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Research paper

4Hz mechanical vibration relieves pain through Na^+/K^+-ATPase α_3 isoform-dependent brain tissue dehydration

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Abstract

In this work the effect of 4Hz 30dB horizontal mechanical vibration (MV) on thermal pain threshold, hydration and [^{3}H]-ouabain binding in brain and heart muscle tissues of rats was studied. It was revealed that 4Hz MV treatment for 10 minutes increased pain threshold, which was accompanied by brain and heart muscle tissue dehydration. In vitro state, hydration of brain and heart muscle tissues of sham animals was increased, while in 4Hz MV-treated animals the increase of brain tissue hydration was more pronounced and heart muscle tissues were dehydrated. The fact that 4Hz MV treatment also impacted heart muscle tissue hydration indicates that 4Hz MV effect on brain and heart muscle tissues is realized through a common messenger circulating in blood. The incubation of brain and heart muscle tissues in PS containing 10^{-4}M and 10^{-9}M ouabain led to tissue hydration in sham and 4Hz MV-treated animals. However, tissues of 4Hz MV-treated animals were less hydrated, and this hydration was accompanied by the decrease and increase of membrane receptors' affinity at 10^{-4}M and 10^{-9}M ouabain concentrations, respectively. Based on the obtained data, it is suggested that pain-relieving effect of 4Hz MV is due to α_3 isoform-dependent brain tissue dehydration.

Keywords: *Brain; Cerebellum; Cortex; Mechanical Vibration; Tissue Hydration.*

1. Introduction

The effects of infrasound frequencies (ISF) of mechanical vibration (MV) on organism can either be hazardous or beneficial depending on its intensity, frequency, physicochemical characteristics of environmental medium and the initial functional state of organism. Since the last century, non-traditional medicine has been using ISF MV as a therapeutic tool for the treatment of a variety of diseases, including injuries, arthritis, osteoporosis, dysfunction of lymphatic system as well as for the improvement of many metabolic processes within the body in general [1].

It is claimed that whole-body vibration can increase heart rate, oxygen uptake, respiratoryrate, produce changes in blood and urine and cause fatigue, insomnia, stomachproblems, headache, "shakiness," etc. [2].Therefore, the elucidation of the cellular and molecular mechanism of ISF MV effect on organism is one of the frontier problems in modern public health. The main barriers for adequate estimation of MV effects on organism are the facts that MV effect highly depends on the initial functional state of organism, and it has non-linear dose-dependent character. Therefore, there is a necessity to find out a biomarker, which is sensitive to different environmental factors and reflects the initial functional state of the organism. Thus, because of the absence of such a biomarker, at present, World Health Organization (WHO) and other international and national health control organizations base their instructions only on thermodynamic characteristics of MV while estimating beneficial and hazardous effects of MV.

It is known that metabolically controlled cell hydration is a dynamic parameter determining its functional activity, which is realized by hydration-induced changes of intracellular macromolecules activity by folding-unfolding mechanism [3] and by surface-dependent changes of the number of functionally active membrane proteins, having enzymes [4], receptors [5] and ionic channels-forming properties [6]. Based on this, cell hydration has been considered as a novel biomarker for estimation of the biological effect of non-ionizing physical factors, including MV [7].

As it has been previously shown that metabolic dysfunction-dependent cell swelling brings about the increase of membrane excitability and chemo-sensitivity, neuronal overhydration-induced hyper-excitability of neuronal membrane has been suggested as a cellular mechanism for generation of nociceptive signals [8]. This suggestion is supported by the data revealing that the factors, such as hypertonic solution [9], ketamine [10], magnetic fields [11], which bring to dehydration of cells, have pain-relieving effect on the organism. It has also been shown that 4Hz MV has a noticeable depression effect on water molecule dissociation leading to the decrease of H_2O_2 formation and CO_2 solubility [12]. Such a treatment of aqua medium depresses the growth and development of microbes [13], dehydrates snail neurons and heart muscle tissues and inhibits their functional activity [7]. On the basis of these data, it is assumed that 4Hz MV could have pain-relieving effect on organism as a result of brain tissue dehydration. Thus, the aim of the present work was to check this hypothesis. For this purpose, the effects of 4Hz 30dB horizontal MV on thermal pain threshold, tissue hydration and [^{3}H]-ouabain binding with cell membrane in brain cortex, cerebellum and heart muscle tissues of rats were studied.

