties and hydrogen peroxide (H_2O_2) formation in water and water solution has elucidated that 4 Hz and 8 Hz frequencies depress water molecule dissociation and H_2O_2 formation in PS. It has also been shown that 8 Hz EMF has pronounced inhibitory effect on bacterial growth and development [21] [22] [23].

Thus, the aim of the present work was to perform a comparative study of 4 Hz and 8 Hz EMF exposure effect on heart muscle tissue hydration, 10^{-9} M and 10^{-4} M ouabain binding with cell membrane in young and old rats.

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 45 young (6 weeks old) and 45 old (18 months old) Wistar 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˚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 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) at 10^{-9} M and 10^{-4} M concentrations dissolved in PS were used for tissue incubation.

2.3. Source of EMF Radiation

The background of magnetic field in the area of experimental setup in the laboratory, due to the 60 Hz electricity system, was less than 0.001 mT. The holder of the exposure tube and the coil holder were placed on two neighboring tables to exclude the vibration during the exposure. The room temperature was 23˚C. The exposure set up is presented in Figure 1.

The coil system has the diameter of 154 mm. The system consists of two Helmholtz rings with the distance of 77 mm that generate homogeneous magnetic field. Coils of Helmholtz are formed by two equal ring coils located coaxially and in a parallel way. The distance between ring coils is equal to their radius of 77 mm. The magnetic field created by these coils has high homogeneity. On distance of 0.25 R from the center of an axis, measured field strength differs from computed by formula only by 0.5% ($H = 71.6\ \omega I/R$). Here, the intensity of generated EMF is equal to 2.5 mT at 4 Hz and 8 Hz. The 4Hz and 8Hz exposures are generated by a special rectangular pulsing generator having output amplifier connected to the coil. The instrument used for measurement of magnetic field intensity was a Teslometer W1-8 (Armenian Radiophysical Institute, Yerevan, Armenia). This instrument measures magnetic fields in the range 10^{-3} T to 1.6 T (±5%). The magnetic induction converter was a crystal, type X511-1, 1.5 × 0.2 mm^2 and was fixed on non-magnetized material (PX13-1).

Animals were placed into the box and then in the setup and exposed by EMF for 15 min. After this procedure animals were sharply immobilized and decapitated. The same was done for sham group without EMF radiation.

2.4. Tissue Preparation

It is well known that anesthetics with different chemical and pharmacological

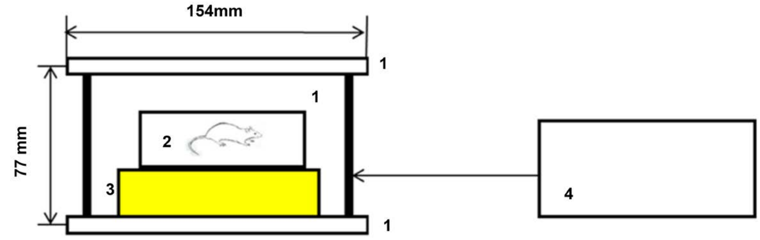

Figure 1. The exposure set up. 1—Helmholtz coil (D = 154 mm, H = 77 mm), 2—Plexiglas Box (134 mm × 105 mm × 55 mm), 3—Wooden table, 4—4 Hz and 8 Hz generator having output amplifier connected to coil.

profiles significantly affect metabolic processes, which play an important role in regulation of cell volume [24] [25]. Therefore, in the present experiments animals were sharply immobilized by freezing method (dipping their noses into liquid nitrogen for 3 - 5 sec) and decapitated. After such a procedure the full absence of somatic reflexes on extra stimuli was recorded.

Experiments were performed on 45 young and 45 old animals. From each group 15 young and 15 old animals were considered as sham animals, while 15 young and 15 old animals were exposed to 4 Hz or to 8 Hz EMF. Six pieces with 50 - 60 mg wet weight (w.w.) per piece were taken from each tested heart muscle. The obtained 90 samples from 15 sham animals were divided into 3 groups: 30 samples were incubated in PS, 30 samples—in PS containing 10^{-4} M [^{3}H]-ouabain and 30 samples—in PS containing 10^{-9} M [^{3}H]-ouabain. The same procedure has been performed on 15 animals exposed to 8 Hz and on 15 animals exposed to 4 Hz EMF. Thus, each column on the figures presents the mean value of the data from 30 samples.

