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# QUANTUM MECHANICAL NATURE OF PAIN SENSITIVITY

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## ABSTRACT

Previously, we have suggested the metabolic controlling of cell hydration as a common quantum mechanical sensitive target through which different factors could modulate membrane excitability and impairment of metabolic driving of water efflux from the cells being a key mechanism, which inhibits inward $Na^+$ currents ($I_{Na}$), considering as a primary mechanism for generation of membrane excitation. The $Na^+/K^+$ pump dysfunction, which is consequence of cell pathology, has a central role in generation of water efflux from the cells and controls membrane excitability for $Na^+$ ions. It is known that the dysfunction of $Na^+/K^+$ pump leads to the increase of intracellular $Ca^{2+}$ ($[Ca^{2+}]i$) as a result of activation of $Na^+/Ca^{2+}$ exchange in the reverse (R) mode. Our previous study has shown that $RNa^+/Ca^{2+}$ exchange can be activated also through the activation of G-proteins in the membrane leading to elevation of intracellular cAMP, which activated by different weak chemical and physical factors is unable to activate ionic channels in the membrane and modulate $Na^+/K^+$ pump activity but is able to stimulate synaptic transmitters' release from presynaptic ending. The fact that the increase of $[Ca^{2+}]i$ leads to contraction of muscle and to nerve ending hydration suggests that the mechanical deformation of junction between nerve ending and endplate could serve as sources for pain signal generation. To check this hypothesis, the comparative study of the effects of intraperitoneal injections with PS containing $^{40}Ca^{2+}$ and PS containing $^{45}Ca^{2+}$ on pain threshold and tissue hydration depending on $Na^+/K^+$ pump and $Na^+/Ca^{2+}$ exchange activities have been performed. The obtained data bring to suggestion that the activation of $Na^+/Ca^{2+}$

exchange has quantum mechanical sensitive mechanism serving as a primary mechanism for pain signal generation.

**KEYWORDS:** Rat, Thermal Sensitivity, Brain Cortex, Cerebellum, Heart Muscle.

## 1. INTRODUCTION

Pain can be provoked by different phenomena starting from mechanical damage to the dysfunction of different metabolic pathways. It could be modulated by weak physical factors, intensity of which is less than thermal one's and by very low concentrations of chemical compounds unable to activate ionic channels in the membrane. This fact indicates that in cell membrane so called nociceptor is distinct metabolic mechanism, which has quantum mechanical sensitivity and is involved in the activation of ionic channels in the membrane during cell pathology. Despite of marked advances in pain study, the cellular mechanism of nociception, having quantum mechanical sensitivity is not evaluated yet, which could warrant opening of a new avenue to discover a novel physical and chemical pain therapy methods free of adverse effects on the organism. From the biophysical point of view, nociception can be considered as a hyper excitation of nerve ending leading to short or long lasting. Its morphological changes cause sustainable generation of action potentials and conduct these excitation signals toward the spinal cord into pain sensing center of brain cortex.

Our previous data have shown that net water uptake by cells leads to the increase of membrane excitation by direct activation of ionic channels for inward ionic currents as well as by membrane-surface-dependent increase of functional ionic channels in the membrane.[1,2] Therefore, the metabolic generation of water efflux from the cells is considered to be a key mechanism, which is controlled low permeability of membrane for $I_{Na}$ and membrane excitability. On the basis of the data that the sustainable impairment of metabolic water efflux from the cell leads to overhydration of neurons, we have suggested cellular mechanism in neurons of CNS as a result of hyperexcitation, which is considered as pain sensation. The electrogenic $Na^+/K^+$ pump is a key mechanism in generation of water efflux from the cell, which is due to the increase of osmotic gradient on the membrane and the stimulation of intracellular oxidative phosphorylation–induced water molecules. It is known that $Na^+/K^+$ pump inhibition induced the increase of intracellular $Na^+$ ($[Na^+]_i$), which leads to the activation of $Na^+/Ca^{2+}$ exchange in the reverse (R) mode.[3] The next mechanism, which controls membrane excitability due to the activation of water efflux from the cell functioning in stoichiometry $3Na^+$:$1Ca^{2+}$ and $[Ca^{2+}]_i$-induced stimulation of mitochondrial

