Խ. ԱԲՈՎՅԱՆԻ ԱՆՎԱՆ ՀԱՅԿԱԿԱՆ ՊԵՏԱԿԱՆ ՄԱՆԿԱՎԱՐԺԱԿԱՆ ՀԱՄԱԼՍԱՐԱՆԻ ԳԻՏԱԿԱՆ ՏԵՂԵԿԱԳԻՐ
УЧЕНЫЕ ЗАПИСКИ АРМЯНСКОГО ГОСУДАРСТВЕННОГО ПЕДАГОГИЧЕСКОГО УНИВЕРСИТЕТА ИМ. Х. АБОВЯНА

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THE EFFECT OF MECHANICAL VIBRATION ON THE CELL HYDRATION IN RAT BRAIN CORTEX AND CARDIAC TISSUES AND PAIN THRESHOLD

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The effect of horizontal mechanical vibration 4Hz, 30dB of 10 minutes duration on pain threshold and hydration rate of brain cortex and cardiac tissues samples has been examined on rats. The experiments were performed in vivo and in vitro on the intact and experimental groups of the animals to check the changes in cell metabolic regulation. Additional the changes in the rats' fecal pH gradient have been assessed. For in vitro experiments the studied tissue samples were incubated to 30 minutes in Tiroide physiological solution. Also for estimation of the number of the membrane receptors [Na^+/K^+ ATPases] the studied tissues were incubated in the Na^+/K^+ exchange pump specific inhibitor [^3H]-ouabain of 10^{-9} and 10^{-4} M solutions for 30 minutes. Obtained results revealed that treatment with mechanical vibration (4Hz, 30dB, 10min) resulted in a 105% increase in pain threshold value, which is the reduction in pain sensitivity, directly correlating with the rate of hydration changes, in particular of 14.6% dehydration.

The experimental data revealed certain orderliness between the changes in cell hydration level and pain threshold in brain studied tissues and cardiac tissue, which is set in between the functional activity of the hydration of the nervous and muscular systems. This can be considered as the basis for the hypothesis according to which the cell volume can be used as a cell marker to characterize the functional state of the different tissue cells.

Keywords: *mechanical vibration, pain threshold, brain cortex, cardiac tissues, cell hydration.*
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Introduction

Since last century non-traditional medicine used mechanical vibration (MV) as a therapeutic measure in a variety of diseases, including injuries, arthritis, osteoporosis, and also of the lymphatic system, and the improvement of many metabolic processes in the body in general.

Early studies demonstrated that MV of 4Hz frequency of 30 minutes duration together with intra-abdominal injection of hypertonic solution of mannitol (well known diuretic osmo-regulator) causes the diaphragm to reduce the number of protein molecules active functional nature and therefore reduction of pain sensitivity [1].

Although, the physiological significance of intracellular water in the cell and cell membrane is well accepted, there were not enough investigations toward it's functional activities.

In earlier studies it has been shown that cell hydration is a dynamic marker to be used to regulate the functional activity of the protein units, chemo-testers [2], ion channels [4], enzymes [3] located in/on the surface of cell membrane. In 1980 Ayrapetyan demonstrated that the cell membrane swollen amplifies the number of active functional units, mean while shrinking causes

the opposite effect [5]. Based on this data cell hydration is recommended as a marker to identify the regulations of the diaphragm activity [5].

As it was mentioned before, although the physiological significance of intracellular water is widely accepted, the correlation between cell hydration and nociceptive signal generation has not been studied yet. Therefore, the experiments to check this hypothesis, to reveal the mechanisms of pain generation regulated by cell volume changes accompanied by metabolic processes, and to study the cellular molecular mechanisms of the influence of environment, have been performed on the young animals treated by MV (4Hz frequency, 30dB for 10 minutes duration) to analyze the pain threshold values. In parallel, the hydration rates for *in vivo* and *in vitro* conditions have been preceded with the cerebral cortex and cardiac tissues samples.

We aimed to carry out the experiments on young animals with MV of 4Hz, 10 minutes to check the effects on hydration level of different samples and tissues, to try to identify the cellular level, molecular basis of cell volume regulation and cell pain generation mechanisms, accompanied by metabolic processes. In parallel the pain thresholds have been measured.