2.5. Definition of Water Content of Heart Tissues

Water content of heart muscle tissues was determined by traditional "tissue drying" method [26]. After measuring the wet weight (w.w.) of heart muscle tissue samples it was 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). For investigation of water content variations and ouabain effects each animal group was divided into 3 subgroups: in the first (sham) subgroup there were animals without any radiation, in the second subgroup the animals were radiated with 8 Hz EMF and in the third subgroup the animals were radiated with 4 Hz EMF.

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

Heart muscle tissue samples were incubated in 10 ml of 10^{-9} M or 10^{-4} M [^{3}H]-ouabain solutions for 30min. Then they were washed three times (10 min-5 min-5 min) in normal PS (ouabain-free) for removing [^{3}H]-ouabain from tissue. After determination of dry weights of samples, they were homogenized in 50 μl of 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 of dry weight by Wallac 1450 liquid scintillation and luminescence counter (Wallac Oy, Turku, Finland).

2.7. Statistical Analysis

Microsoft Excel and Sigma-Plot (Version 8.02A, NY, USA) were used for data analyses. 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

In our previous works cell hydration has been suggested as a primary messenger through whi3ch the biological effects of EMF are realized [11] [17] [27] [28] [29].

As it can be seen in Figure 2(A), Figure 2(B), the level of heart muscle hydration was significantly increased upon the effects of 4 Hz (1.4%-in young; 1.8%-in old) and 8 Hz (5.7%-in young; 4%-in old) EMF exposures as compared to sham group.

The dysfunction of Na^+/K^+ pump, which is a common consequence of ageing, has a key role in metabolic regulation of cell hydration and intracellular Ca homeostasis ($[Ca^{2+}]_i$). The Na^+/K^+-pump, being a high metabolic energy (ATP) utilizing mechanism and working with high intensity in cardiomyocytes, has a great intracellular signaling role in controlling Ca^{2+} sorption properties by intracellular structure as well as in generation of endogenous H_2O in cytoplasm. Therefore, Na^+/K^+-pump could be considered not only as an ion transporting mechanism but also as a powerful intracellular signaling system controlling cell hydration and $[Ca^{2+}]_i$ in myocytes.

To evaluate the role of Na^+/K^+ pump in realization of biological effects of 4Hz and 8Hz EMF on heart muscle hydration, after EMF exposure the heart muscle samples of animals are incubated in 10^{-4} M ouabain solution, which has inhibitory effect on Na^+/K^+ pump activity [30].

As it can be seen in Figure 3(A), Figure 3(C), in sham groups heart muscle sample incubation in 10^{-4} M ouabain brings to tissue hydration (11%) in young animals and dehydration (2.1%) in old ones (sham groups). In experimental groups (after 4 Hz EMF radiation) sample incubation in 10^{-4} M ouabain containing PS leads to tissue hydration (5.4%) in old animals, while in young animals muscle

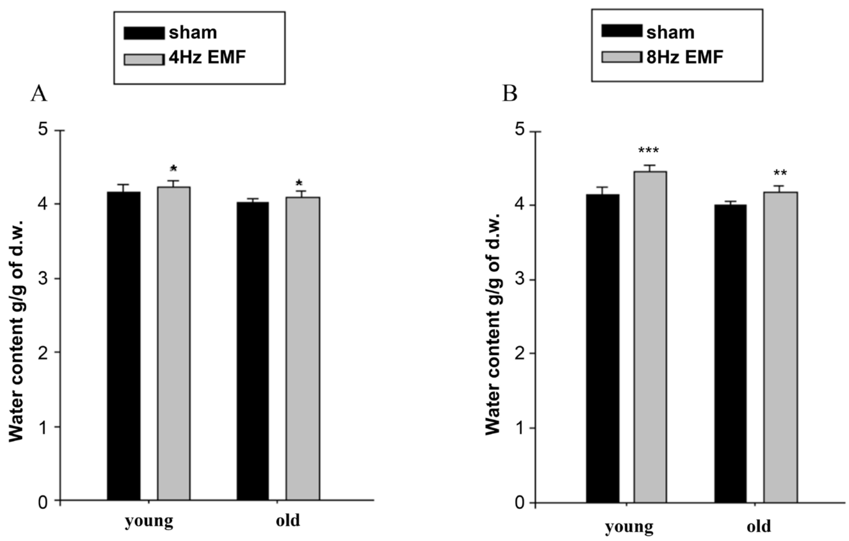

Figure 2. 4 Hz (A) and 8 Hz EMF (B) exposures on hydration of heart muscle tissues of young and old animals. Each bar on figure represents the mean ± SEM of 30 samples. The symbols (*), (**) and (***) indicate p < 0.05, p < 0.01 and p < 0.001, respectively.