function releases the endogen water molecules in cytoplasm.[4] Previously, we have shown that the activation of $RNa^+/Ca^{2+}$ exchange takes place also by $Na^+/K^+$ pump independent mechanism, such as activation of G proteins in the membrane leading to elevation of the intracellular cAMP-content, having activation effect on $Ca^{2+}$-pump in the membrane of endoplasmic reticulum (ER), which pushes $[Ca^{2+}]_i$ from cytoplasm into ER. The cAMP-dependent $RNa^+/Ca^{2+}$ exchange is much more sensitive to weak chemical and physical factors than the $Na^+/K^+$ pump inhibition-induced activation of $RNa^+/Ca^{2+}$ exchange.[2,5-7] The activation of $RNa^+/Ca^{2+}$ exchange leading on the one hand to the increase of membrane excitation by intracellular $Ca^{2+}$ ($[Ca^{2+}]_i$ )-induced inhibition of $Na^+/K^+$ pump activity leads to neuronal hydration[4] and on the other hand the elevation of $[Ca^{2+}]_I$ activates the release of synaptic transmitters from presynaptic terminals.[4] Considering the fact that over 50% of the volume of myocytes is occupied by myosin, the activation of $RNa^+/Ca^{2+}$ exchange-induced increase of $[Ca^{2+}]_i$ leads to contraction of endplate and to micromechanical deformation of junction between presynaptic and postsynaptic membranes, which is suggested as a source for generation of sustainable hyperexcitation of nerve ending (pain signal). Therefore, the activation of $RNa^+/Ca^{2+}$ exchange-induced hydration of nerve ending and dehydration of endplate are suggested as a primary quantum mechanical sensitive mechanism generating pain signals. To check this suggestion, the comparative study of the effects of $^{40}Ca^{2+}$ (cold) and $^{45}Ca^{2+}$ (radioactive) containing physiological solution (PS) intraperitoneal injections on thermal pain thresholds and brain and heart tissues hydration as well as the $^{45}Ca^{2+}$ uptake by these tissues in different experimental conductions were performed.

## 2. MATERIALS AND METHODS

**2.1 Animals:** All the 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 adult (8 weeks old) male albino rats. The animals were regularly examined, kept in the control of the veterinary in LSIPEC and reserved in a specific pathogen-free animal room under optimum conditions of 12-h light/dark cycle, at temperature of 22 ± 2$^o$C, with a relative humidity of 50% and were fed *ad libitum* on a standard lab chow and water.

**2.2 Chemicals:** Tyrode's physiological solution (PS) containing (in mM) 137 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1.05 $MgCl_2$, 5 $C_6H_{12}O_6$, 11.9 $NaHCO_3$, and 0.42 $NaH_2PO_4$ and adjusted to pH 7.4 with NaOH was used. All chemicals were obtained from ''Medisar'' Industrial Chemical

Importation Company (Yerevan, Armenia). The cold (non-radioactive) ouabain (PerkinElmer, Massachusetts, USA) at $10^{-9}$M and $10^{-4}$M dissolved in PS were used for intraperitoneal (i/p) injection. PS with the radioactive $^{45}Ca^{2+}$ (PerkinElmer, Massachusetts, USA) was received by substituting 0.0115mM of $CaCl_2$ from 1.8 mM $CaCl_2$ by radioactive one (with 11.2 mCi/l activity). The volume of injected solutions was adjusted according to the weight of animals (0.02ml/g).

All the experiments were carried out in ***in vivo*** conditions. The 1st series was carried out on three animal groups (6 animals in each) and the pain sensitivity latent period was defined in all rats. The rats of the first group were intact (sham). The animals of the 2nd and 3rd groups were i/p injected with PS containing $^{40}Ca^{2+}$ and PS containing $^{45}Ca^{2+}$, respectively. In the figures the names of these solutions are indicated as $^{40}Ca^{2+}$ and $^{45}Ca^{2+}$, respectively. After this procedure the animals were decapitated and 5 samples were taken from each animal's brain cortex, cerebellum and heart muscle tissues. Thus, from each tissue were received 30 samples of brain cortex, cerebellum and heart muscle. Then these tissue samples were three times washed in PS and the definition of water content and $^{45}Ca^{2+}$ uptake were made.

In the 2nd series of experiments there were also taken three animal groups (n=6). Animals of first group (sham) were i/p injected with PS. The rats of the 2nd and 3rd groups were i/p injected with PS containing $^{40}Ca^{2+}$ (with addition of ouabain at$10^{-4}$M) and PS containing $^{45}Ca^{2+}$ (with addition of ouabain at $10^{-4}$M), respectively. The pain sensitivity latent period was defined in all rats. The rest of the procedures were the same as in the 1st series. The 3rd series of experiments was identical to the 2nd one. Animals of the first group (sham) were i/p injected with PS. The animals of the 2nd and 3rd groups were i/p injected with the same manner but in this case the cold ouabain at $10^{-9}$M was used. The rest of the procedures were the same as in the 1st series.