For that purpose we compared the values of water content and pain thresholds of intact and experimental groups (after MV of 4 Hz, 10 minutes) of animals. The same parameters have been checked for control and experimental groups of animals after incubation in 10^{-4} and 10^{-9} ice-cold ouabain solution (for 30 minutes). Additionally, the values of hydration and pain threshold of intact and experimental groups of animals were measured for the control and experimental groups of animals after incubation in physiological solution for 30 minutes.

Material and methods

Methods

The study was conducted on adult rats (90-110g weight females breed: *Wistar albino.* From our experience the animals (n=24) were kept in optimal conditions at 12 hours of day light and implementing temperature of 22-24°C, food and water were available to the all animals. The research group included 3 animals each. Research was carried out in accordance with the animal acts committed by animal care and protection established by the International Center for Postgraduate Education Regulations (LSIPEC, Yerevan, Armenia).

In the experiments a mechanical vibrating device and thermal platform were used. The statistical analyses of the experimental results were conducted with Sigma Plot (version 12.5) software (with the data verifiability of * when $P < 0.5$; of ** when $P < 0.1$; and of *** when $P < 0.01$).

Chemicals and equipment

For measurements 12 Ci/mM [H^{3+}]-ouabain (Amersham, Bucks, UK) in 10^{-8}M of 0.9% NaCl diluted solution was used. The quantification of radioactive material in the tissue samples was performed by Wallac 1450-001 (Wallac Oy, Turku, Finland) liquid scintillation counter.

The tissue samples from both experimental groups were incubated for 30 minutes in the [H^{3+}]-ouabain 10^{-4} M and 10^{-9} M solutions. The tissue samples were grouped into separate containers of 10ml 10^{-4} M and 10^{-9} M [H^{3+}]-ouabain solutions. The tissue samples were incubated three times for 10, 5, and 5 minutes each and washed afterwards in 100 ml of normal physical ring solution to remove extra radioactive molecules from membrane and intracellular environment. Then the tissue wet samples were gently dried on the filter paper and homogenized in 50 µl 68% - nitric acid and stored for 24 hours. Then 2 ml of Bray scintillation liquid was added. Two hours later, Wallac 1450 liquid scintillation computer device (Wallac Oy, Turkey, Finland) counted the number of irradiating molecules in the tissue samples.

The radioactivity per mg of dry tissue was calculated according to the following formula: *cpm / mg*, where cpm is the number of nuclear transformations per minute, mg is a dry weight of the tissue sample.

Washing the tissue samples was done with scintillation cocktail sq/m of pure HNO_3, dioxin, the Tirode solution for tissue samples washing for the warm-blooded animals prepared according to the following recipe (for volume of 1 liter: 137g of NaCl, 5.4g of KCl, 1.8g of $CaCl_2$, 1.05g of $MgCl_2$, 5.0g of $C_6H_{12}O_6$, 11.9g of $NaHCO_3$, 0.42g of NaH_2PO_4, with pH-7.4 (PHM-22r, Copenhagen, Denmark). All the chemicals were purchased by the Medisar Center (Yerevan, Armenia).

The value of hydration of the tissue samples

The water content of the samples of animals of all research groups was measured in brain cortex and cardiac tissues. To avoid the experimental animals any emotional tension and pain, as well as to fix the water exact level in the brain cortex and cardiac in particular), the animal's head was directly frozen in liquid nitrogen and kept there in for 3-4 sec to ensure water rapid freezing and animal immobility. The samples were taken from cortex and cardiac tissues of each animal (n=3). Radioactivity of the tissue samples were measured after 30 minutes incubation in 10ml of ice-cold [H^{3+}]-ouabain (10^{-4} M and 10^{-9} M). The samples were incubated for 5 and 10 minutes, after washed with 100 ml physiological solution and then carefully dried on filter paper. Afterwards, the samples wet and dried in thermostat (105^0C, 24 hours) weight were determined. The 1 gram of tissue was calculated by the following formula: *(A-B) / B x 1000 mg*, where A is the tissue wet weight, B is the tissue weight after drying.

Measurements of the pain thresholds

The pain threshold was determined for the intact and experimental group's rats (mechanically vibrated for 4 Hz 10 minutes). The rats were placed on the heating stage separately. Pain threshold determination was performed on the thermal platform created by the center, covered with a Zn-based and Plexiglas device (to keep the platform temperature stability). The temperature of the platform (52.4°C) was monitored through the thermometer (Figure 1). The limbs licking or rapid lift up, as well as the try to skip from the platform were registered as the pain stress.