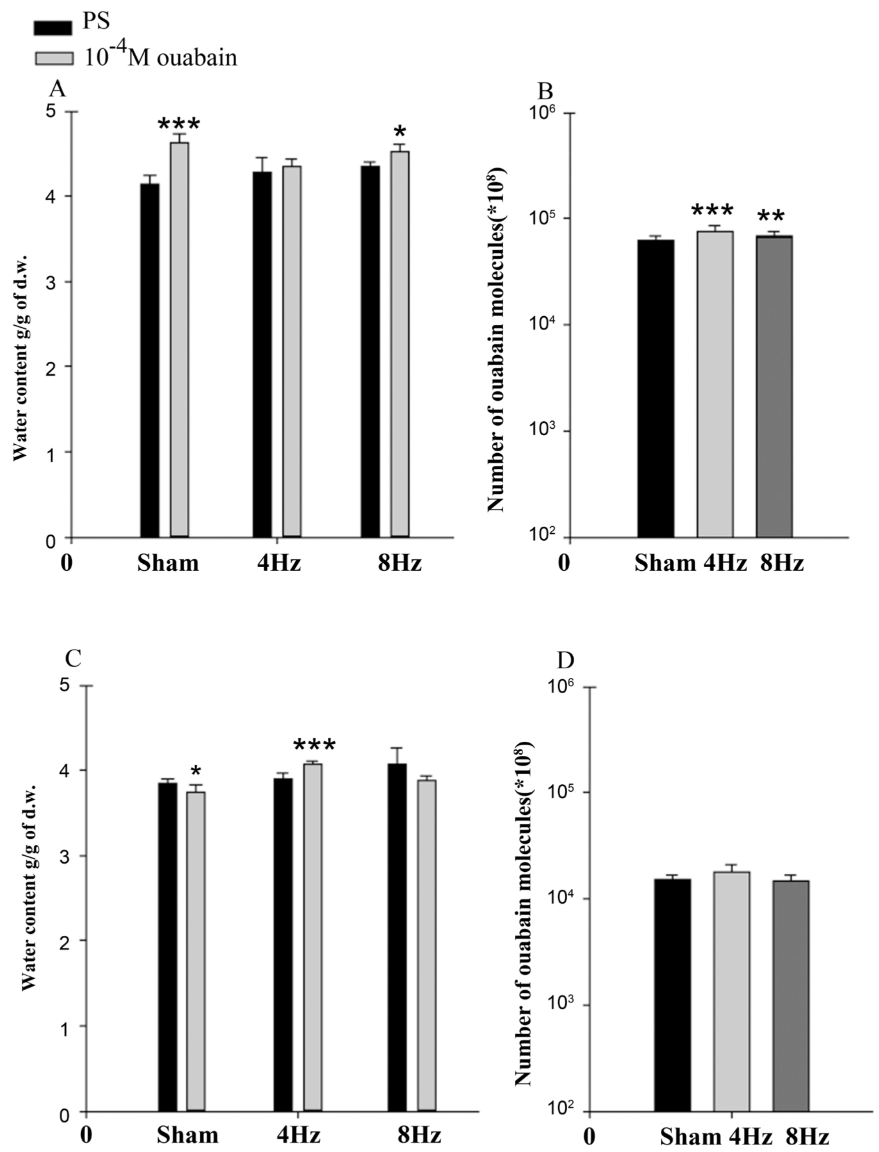

Figure 3. The changes of heart muscle tissue hydration after incubation in 10^{-4} M ouabain in sham, 4 Hz EMF and 8 Hz EMF-exposed young (A, B) and old (C, D) animals. Ordinates on A, C indicate the mean value of water content in heart muscle tissues. Ordinates on B, D are logarithmic and define the number of [^{3}H]-ouabain binding molecules with cell membrane in heart muscle tissues. Each bar represents the mean ± SEM of 30 samples. The symbols (*), (**) and (***) indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

hydration is not significantly changed compared with muscle hydration incubated in ouabain-free PS. After 8 Hz EMF radiation 10^{-4} M ouabain leads to muscle hydration (4.6%) in young animals and dehydration (8%) in heart muscle tissues in old animals. The previous studies have revealed that cell swelling increases the number of ouabain binding sides in membrane [6], while the affinity of ouabain receptors is depressed as a result of $[Ca^{2+}]_i$ increase [20] [31].