### 2.4. Determination of Latent Period of Pain Sensitivity (LPPS)

This test was conducted by a specific hot plate setup constructed in LSIPEC and approved by the Ethic Committee of UNESCO Chair in Life Sciences. It consists of Plexiglas cage with a brass bottom. The bottom temperature (52.2$^{o}$C) was controlled with the thermometer (accuracy of measurement 0.01$^{o}$C). Brass bottom was completely covered for keeping the temperature constant. Latent period of pain sensitivity was recorded 15 min after injection. 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 defined visually (in sec)

as the time elapsed to obtain one of the following responses: licking the feet, jumping or rapidly stamping the feet. The tissue damage prevention was nearly 10 sec. In our experiments the latency periods were measured in intact and injected groups. Decapitation was made just after the measurement of latent period. Statistic significance was defined between data of sham and experimental groups.

**2.5. Tissue Preparation:** The tissue samples from each experiment were investigated after decapitation. Since the anesthetics with different chemical and pharmacological profiles significantly affect the metabolic processes in tissues[4], in our experiments the animals were sharply immobilized by freezing method[4] and decapitated. Full absence of somatic reflexes was recorded after this procedure. The heart muscle, brain cortex and cerebellum tissues were isolated and dissected according to the corresponding experiments.

**2.6. Definition of Water Content:** The water content of heart muscle, brain cortex and cerebellum tissues was determined by traditional "tissue drying" method.[4] After measuring the wet weight (w.w.) of tissue samples 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. Measurement of $^{45}Ca^{2+}$ uptake**

In ***in vivo*** experiments the tissue samples, which were subjected to $^{45}Ca^{2+}$ were homogenized in 50 µl of 68% $HNO_3$ solution after determination of wet and dry weights. Then 2 ml of Bray's scintillation fluid was added and chemiluminescence of samples was quantified with 1450-MicroBeta liquid scintillation counter (Wallac, Turku, Finland). The number of $^{45}Ca^{2+}$ 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. The statistical significance in comparison with the control group was calculated with Student's t-test with the following symbols (*p<0.05; **p<0.01; ***p<0.001).

## 3. RESULTS

To evaluate the possible difference between effects of PS containing $^{40}Ca^{2+}$ and PS containing $^{45}Ca^{2+}$ on the latent period of pain sensitivity (LPPS) the animals have been i/p injected with

these solutions and 15 min after their LPPS has been measured on hot plate. These results have been compared with that of sham animal group. As can be seen in Fig. 1, LPPS of animal group i/p injected with PS containing $^{40}Ca^{2+}$ is higher (by 107.4%) than that of sham animal group.

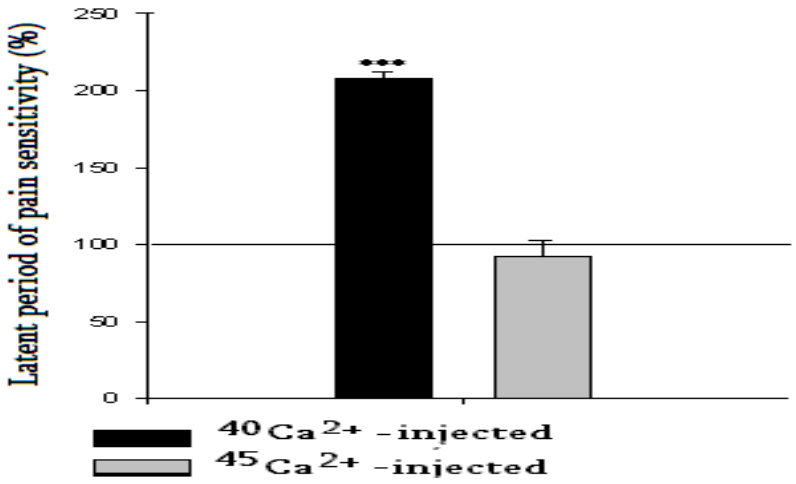


**Fig. 1: The effect of i/p injection with PS containing $^{40}Ca^{2+}$ (black column) and PS containing $^{45}Ca^{2+}$ (gray column) on latent period of pain sensitivity. Horizontal line indicates the data of latent period of pain sensitivity of sham (non injected) animals. Each bar represents the mean ±SEM (n=54). Data were expressed in % and compared to that of intact animal group. The symbol (***) indicates p<0.001. All data were obtained from three independent experiments.**

Meanwhile, in animals of experimental group injected with PS containing $^{45}Ca^{2+}$ LPPS leads to the decrease (by 8%) compared with that of sham animal group. After the definition of LPPS animals of all groups have been decapitated and the water content in their tissues has been indicated.