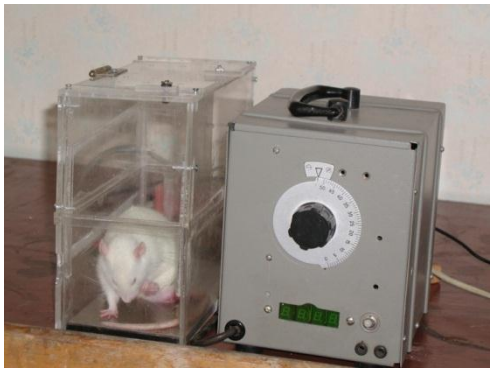

Fig.1. The thermal platform

Results

The obtained results revealed tendency to register dehydration in brain cortex (mean on 14.6 %) (Figure 2.A) and cardiac (mean on 11.5 %) tissues (Figure 2.B) for the *in vivo* experiments treated with the MV (4 Hz for 10 minutes) compared to the intact group of animals.

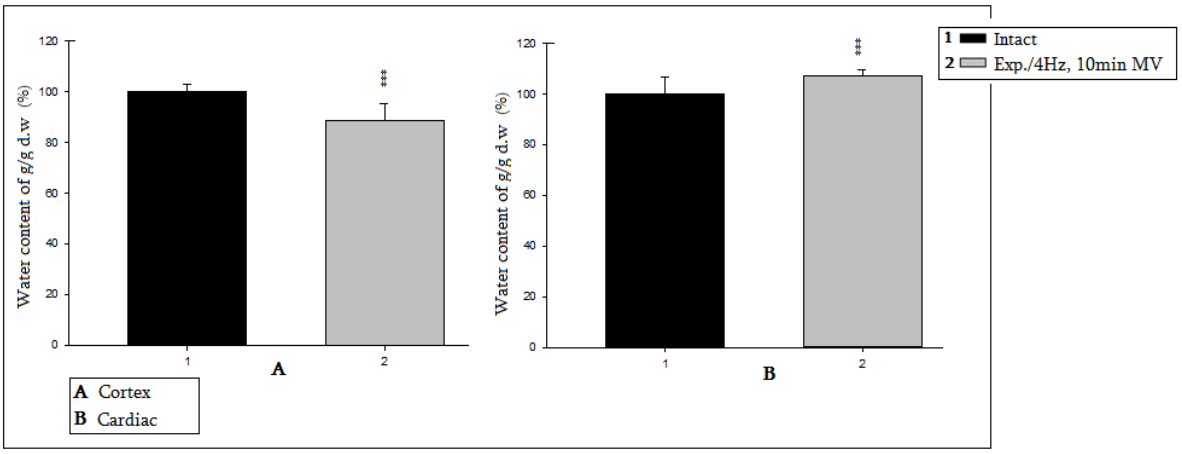

Fig.2. Mechanical vibration (4Hz, 30 dB, 10min) effects on brain cortex (A) and cardiac (B) tissues slices in vivo condition. Verifiability (***), which corresponds to, p <0.01

Increase of hydration level of the cortex tissues after MV (4 Hz for 10 minutes) (mean on 38.8%) during the incubation in PS solution (*in vitro* experiments, incubation for 30 minutes) was fixed (Figure 3.B), as well as in the samples of cardiac during incubation in PS solution (for 30 minutes) (mean on 23.5 %) (Figure 3.D).

As can be seen *in vivo* experiments MV leads to the studied tissues dehydration, while *in vitro* experiments the level of hydration of the studied tissues was increased (Figure 3.A, B, C, D).