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).
Experiments were performed on 120 adult (4-month-old) Wistar albino rats, from which 30 animals were used during the study of pain threshold, and 90 animals were used throughout the study of tissue hydration and ouabain binding with cell membrane. 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 ± 2ºC, 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 CaCl2, 1.05 MgCl2, 5 C6H12O6, 11.9 NaHCO3, and 0.42 NaH2PO4 and adjusted to pH 7.4 with NaOH was used. All chemicals were obtained from "Medisar" Industrial Chemical Importation Company (Yerevan, Armenia). [^{3}H]-ouabain with specific activity (25.34 Ci/mM) and non-radioactive ouabain (PerkinElmer, Massachusetts, USA) at 10^{-9}M and 10^{-4}M concentrations dissolved in PS were used for tissue incubation.

2.3. Method for horizontal mechanical vibration

The vibration was performed using the setup constructed in the laboratory of LSIPEC (Fig. 1). Plexiglas chamber (1) was fixed under the table of the vibrator (2). The vibrator was driven by the sine-wave generator (4) (GZ-118, Russia) through the power amplifier (3). To match the output power of the sine-wave generator with the driving power vibration, a special power amplifier (3) (IRPhEA NAS, Armenia) was used. The frequency of vibration was measured by a frequency meter-Ch 3–47 (Russia, not shown in Fig. 1).

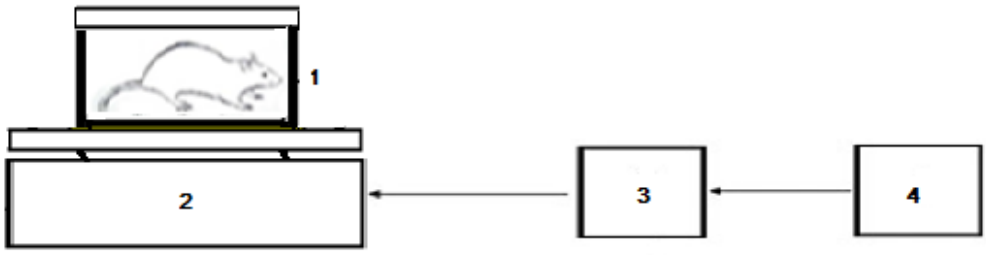

Fig. 1: The Schematic Diagram of A Device for Investigation of MV Treatments: 1-Plexiglas Chamber, 2-Vibrator, 3-Power Amplifier, 4-Sine-Wave Generator.

2.4. Measurement of pain threshold

The pain threshold was determined for sham and experimental groups of rats (mechanically vibrated with 4Hz for 10 min). Pain threshold determination was performed on the thermal platform created by the center. It consisted of org-glass chamber with the brass bottom. The bottom temperature (51°C) was controlled by the thermometer (accuracy of measurement 0.01°C). Brass bottom was completely covered by the Plexiglas box keeping the temperature constant (Fig. 2).
Latent period of pain sensitivity was recorded 30 min before and immediately after the treatment with 4Hz MV for 10 min. This procedure was made consequently, every time only on one animal. Rats were placed individually on brass bottom and latent period of pain sensitivity was recorded as the time elapsed to obtain one of the following responses: licking the feet, jumping or rapidly stamping the feet. Statistic significance was defined between the data of 10 animals before and after treatment. All data were received from three independent experiments. The interval between pain threshold measurements of sham and 4Hz MV-treated rats was 4 hours, and a new animal was chosen for each experiment.

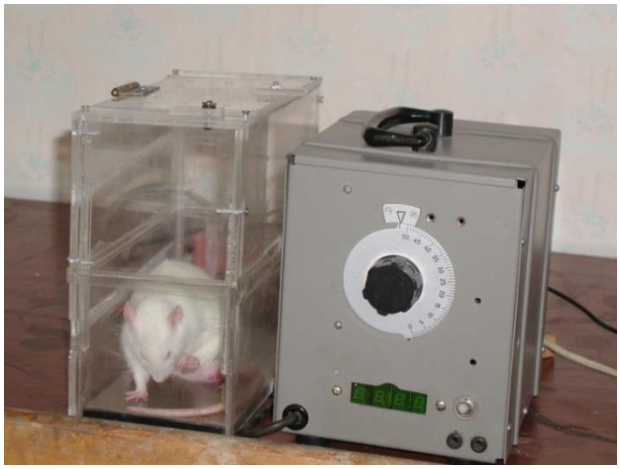

Fig. 2:"Hot Plate" Setup for Determination of Pain Threshold of Rats.