As shown in Figure 3(B), Figure 3(D) after 4 Hz EMF exposure at 10^{-4} M ouabain concentration the number of [^{3}H]-ouabain binding receptors in heart muscle are slightly increased in young and old animals compared to sham groups of rats. After 8 Hz EMF exposure at 10^{-4} M ouabain concentration, the number of [^{3}H]-ouabain binding receptors with cell membrane in heart muscle tissues is significantly increased in young and is not changed in old rats.

Previously it has been shown that 10^{-9} M ouabain brings to stimulation of cAMP and activation of Na^+/Ca^{2+} exchange in reverse mode (R Na^+/Ca^{2+} exchange) without inactivation of Na^+/K^+ pump activity [32]. Therefore, in order to find out the role of Na^+/Ca^{2+} exchange in EMF-induced modulation of heart muscle hydration, in the next series of experiments the above presented protocol of experiments with 10^{-4} M ouabain is repeated with 10^{-9} M ouabain.

The data presented in Figure 4 (A), Figure 4(C) indicate that 10^{-9} M ouabain has hydration effect on heart muscle tissues in both young (3.7%) and old (5.4%)

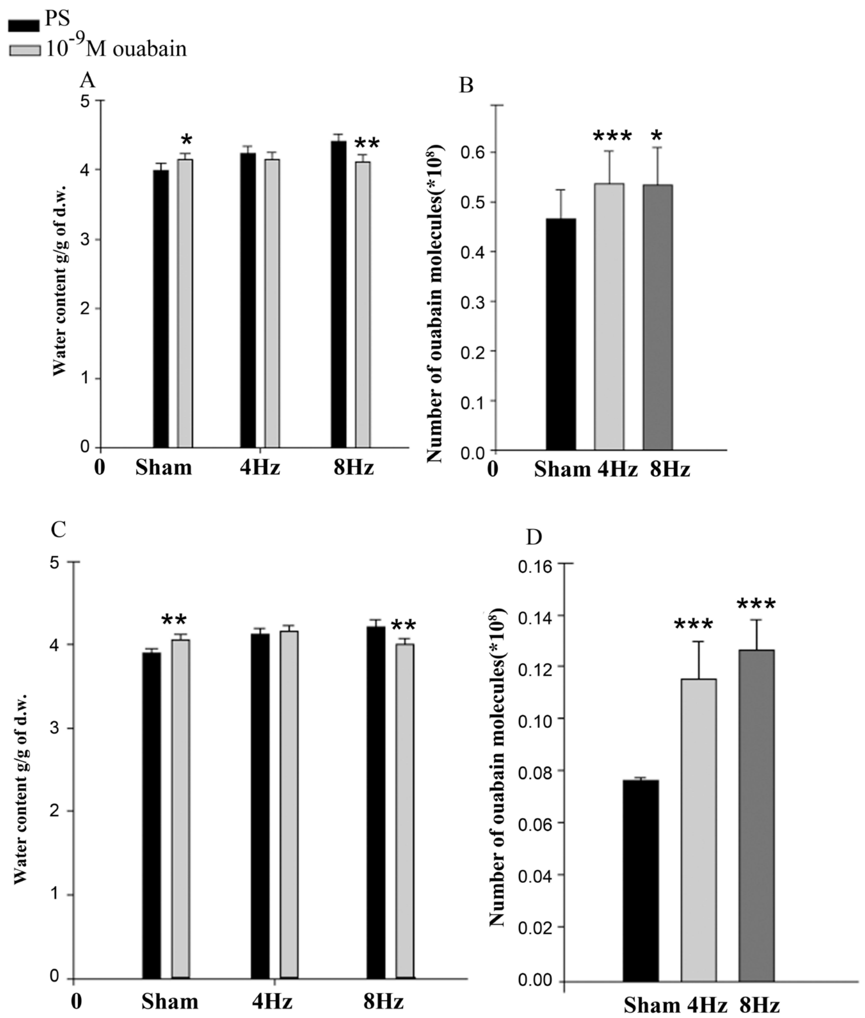

Figure 4. The changes of heart muscle tissue hydration after incubation in 10^{-9} M ouabain in sham, 4 Hz EMF and 8 Hz EMF-exposed young (A, B) and old (C, D) animals. Ordinates on A, C indicate the mean value of water content in tissues. Ordinates on B, D are logarithmic and define the number of [^{3}H]-ouabain binding molecules in tissues. Each bar represents the mean ± SEM of 30 samples. The symbols (*), (**) and (***) indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