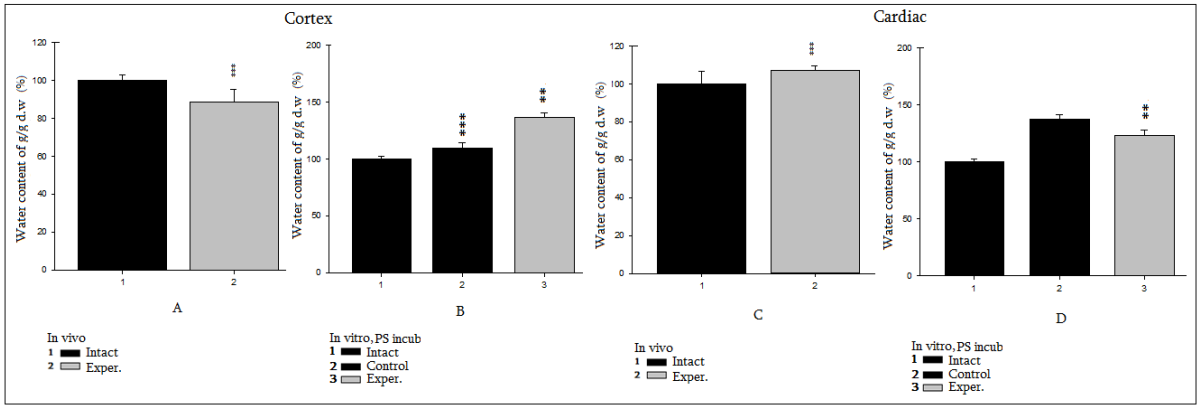

Figure 3. Mechanical vibration (4Hz, 30 dB, 10min) effects on brain cortex (A) and cardiac (B) tissues slices tissue hydration immediately after (<3 min) animal decapitation, and after 30min of tissue slices' incubation in PS at room temperature in vivo and in vitro condition in all the studied groups. Verifiability (***), which corresponds to, p <0.01

In *vitro* incubation in 10^{-4} M and 10^{-9} M [H^{3+}]-ouabain caused dehydration in all the tissues (Figure 4). These results are opposite to the results of PS-incubation when tissues hydration has been observed for brain tissues, meanwhile for cardiac tissue dehydration was registered (Figure 3.B, D).

Data of hydration level of cortex and cardiac tissues for both experimental groups (intact and vibrated) after incubation in [H^{3+}]-ouabain (10^{-4} and 10^{-9} M) for 30 minutes (*in vitro* experiments) demonstrated reverse tendency. Increased level of water content was registered for all the analyzed samples (*in vitro* experiments) for incubation in [$^{3+}$H]-ouabain 10^{-9} M (mean on 23.8% for cortex; 28.5% for cardiac tissues) (Figure 4.B, D). The similar situation was observed after

incubation in [$^{3+}$H]-ouabain 10^{-4} M (mean on 22.8% for cortex; 22.1% for cardiac tissues) (Figure 4.A,C).

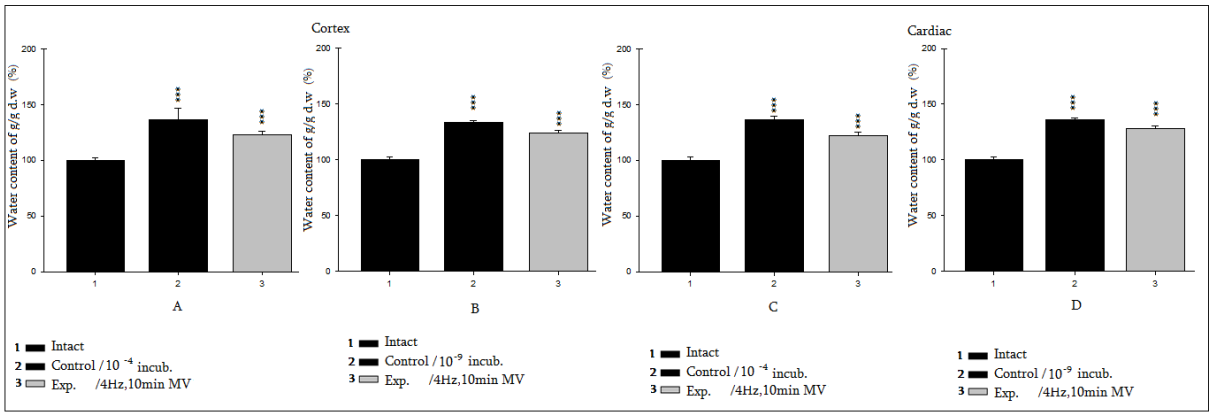

Fig.4. Mechanical vibration (4Hz, 30 dB, 10min) effects on tissue hydration immediately after (<3 min) animal decapitation, and after 30min of tissue slices' incubation in [H^{3+}]-ouabain 10^{-4}M and [H^{3+}]-ouabain 10^{-9}M at room temperature in vitro condition in all the studied groups. Verifiability (***), which corresponds to, p <0.01

Studies of the effect of MV of 4Hz 30 dB 10 minutes on [H^{3+}]-ouabain binding with cell membrane in cortex and cardiac muscle tissues revealed increased number of [H^{3+}]-ouabain binding molecules with membrane for [H^{3+}]-ouabain of 10^{-9} M concentration (Figure 5.A), meanwhile after incubation in [H^{3+}]-ouabain of 10^{-4} M concentration, the number of [H^{3+}]-ouabain binding molecules decreased in all the studied tissues (Figure 5.B).