2.5. Tissue preparation

It is well known that anesthetics with different chemical and pharmacological profiles significantly affect metabolic processes, which play an important role in regulation of cell volume [10], [14]. Therefore, in the experiments animals were sharply immobilized by freezing method (dipping their noses into liquid nitrogen for 3-5 sec) and decapitated [15]. After such a procedure, the full absence of somatic reflexes on extra stimuli was recorded.
Experiments on determination of tissue hydration and ouabain binding with cell membrane were performed on 90 adult animals (30 x 3 independent experiments). 15 animals were considered as sham, 15 were treated with 4Hz MV and 9 cortex, 9 cerebellum and 9 heart muscle samples were dissected from each animal,

weighing from 50 to 70 mg. The obtained 135 samples from 15 sham animals were divided into 3 groups. 45 samples were incubated in PS, 45 samples – in PS containing 10^{-4}M [^{3}H]-ouabain and 45 samples – in PS containing 10^{-9}M [^{3}H]-ouabain. The same procedure was performed on 15 animals treated with 4Hz MV. Thus, each column on the figures presents the mean value of the data from 45 samples. For each experimental group, five animals were chosen. All data were received from three independent experiments.

2.6. Definition of water content of brain and heart muscle tissues

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

2.7. Counting of [3H]-ouabain receptors in membrane

Brain cortex, cerebellum and heart muscle tissue samples were incubated in 10ml PS containing 10^{-9}M or 10^{-4}M [^{3}H]-ouabain for 30min. Then they were washed three times. Each wash was about 5min in duration in normal PS (ouabain-free) for removing [^{3}H]-ouabain from tissues. After determination of wet and dry weights of samples, they were homogenized in 50 µl of 68% HNO_3 solution. Then 2ml of Bray's scintillation fluid was added, and chemiluminescence 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.

2.8. 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

It is known that pain threshold is one of the parameters, which is used to determine mechanical factor-induced changes in health condition. Therefore, in order to estimate MV effect on pain threshold, the impact of 4Hz 30 dB MV for 10 min on "Hot plate" thermal threshold (duration of latent period for reaction of legs to heating) was studied. As can be seen in Fig. 3, 4Hz MV significantly increased pain threshold of rats (Fig. 3).

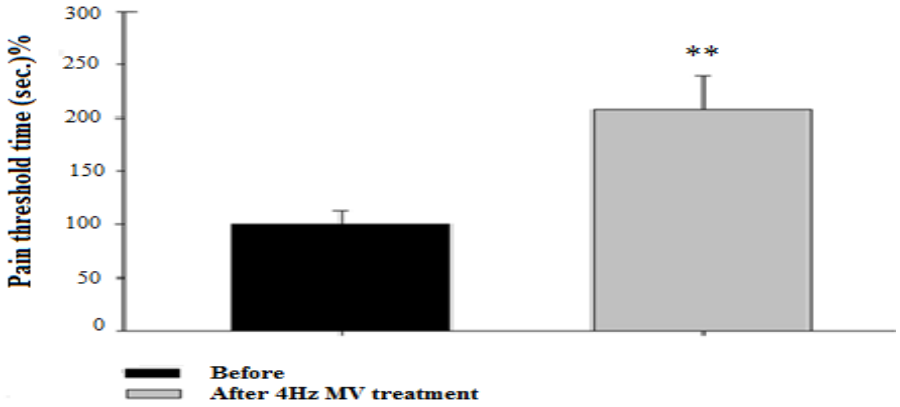

Fig. 3: The Effect of 4 Hz 30 Db MV Treatments for 10 Min on "Hot Plate" Thermal Threshold in 30 Rats. TheBlack Column Represents Pain Threshold of Rats before Treatment (Sham); the Grey Column Shows Pain Threshold after 4Hz MV Treatment for 10 Min.