Our previous study has shown that painful heating by “hot plate” leads to dehydration of heart muscle tissue and hydration of brain cortex and cerebellum tissues. Nevertheless, leg movement because of heating leads to hydration of cerebellum tissue, which is considered as a marker for painful heating.[8]

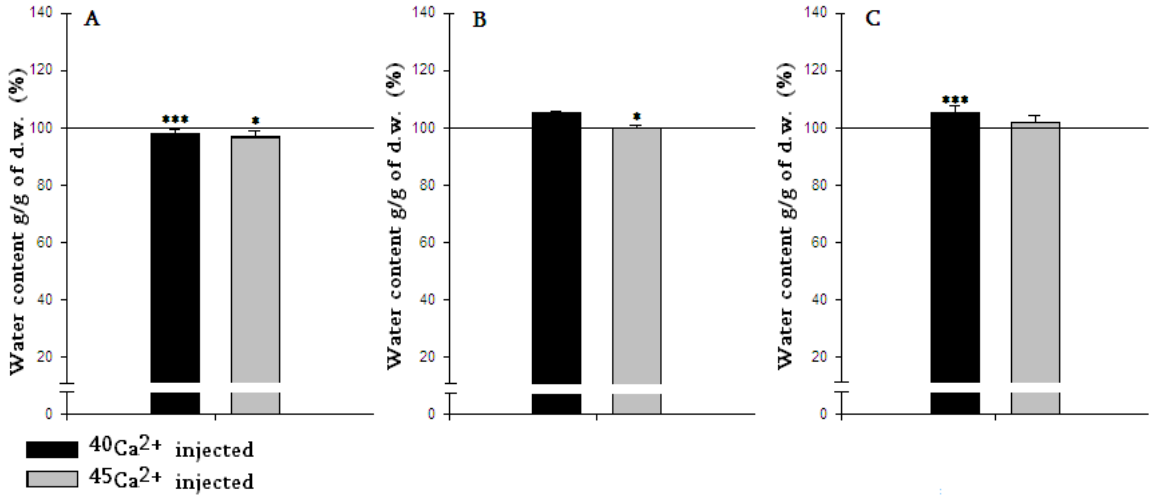


**Fig. 2: The effect of i/p injection with PS containing $^{40}Ca^{2+}$ (black column) and PS containing $^{45}Ca^{2+}$ (gray column) on water content in heart muscle (A), brain cortex (B) and cerebellum (C) tissues. Each bar represents the mean ±SEM (n=90). Horizontal line indicates the water content in corresponding data of non injected (sham) animals. All data were expressed in %. Data of injected animals compared to that of sham group. The symbols (*) and (***) indicate p< 0.05 and p<0.001, respectively. All data were obtained from three independent experiments.**

In Fig. 2 the data of water content in heart muscle, brain cortex and cerebellum tissues are presented after animals' injection with PS containing $^{40}Ca^{2+}$ and PS containing $^{45}Ca^{2+}$, respectively. These results are compared with that of sham (non-injected) animal group. As can be seen in Fig. 2A the water content in heart muscle tissue (compared with that in sham animals) has been decreased both after injection with PS containing $^{40}Ca^{2+}$ (by 1.6%) and PS containing $^{45}Ca^{2+}$ (by 2.6%), respectively. In case of brain cortex tissue there has been observed non-significant increase (by 5%) of water content after injection with PS containing $^{40}Ca^{2+}$ and the water content after injection with PS containing $^{45}Ca^{2+}$ is equaled to that of sham animals'. On the contrary, the water content in cerebellum tissue has been increased after both types of injections. After injection with PS containing $^{40}Ca^{2+}$ it has been increased by 6% and after injection with PS containing $^{45}Ca^{2+}$ by 3%.