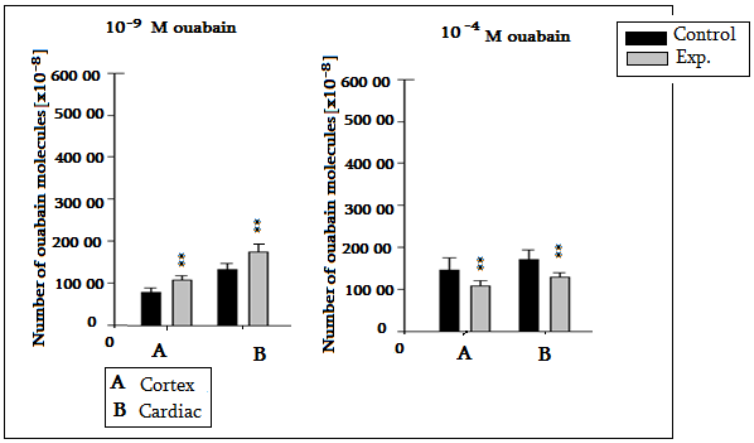

Fig. 5. Effect of MV of 4Hz 30 dB 10 minutes on [H^{3+}]-ouabain 10^{-9}M (A) and [H^{3+}]-ouabain 10^{-4}M (B) binding with cell membrane in cortex and cardiac muscle tissues in vitro condition in all the studied groups. Verifiability (**), which corresponds to, p <0.1

Measurements of changes of pain thresholds for intact and experimental groups of animals all together demonstrated doubled level (mean on 100 %) of the range of pain sensitivity after treatment with MV (4 Hz, 30 dB for 10 minutes) (namely increases latent period of animal response to 52.4^{o}C degree heating) (Figure 6.).

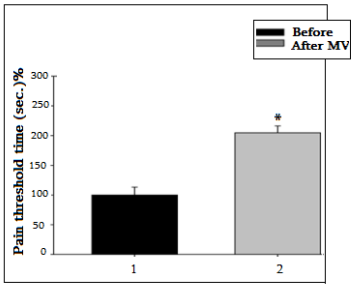

Fig.6. Effect of MV of 4Hz 30 dB 10 minutes on pain threshold all the studied groups. Verifiability (*), which corresponds to, p <0.5

Discussion

Presented data of the experiments on changes in pain threshold shows that MV of 4 Hz frequency, 30 dB of 10 minutes duration reduced the pain sensitivity and thus increased the pain threshold for all the studied animals. The observed lowering of the level of pain sensitivity in our experiments correlated with the data of reduced hydration level (dehydration) of brain cortex and cardiac tissues samples.

In our previous research in order to check whether MV-induced (4Hz, 30 dB 30 min duration) cell hydration is a determining factor for MV-induced increase of pain threshold, a comparative study of the effect of MV and intra-peritoneal injection of mannitol on hydration of brain tissue and pain threshold were studied [1]. According to the Ayrapetyan's hypothesis [6; 8] the changes in pain threshold can be explained by the changes in the sensitivity of brain tissue, in response to a painful stimulus impact.

As it is known cell hydration can be a result of either diffusion of water uptake by the cell, or the metabolic release of endogenous water. Therefore, in order, to find out, which mechanism out of these two is responsible for MV-induced cell hydration, we have studied the effects of MV on brain cortex and cardiac tissues hydration.

Since sodium-potassium (Na^+/K^+) pump has a central role in cell volume regulation mechanisms via metabolic activities, to assess the effect of MV on pump active and inactive states, we have used the 10^{-4} and 10^{-9} Molarity solution of [H^{3+}]-ouabain - a classic inhibitor [9] for ATPase, where the last ones are the working molecules for the pump. Our previous studies have shown that 10^{-9} M [H^{3+}]-ouabain has stimulation effect on the metabolic release of endogenous water in cytoplasm, while 10^{-4} M [H^{3+}]-ouabain-induced poisoning of sodium/potassium pump (Na/K+) leads to cell swelling as a result of diffusion of water uptake by cell [8].