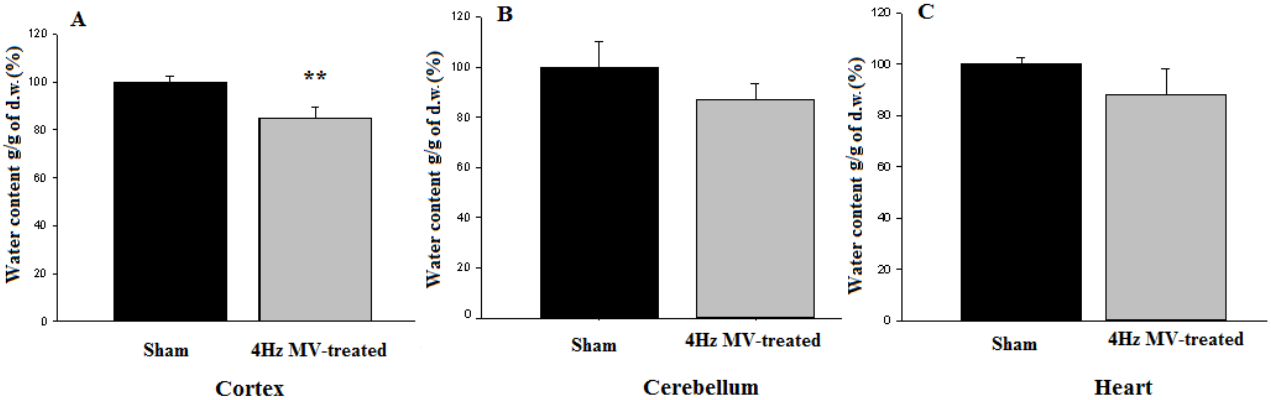

Fig. 4: The Effect of 10 Min 4Hz MV Treatment on Brain Cortex (A), Cerebellum (B) and Heart Muscle Tissue (C) Hydration. Black Columns Indicate Water Content of Tissue (Water Content G/G D.W.) of Sham Animals; Grey Columns Indicate Tissue Hydration of 4Hz MV-Treated Animals.

By our previous study we have shown that hypertonic solution (mannitol)-induced brain cortex tissue dehydration increases pain threshold [9]. In order to elucidate whether 4Hz MV-induced increase of pain threshold is due to brain tissue dehydration, in the next experiments the effect of 4Hz MV on brain tissue hydration was studied. Considering the fact that cerebellum plays an important role in motor control (coordination, precision, and accurate timing), in the present work, the comparative study of MV effects on brain cortex and cerebellum hydration was performed. The determination of tissue hydration of sham and 4Hz MV-treated animals was done immediately (within 3 min) after decapitation of rats.

Fig. 4A and 4B show that 4Hz MV treatment for 10 min had dehydration effects on brain cortex and cerebellum tissues. In order

to evaluate whether the observed 4Hz MV-induced dehydration effect is specific for brain tissues, the similar protocol of experiments was performed on heart muscle tissues, as the latter is one of the stress-sensitive tissues of the organism. The data presented in Fig. 4C indicate that 4Hz MV had dehydration effect on heart muscle tissues. Therefore, it can be suggested that 4Hz MV-induced dehydration effect on brain and heart muscle tissue hydration is realized through an unknown common messenger circulating in blood.

It is known that tissue hydration can be changed either by variation of osmotic water uptake by cell or by metabolic release of water from the cell. It is obvious that in *in vitro* condition, when tissues are incubated in PS at room temperature, there is a time-dependent dysfunction of metabolic activity of tissues bringing to cell death. Therefore, it was assumed that the comparative study of the changes of tissue hydration of sham and 4Hz MV-treated animals in the same period and conditions would make it possible to evaluate the nature (osmotic or metabolic) of the mechanism through which the effect of 4Hz MV on tissue hydration is realized.

As can be seen from the data presented in Fig. 5, the hydration in all three types of sham tissues was increased after 30 min incubation in PS at room temperature. However, compared with sham animals, brain cortex and cerebellum tissue hydration of 4Hz MV-treated animals were more increased, while heart muscle tissue hydration was decreased.