To clarify the influence of $Na^+/K^+$ pump on pain sensitivity as well as in thermal pain-induced tissue hydration in the next series of experiments animals have been i/p injected with the same solutions with addition of ouabain, a well known specific inhibitor for $Na^+/K^+$ pump activity.[4]

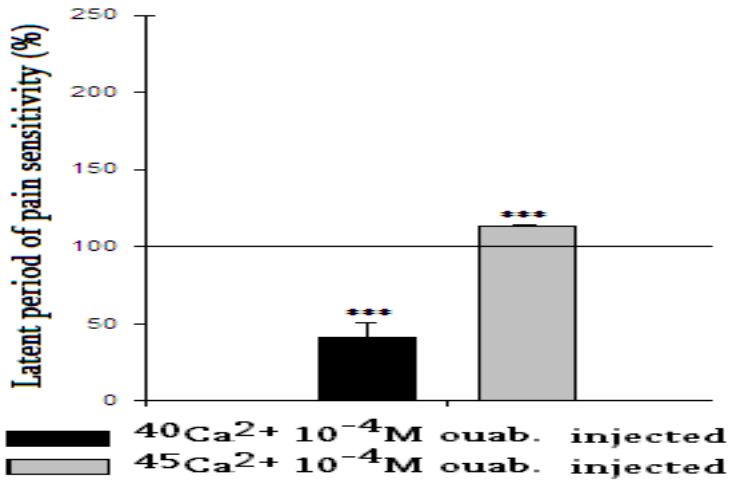


**Fig. 3: The effect of i/p injection with PS containing $^{40}Ca^{2+}$ (with addition of ouabain at $10^{-4}$M, black column) and PS containing $^{45}Ca^{2+}$ (with addition of ouabain at $10^{-4}$M, gray column) on latent period of pain sensitivity. Horizontal line indicates the data of latent period of pain sensitivity of sham animals i/p injected with PS. Each bar represents the mean ±SEM (n=54). Data were expressed in % and compared to that of intact animal group. The symbol (***) indicates p<0.001. All data were obtained from three independent experiments.**

As can be seen in Fig. 3 in animals injected with PS containing $^{40}Ca^{2+}$ (with addition of ouabain at $10^{-4}$M) LPPS has been extensively decreased (by 59%) compared with that of animals of sham group injected with PS. It must be noted that in this condition the animals become more sensitive to thermal pain. Meanwhile, the opposite data have been observed when the animals of the next group have been injected with PS containing $^{45}Ca^{2+}$ (with addition of ouabain at $10^{-4}$M). Their LPPS has been significantly increased (by 13%) compared with that of sham animals. Thus, in this case the animals become less sensitive to thermal pain. After decapitation of animals the water content in investigated tissues has been strongly changed.

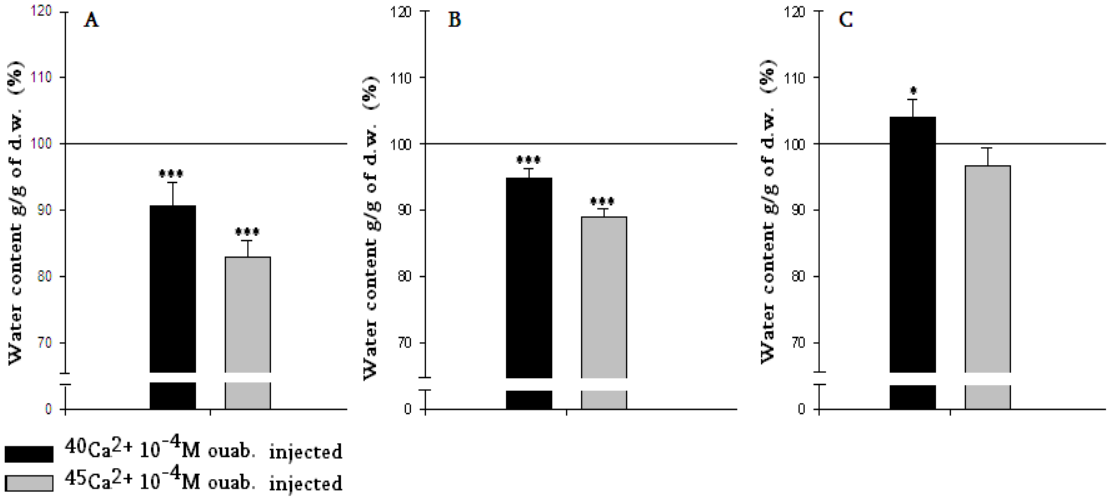


**Fig. 4: The effect of i/p injection with PS containing $^{40}Ca^{2+}$ (with addition of ouabain at $10^{-4}$M, black column) and PS containing $^{45}Ca^{2+}$ (with addition of ouabain at $10^{-4}$M, gray column) on water content in heart muscle (A), brain cortex (B) and cerebellum (C) tissues. Each bar represents the mean ±SEM (n=90). Horizontal line indicates the water content in corresponding data of sham animals i/p injected with PS. All data were expressed in %. Data of injected animals compared to that of sham group. The symbols (*) and (***) indicate p< 0.05 and p<0.001, respectively. All data were obtained from three independent experiments.**