animals. As it can be seen in Figure 4(A), Figure 4(C), the 10^{-9} M ouabain has dehydration effect on heart muscle tissues of 4 Hz EMF-exposed young (2.4%) animals compared to sham group of animals and has no effect in old animals. After 8 Hz EMF exposure 10^{-9} M ouabain has dehydration effect (7%) on heart muscle tissues of young animals as compared to sham group of animals and has no effect in old ones. The dehydration effect in 4 Hz and 8 Hz EMF-exposed young animals are accompanied by the increase (15.2%) of ouabain binding with cell membrane in heart muscle tissues (Figure 4(B)). In old animals the exposure with 4 Hz and 8 Hz EMF are also accompanied by the increase of ouabain binding with membrane (37.5% and 50%, respectively) (Figure 4(D)).

4. Discussion

It is known that the permeability of cell membrane for water molecules is much higher than the permeability of cell membrane for ions [12] [13] and that intracellular osmotic pressure exceeds the extracellular one [33]. Therefore, to keep cell volume in a steady state, the osmotic water uptake must be balanced by water efflux from the cell.

Our study performed on intracellular dialyzed squid axons and intact neurons of snails has shown that water fluxes through membrane have a crucial role in regulation of cell membrane permeability for Na^+, Ca^{2+}, K^+: water influx and efflux through cell membrane have activation and inactivation effects on inward ionic currents on Na^+ and Ca^{2+} current, and opposite effect on outward K^+ current, respectively [8] [34] [35] [36].

The Na^+/K^+ pump is a fundamental metabolic mechanism in cell membrane which controls cell functional activity. The activation of Na^+/K^+ pump leads to generation of water efflux from the cells by 1) push out of $3Na^+$ and uptake of $2K^+$ and 2) release of H_2O in cytoplasm (42 H_2O for one molecule glucose oxidation) as a result of activation of intracellular oxidative phosphorylation [37]. Previously we have shown that Na^+/K^+ pump-dependent regulation of cell volume is a powerful metabolic mechanism through which both the auto-regulation of Na^+/K^+-pump and the regulation of membrane chemo sensitivity [7] and excitability [8] are realized by changing surface-dependent number of functionally active proteins in membrane.

The data presented in Figure 2(A) and Figure 2(B) indicate that heart muscle tissue hydration in both group of animals is sensitive to 4 Hz and 8 Hz EMF. By our previous study it has been shown that static and pulsing magnetic fields activate cGMP-dependent Na^+/Ca^{2+} exchange in forward mode (F Na^+/Ca^{2+}) pushing out Ca^{2+} from the cell [16]. As Na^+/Ca^{2+} exchange functions in stoichiometry of $3Na^+:1Ca^{2+}$ [38] it was predicted that F Na^+/Ca^{2+} exchange should have hydration effect on cells. This effect was presented in Figure 2(A), Figure 2(B). As it can be seen in Figure 2(A), Figure 2(B) the 8 Hz EMF leads to more pronounced effect on hydration in both group of animals compared to 4 Hz EMF.

The obtained data (Figure 3(A), Figure 3(C) on the effects of 10^{-4} M ouabain (Na^+/K^+ pump is in inactive state) on heart muscle tissue hydration indicate that in sham animals 10^{-4} M ouabain-induced hydration has age-dependent (metabolic-dependent) character (in young animals it brings to hydration, while in old animals it has dehydration effect). Previously it has been shown that Na^+/K^+ pump inactivation-induced hydration is due to both Na^+ uptake and cAMP-dependent R Na^+/Ca^{2+} exchange-induced activation of intracellular oxidative processes leading to the release of endogenous water molecules, and the hydration of heart muscle tissue in young sham animals (Figure 3(A)) is considered as a result of these processes.