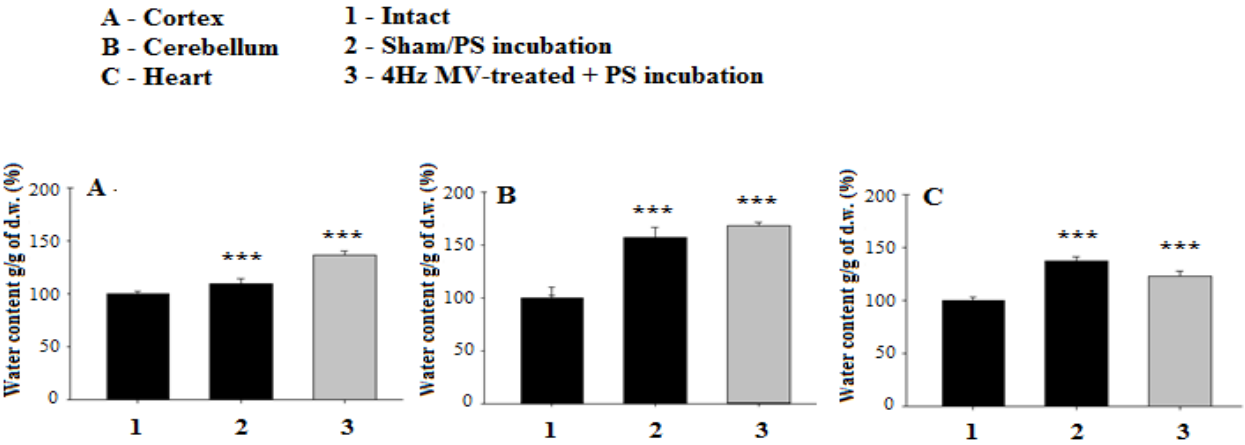

Fig. 5: The Effect of in Vitro Incubation on Brain Cortex (A), Cerebellum (B) and Heart Muscle (C) Tissue Hydration Immediately after Decapitation (Intact) (1), 30 Min Incubation of Sham (2) and 4Hz MV-Treated Animals (3).

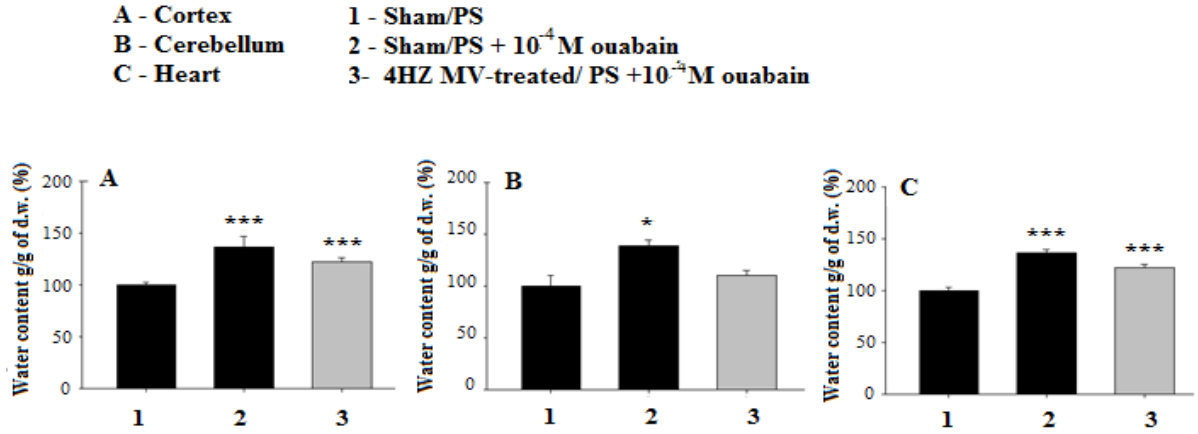

Fig. 6: The Sensitivity of Brain Cortex (A), Cerebellum (B) and Heart Muscle (C) Tissue Hydration of Sham and 4Hz MV-Treated Animals to 10^{-4}M Ouabain. Hydration of Sham Animals' Tissues Incubated in Ouabain-Free PS (1); Hydration of Sham (2) and 4Hz MV-Treated (3) Animals' Tissues Incubated In PS Containing 10^{-4}M Ouabain. Tissue Incubation Time was 30 Min.

From these data it can be concluded, that there is a metabolic mechanism which is responsible for the increase of brain tissue hydration and decrease of heart muscle tissue hydration in 4Hz MV-treated animals.

It is known that electrogenic Na^+/K^+pump has a crucial role in metabolic control of cell hydration [16], [17], [18]. In order to find out the role of Na^+/K^+pump in realization of 4Hz MV-induced hydration changes in the aforementioned three types of tissues (cortex, cerebellum and heart muscle), in the next series of experiments, the sensitivity of 4Hz MV-induced changes of tissue hydration to 10^{-4}M ouabain (inhibiting Na^+/K^+pump) was studied. For this purpose, after decapitation of sham and 4Hz MV-treated animals, theirtissue samples were incubated in ouabain-free and 10^{-4}M ouabain containing PS for 30min, respectively. Then tissue hydration and [^{3}H]-ouabain binding with cell membrane were detected.