The data of the current *in vivo* experiments on intact animals demonstrate dehydration of brain cortex tissue on 14.4% after treating with MV (4 Hz, 30 dB, 10 min) compared to the control group. This indicates that MV of certain frequency and duration increased the Na^+/K^+-pump activity and contributed to the activation of membrane and metabolic mechanism pushing water from the cell. This allows us to suggest that MV-induced (4Hz, 30dB of 10 minutes) changes of tissue hydration, cannot be explained by the changes of sodium/potassium pump (Na^+/K^+) activity only. It is known that the second ionic transporting mechanism involved in cell volume regulation, is electrogenic sodium/calcium exchange (Na^+/Ca^{2+} exchange) and that 10^{-9} M [H^{3+}]-ouabain, which is unable to activate sodium/potassium pump (Na^+/K^+) has activation effect on sodium/calcium exchange (Na^+/Ca^{2+}) in reverse mode.

However, the MV has depression effect on tissue hydration induced by 10^{-9} M [H^{3+}]-ouabain. This difference can be explained by the fact that the metabolic activity is depressed *in*

vitro conditions, which brings to the increase of intracellular Na^+ and Ca^{2+} ions content. As sodium (Na^+) uptake is more pronounced in brain cortex cells, the increase of intracellular sodium (Na^+) in brain cortex cells leads to hydration, while in cardiac muscle tissues it brings to dehydration, due to the increase of intracellular Ca^{2+}-induced contraction of myosin.

As it was mentioned above, 10^{-4} M of [H^{3+}]-ouabain has poisoning effect on the cell membrane mechanisms by increasing the cell inner osmotic activity and by stimulating the extracellular water quick penetration into the cell. As a result, endogenous water level increases and consequently enlarges cell volume. This enhances penetration of the large amount of sodium (Na^+) ions due to pump suppressed activity, to which metabolic processes counteract.

Studies of the effect of MV of 4Hz, 30dB 10 minutes on [H^{3+}]-ouabain binding with cell membrane in cortex and cardiac muscle tissues revealed increased number of [H^{3+}]-ouabain (10^{-9} M) binding molecules with membrane. Although MV has dehydration effect on brain cortex and cardiac tissues, this leads to the decrease of [H^{3+}]-ouabain (10^{-4} M) receptors in membrane [4]. As the results of the current experiments shows that the cell poisoning by 10^{-4} M [H^{3+}]-ouabain MV-induced dehydration leads to the decrease in the number of [H^{3+}]-ouabain receptors.

From these data it can be concluded that MV-induced tissue dehydration is due to activation of sodium/calcium (Na^+/Ca^{2+}) exchange in reverse mode pushing Ca^{2+} ions from the cell.

Previous study of our laboratory has shown that 10^{-9} M [H^{3+}]-ouabain which is agonist for α_3-isoform of sodium/potassium-ATPase (Na^+/K^+-ATPase) and has only signaling function, activates cGMP formation and increases [H^{3+}]-ouabain receptors affinity as a result of decrease of intracellular [8] Ca^{2+}. All these data together confirm the Ayrapetyan's theory [5].

Conclusions.

1. Studies of hydration level changes, the number of [H^{3+}]-ouabain binding molecules, as well as a negative correlation of pain threshold value to tissue hydration/dehydration, all together confirms the hypothesis according to which increase cell volume (hydration) leads to increase in pain sensitivity, and dehydration causes the opposite effect.
2. Mechanical vibration has the analgesic effect, due to certain cell regulation mechanisms, which lead to pain threshold increase/decrease via hydration/dehydration changes. This again confirms the hypothesis.
3. Mechanical vibration causing dehydration of the brain and cardiac tissue samples is due to the Na: Ca pump activation. It has been shown that mechanical vibration (4Hz) causes increase in c-GMP, which leads to the Na^+:Ca^{2+} pump activation. The last changes bring removing of Ca^{2+} ions from the cell, which, in turn, contributes to the increased Na^+/ K^+ pump activity. These processes cascade causes the cell dehydration.