It is known that the dysfunction of Na^+/K^+ pump, which is accompanied by the increase of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), is a common consequence of any cell pathology (including ageing). The dehydration of heart muscle in old sham rats (Figure 3(C)) is considered as a result of initial high level of $[Ca^{2+}]_i$. The increase of $[Ca^{2+}]_i$, which is accompanied by Na^+/K^+ pump inactivation, is considered as a result of intracellular Na^+ concentration ($[Na^+]_i$) increase which stimulates Ca^{2+} uptake through R Na^+/Ca^{2+} exchange.

In heart muscle tissues of 4 Hz and 8 Hz EMF-exposed young animals 10^{-4} M ouabain has dehydration effect on tissues compared with sham group (the bar on 10^{-4} M ouabain) and is accompanied by the increase in the number of ouabain binding molecules with membrane. These results can be interpreted by the activation of cGMP-dependent F Na^+/Ca^{2+} exchange pushing out Ca^{2+} from the cell and reactivating electrogenic Na^+/K^+ pump, which leads to the increase of ouabain receptors affinity [20] [39].

In case of 4 Hz EMF and 8 Hz EMF-exposed old animals, the 10^{-4} M ouabain leads to the increase of heart muscle tissue hydration without changes in the number of ouabain binding molecules with membrane (Figure 3(C), Figure 3(D)). It can be explained by high $[Ca^{2+}]_i$ in old animals, which is more increased by applying 10^{-4} M ouabain leading to activation of "Ca^{2+}-calmodulin-NO synthase-cGMP" metabolic chain, which stimulates F Na^+/Ca^{2+} exchange having hydration effect on cells.

By our previous experiment performed on snail neurons it has been shown that $<10^{-9}$ M ouabain has activation effect on $^{22}Na^+$ efflux in exchange to Ca^{2+} uptake (R Na^+/Ca^{2+} exchange), which is accompanied by elevation of intracellular cAMP, without changing Na^+/K^+-pump activity [32].

The fact that the nM ouabain can elevate the intracellular cAMP is demonstrated in different tissues including dog renal cortex, gold fish intestinal mucosa, mouse pancreatic islets, murine epithelioid and fibroblastic cell lines, rat brain, rat renal collecting tubule cells in culture and astrocytes [40].

The obtained data (Figure 4(A)) of the effects of 10^{-9} M ouabain (Na^+/K^+ pump is in active state) on heart muscle tissue hydration indicate that in sham group of young animals 10^{-9} M ouabain-induced hydration is due to stimulation of R Na^+/Ca^{2+} exchange as a result of cAMP-dependent Ca^{2+} pump activation in the membrane of endoplasmatic reticulum, which brings to activation of mitochondrial function and release of endogenous H_2O.

As in heart muscles of old animals $[Ca^{2+}]_{i,}$ is high, phospholipase activity in membrane is increased and 10^{-9} M ouabain through activation of inositol 1,4,5-trisphosphate receptors brings to activation of $[Ca^{2+}]_i$-Calmodulin-NO-cGMP cascade leading to stimulation of Ca^{2+} efflux and leads to hydration (**Figure 4(C)**).

The data that 4 Hz EMF causes no hydration changes in young and in old rats compared with sham group (the bar on 10^{-9} M ouabain), while 8 Hz EMF exposure (**Figure 4(A)**, **Figure 4(C)**) brings to dehydration effect and both cases are accompanied by the increase of ouabain binding (**Figure 4(B)**, **Figure 4(D)**) clearly indicate that membrane receptors affinity to ouabain is increased. This effect can be explained by the activation of cGMP-dependent F Na^+/Ca^{2+} exchange leading to decrease of $[Ca^{2+}]_i$ [41].

Thus, from the obtained data it can be concluded that heart muscle tissue hydration of sham animals is sensitive to 4 Hz and 8 Hz EMF exposure and this sensitivity has metabolic and age-dependent character. The cGMP/cAMP-dependent Na^+/Ca^{2+} exchange controlling intracellular oxidative phosphorylation processes and endogenous release of water molecules in cytoplasm has a major role in regulation of cell hydration and $[Ca^{2+}]_i$. Thus, on the basis of previous and present data we suggest that 8 Hz EMF has more pronounced effect on heart muscle tissue hydration than 4 Hz EMF and this effect is realized through activation of cGMP-dependent F Na^+/Ca^{2+} exchange.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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