The data presented in Fig.6 show that the incubation of sham tissues in PS containing 10^{-4}M ouabain PS led to brain cortex, cerebellum and heart muscle tissue hydration. In case of 4Hz MV-treated animals, the same incubation also increased hydration in all above-mentioned tissues. However, this hydration was less pronounced, i.e. 4Hz MV depresses tissue hydration sensitivity to 10^{-4}M ouabain.

By our previous studies we have shown that there is a direct correlation between cell volume and the number of ouabain binding sides (pump units) in membrane [4], while the affinity of ouabain receptors is depressed as a result of increase of intracellular Ca^{2+} ($[Ca^{2+}]_i$) [19]. Therefore, to evaluate whether the weakening effect of 10^{-4}M ouabain-induced tissue hydration in 4Hz MV-treated animals is due to the decrease of the number of ouabain sides in membrane, [^{3}H]-ouabain binding with cell membrane in brain cortex, cerebellum and heart muscle tissues of sham and 4Hz MV-treated animals was studied.

As can be seen in Fig. 7, in all three types of tissues of 4Hz MV-treated animals, ouabain binding was less expressed than in sham ones. It is worth to note that in cerebellum tissue the expression of ouabain receptors was much higher than in cortex and heart muscle tissues.

Our previous study has shown that 10^{-9}M ouabain, which is unable to inactivate Na^+/K^+pump, leads to activation of cAMP-dependent in reverse mode (R Na^+/Ca^{2+} exchange) [20]. It has also been shown, that although R Na^+/Ca^{2+} exchange functions in stoichiometry of $3Na^+:1Ca^{2+}$[21], 10^{-9}M ouabain-induced activation of R Na^+/Ca^{2+} exchange in *in vivo* experiments brought to metabolic-dependent hydration in brain and heart muscle tissues [22]. Therefore, in the next series of experiments, we studied 10^{-9}M ouabain effect on tissue hydration in sham and 4Hz MV-treated animals to elucidate the role of cAMP-dependent R Na^+/Ca^{2+}exchange in determination of tissue hydration changes in 4Hz MV-treated animals.

The data presented in Fig. 8 indicate that, like 10^{-4}M ouabain, 10^{-9}M ouabain also caused hydration in brain and heart muscle tissues of both sham and 4Hz MV-treated animals, while tissue hydration of 4Hz MV-treated animals was less pronounced. However, 4Hz MV-induced weakening of 10^{-9}M ouabain-dependent hydration of all three tissues was accompanied by the increase of ouabain binding with cell membrane (Fig. 9).

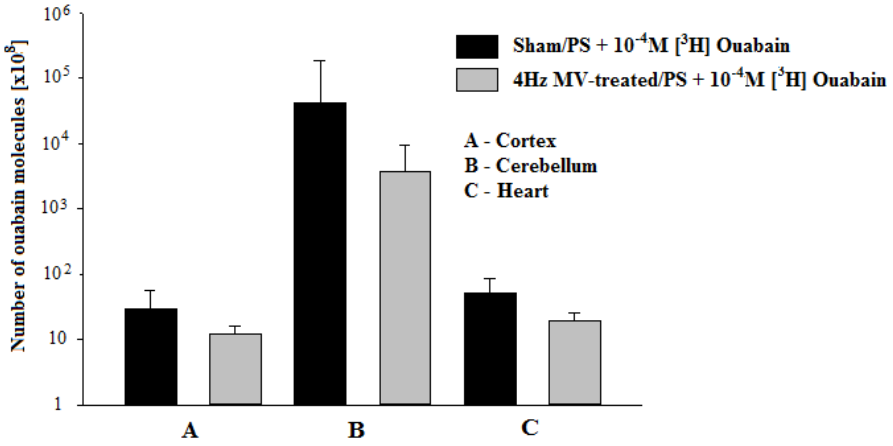

Fig. 7: 10^{-4}M [^{3}H]-Ouabain Binding with Cell Membrane in Brain Cortex (A), Cerebellum (B) and Heart Muscle (C) Tissues of Sham and 10 Min 4Hz MV-Treated Animals.