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ԱՄՓՈՓՈՒՄ

ՄԵԽԱՆԻԿԱԿԱՆ ՎԻԲՐԱՑԻԱՅԻ ԱԶԴԵՑՈՒԹՅՈՒՆՆ ԱՌՆԵՏՆԵՐԻ ԳԼԽՈՒՂԵՂԻ ԿԵՂԵՎԻ ԵՎ ՍՐՏԱՄԿԱՆԻ ՀՅՈՒՍՎԱԾՔՆԵՐԻ ՀԻԴՐԱՏԱՑԻԱՅԻ ԵՎ ՑԱՎԻ ՇԵՄԻ ՎՐԱ

ՄՈՒՇԵՂՅԱՆ Գ.Ք., ՄԱԴՈՅԱՆ Գ.Կ., ԱՋԻՋՅԱՆ Ա.Է., ՄԱՐԳՍՅԱՆ Գ.Վ., ԱՐԱՋՅԱՆ Գ.Մ.

In vivo և in vitro պայմաններում բջջի նյութափոխանակության գործընթացում նկատվող փոփոխությունը գնահատելու նպատակով հետազոտվել է 4Հց հաճախականությամբ, 30դԲ ուժգնությամբ, հորիզոնական դիրքով, մեխանիկականական վիբրացիայի ազդեցությունն առնետների ցավի շեմի արժեքի և գլխուղեղի կեղևի, սրտի հյուսվածքների նմուշների հիդրատացիայի ցուցանիշի վրա: In vitro պայմաններում իրականացվող փորձերում, վերը նշված հյուսվածքների նմուշները ենթարկվել են 30րոպե տևողությամբ ինկուբացիայի Թիրոդեի ֆիզիոլոգիական լուծույթում: Բացի այդ, հյուսվածքների նմուշներում թաղանթի ընկալիչների [Na^+/K^+ ԱԵՖ-ազների] թվի գնահատման նպատակով, նմուշները 30րոպե տևողությամբ ենթարկվել են Na^+/K^+ փոխանակիչի գործունեությունը ընկճող [3^+ H]–օուաբաինի 10^{-9}Մ и 10^{-4}Մ կոնցենտրացիաներով լուծույթներում: Ստացված արդյունքերը վկայում են, որ 4Հց հաճախականությամբ, 30դԲ ուժգնությամբ, մեխանիկականական վիբրացիան 105%-ով հանգեցնում է կենդանիների ցավի շեմի արժեքի բարձրացմանը, այսինքն ցավազգայնության ցուցանիշի իջեցմանը, որը 14,6%-ով կորելացվում է հյուսվածքների ջրազրկմամբ (14,6%-ով):

Հետազոտության արդյունքները հավաստում են բջջային մակարդակով ցավի շեմի արժեքի և հիդրատացիայի ցուցանիշներում նկատվող փոփոխությունները հաստատվում են նյարդային և մկանային համակարգերի միջև առկա ֆունկցիոնալ ակտիվությամբ: Սա հիմք է հանդիսանում այն հիպոթեզի, ըստ որի բջջի ծավալի փոփոխությունը պետք է դիտել որպես մարկեր տարբեր բջիջների ֆունկցիոնալ վիճակը բնութագրելու համար:

РЕЗЮМЕ

ВЛИЯНИЕ МЕХАНИЧЕСКОЙ ВИБРАЦИИ НА ЗНАЧЕНИЕ ШКАЛЫ БОЛИ И ГИДРАТАЦИИ КОРЫ ГОЛОВНОГО МОЗГА И СЕРДЕЧНОГО СЛОЯ У КРЫС

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Для того чтобы оценить изменения в клетках, замечаемые в процессе обмена веществ при условии in vivo и in vitro было изучено влияние механической вибрации при частоте 4Гц, силе 30дБ, в горизонтальном состоянии, на значение шкалы боли и гидратации коры головного мозга и сердечного слоя у крыс. Во время опытов в условии in vitro, приведенные выше примеры слоев были обработаны физиологическим раствором Тироидеи в течение 30 минут. Кроме того, для того, чтобы оценить количество приемников Na^+/K^+ АТФ в оболочке в слоях, они были обработаны 10^{-9}M և 10^{-4}M растворами [$З^+$Н] оуабаина, который улучшает деятельность заменителя Na^+/K^+ в течение 30 минут.

Полученные результаты свидетельствуют, что механическая вибрация при частоте 4Гц, силе 30дБ приводит к увеличению значения шкалы боли у животных, то есть к уменьшению чуствительности, которое кореллируется обезвоживанием на 14,6%. Результаты исследования доказывают, что перемены значения шкалы боли на клеточном уровне и гидратации подтверждаются функциональной активностью между нервными и мышечными системами. Это является основой гипотезы, по которой перемену обьема клетки можно маркировать для описания функционального состояния различных клеток.