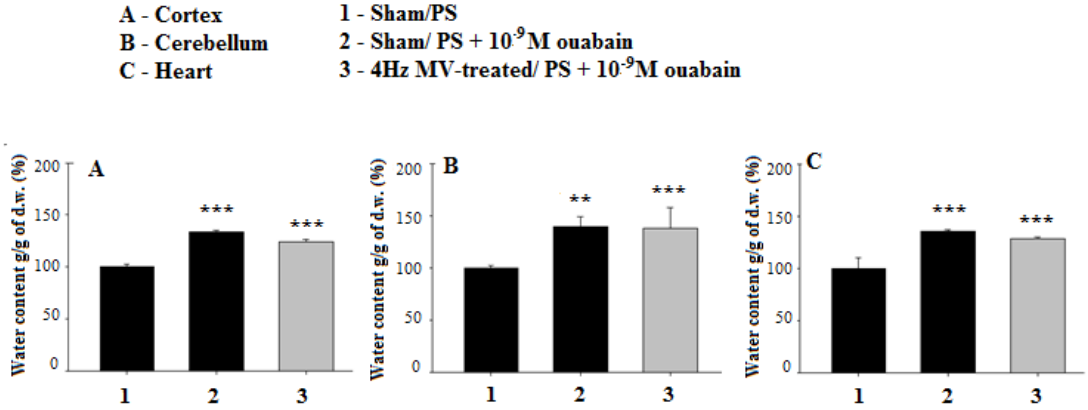

Fig. 8: The Sensitivity of Brain Cortex (A), Cerebellum (B) and Heart Muscle (C) Tissue Hydration of Sham and 4Hz MV-Treated Animals to 10^{-9}M Ouabain. Hydration of Sham Animals' Tissues in Ouabain-Free PS (1), Hydration of Sham (2) and 4Hz MV-Treated (3) Animals' Tissues Incubated in PS Containing 10^{-9}M Ouabain. Tissue Incubation Time was 30 Min.

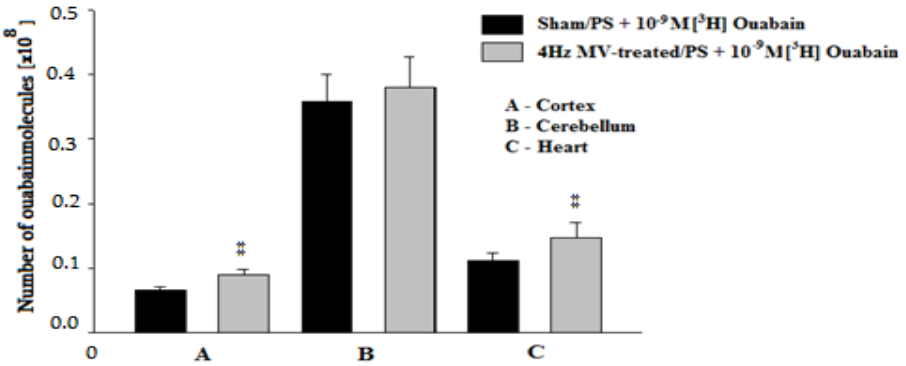

Fig. 9:10^{-9}M [^{3}H]-Ouabain Binding with Cell Membrane in Brain Cortex (A), Cerebellum (B) and Heart Muscle (C) Tissues of Sham and 4Hz MV-Treated Animals.

Thus, the obtained data indicate that 30dB 4Hz horizontal vibration for 10 min increases thermal pain threshold of rats, which is accompanied by brain tissue dehydration and activation of an unknown mechanism leading to the increase of membrane receptors' affinity to nM ouabain.

4. Discussion

Previously, we have shown that in normal living state the activation of electrogenic Na^+/K^+ pump inhibits cell membrane excitability by both membrane hyperpolarization [23] and potential-independent mechanisms [24], [25]. The latter is realized by minimum two pathways: from one side electrogenic Na^+/K^+ pump inactivates inward going ionic currents by generation of water efflux through the membrane, from the other side it leads to cell surface-dependent decrease of the number of active ionic channels in membrane [6], [26], [27]. Considering these data, we have suggested that Na^+/K^+ pump inhibition-induced neuronal overhydration is the main cellular mechanism generating nociceptive signals [8]. The experimental data that the factors, such as hypertonic solution [9], ketamine [10], magnetic fields [11] bringing to cell dehydration, have pain-relieving effect on organism, support this suggestion. The obtained data of the present work showing that 4Hz MV increases pain threshold (Fig. 3), which is accompanied by brain tissue dehydration (Fig. 4) can also serve as additional evidence supporting this hypothesis. The data that 4Hz MV also has dehydration effect on heart muscle tissues (Fig. 4) indicate that 4Hz MV-induced modulation effect on brain and heart muscle tissue hydration is realized through an unknown common messenger circulating in blood.

The difference between the sensitivities of sham and 4Hz MV-treated tissues hydration to 10 min in *in vitro* incubation indicates that 4Hz MV stimulates some metabolic mechanism involved in regulation of cell hydration (Fig. 5). It is known that Na^+/K^+ pump inactivation leads to cell hydration [16], [17], [18]. The obtained results showing that 10^{-4}M ouabain, having inhibitory effect on Na^+/K^+ pump, leads to hydration in all three investigated tissues of sham animals (Fig. 6), allow us to suggest that Na^+/K^+ pump is the main metabolic mechanism through which 4Hz MV modulates tissue hydration. This suggestion is confirmed by the data revealing that compared with sham animals the hydration of all three investigated tissues of 4Hz MV-treated animals is less sensitive to 10^{-4}M ouabain and is accompanied by the decrease of ouabain binding with membrane.

Earlier it has been shown that there is a negative feedback between Na^+/K^+ pump activity and the number of pump units in membrane, which is realized through Na^+/K^+ pump-induced cell shrinkage [4]. Therefore, this impairment of cell hydration in 4Hz MV-treated animals' tissues incubated in 10^{-4}M ouabain can be explained by Na^+/K^+ pump activation during mechanical vibration of intact animals, which brings to the decrease of brain tissue hydration (Fig. 4).

It is known that Na^+/K^+-ATPase (working molecules of Na^+/K^+ pump) has three catalytic isoforms (α_1, α_2, α_3) in neuronal and muscle membranes [28]. These isoforms have different affinities to cardiac glycoside-ouabain and functional activities: α_1 (with low affinity – agonist for $>10^{-4}$M ouabain) andα_2 (with middle affinity) isoforms are involved in transportation of $Na^+$and K^+ through membrane, while α_3 (agonist for $<10^{-9}$M ouabain) isn't directly involved in transporting process of Na^+ and $K^+$and has only intracellular signaling function through which $[Ca^{2+}]_i$ is regulated [28], [29]. Our previous study has shown that the activation of α_3 receptors in both brain and heart muscle tissues of rats by 10^{-9}M ouabain, leads to activation of cAMP-dependent R Na^+/Ca^{2+} exchange which has a metabolic-dependent tissue hydration effect [22]. Thus, the obtained data of this work on 10^{-9}M ouabain-induced hydration effect on brain and heart muscle tissues of sham animals can be considered as a result of activation of cAMP-dependent R Na^+/Ca^{2+} exchange.

The data indicating that the impairment of 10^{-9}M ouabain-induced tissue hydration in 4Hz MV-treated animals are accompanied by the increase of ouabain with α_3 receptors, allow us to suggest that MV activates a mechanism leading to the increase of α_3 receptors affinity to ouabain. It is worth noting that in cerebellum, which is responsible for coordination of animal movements, the expression of ouabain receptors is much higher than in cortex and heart muscle tissues (Fig. 7, 9). This probably underlines the crucial role of cerebellum in regulation of animal movements.

Our previous studies performed on snail isolated neurons, and heart muscles have shown that 4Hz MV activates cGMP-dependent Na^+/Ca^{2+} exchange in forward mode (F Na^+/Ca^{2+} exchange), which pushes out $[Ca^{2+}]_i$ from the cells [7], [30]. Therefore, the obtained data of the present work indicating that 4Hz MV-induced increase α_3 receptors affinity to ouabain (Fig. 9) can be explained by activation of cGMP-dependent F Na^+/Ca^{2+} exchange leading to the decrease of $[Ca^{2+}]_i$, which in its turn activates Na^+/K^+ pump [31]. Therefore, 4Hz MV-induced weakening of hydration generated by 10^{-9}M ouabain (cAMP-dependent R Na^+/Ca^{2+} exchange) is due to activation of Na^+/K^+ pump. This metabolic pathway is responsible for 4Hz MV-induced brain tissue dehydration (Fig.1), which has pain-relieving effect on rats.

The data that 4Hz MV also modulates heart muscle tissue hydration indicate that the impact of MV on organism results in formation of an unknown messenger, which circulates in blood and modulates both brain and heart muscle tissue hydration. The nature of this messenger can be a subject for further investigation.

From the obtained data it can be suggested that α_3 isoform-dependent signaling system controlling pump activity and tissue hydration could serve as a novel target for nociception therapy, which could open a new avenue for discovering new drugs and physiotherapeutic tools having pain-relieving effect.

5. Declaration of interest

There is no conflict of interest concerning the materials presented in the article.

Acknowledgement

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