As can be seen in Fig. 4 in heart muscle and brain cortex tissues the expressed dehydration has been observed (Fig. 4A, B). In heart muscle tissue (Fig. 4A) the water content after injection with PS containing $^{40}Ca^{2+}$ decreased by 9.3%, while after injection with PS containing $^{45}Ca^{2+}$ by 17%. These data were compared with that of sham group animals injected with PS. The identical effect has been demonstrated in brain cortex tissue (Fig. 4B). After injection with PS containing $^{40}Ca^{2+}$ the water content has been decreased by 5%, while the injection with PS containing $^{45}Ca^{2+}$ leads to water decrease by 11%. As for the cerebellum tissue there is pronounced hydration after injection with PS containing $^{40}Ca^{2+}$ (by 23%) and the dehydration after injection with PS containing $^{45}Ca^{2+}$ (by 3%).

Our previous study has shown that the injection with nM ouabain in rats leads to more pronounced hydration effect in cerebellum tissue than the injection with ouabain free PS solution.[13] The effect of nM ouabain is indicated in other experiments where its stimulating effect on cAMP-dependent $RNa^+/Ca^{2+}$ exchanger is demonstrated without changing $Na^+/K^+$ pump activity.[6] Therefore, to estimate the role of cAMP dependent $RNa^+/Ca^{2+}$ exchange in

realization of painless effect and tissue dehydration in the next series of experiments the comparative study of PS containing $^{40}Ca^{2+}$ and PS containing $^{45}Ca^{2+}$ injections' effects (adding nM ouabain) on LPPS and tissue hydration have been studied.

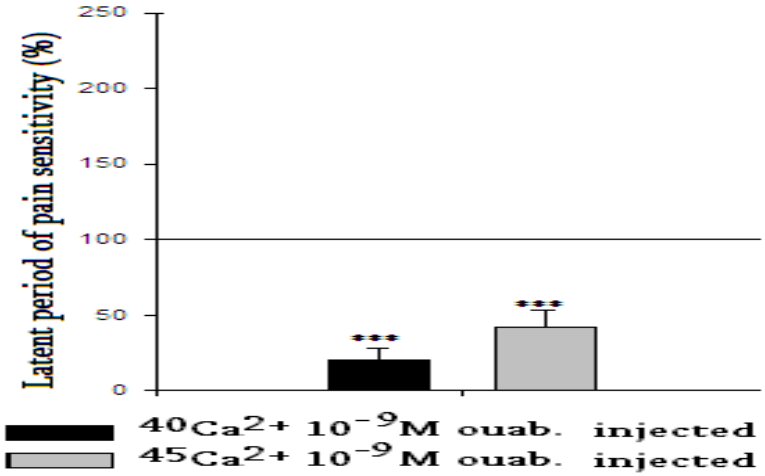


**Fig. 5: The effect of i/p injection with PS containing $^{40}Ca^{2+}$ (with addition of ouabain at $10^{-9}$M, black column) and PS containing $^{45}Ca^{2+}$ (with addition of ouabain at $10^{-9}$M, gray column) on latent period of pain sensitivity. Horizontal line indicates the data of latent period of pain sensitivity of sham animals i/p injected with PS. Each bar represents the mean ±SEM (n=54). Data were expressed in % and compared to that of intact animal group. The symbol (***) indicates p<0.001. All data were obtained from three independent experiments.**

Fig. 5 shows the data of LPPS of sham (injected with PS) and experimental (injected with PS containing $^{40}Ca^{2+}$ and PS containing $^{45}Ca^{2+}$, respectively) animals. Ouabain at $10^{-9}$M has been added in injected solution of experimental animals. In group of animals injected with PS containing $^{40}Ca^{2+}$ LPPS is shorter than that of next animal group being injected with PS containing $^{45}Ca^{2+}$ LPPS of animal group injected with PS containing $^{40}Ca^{2+}$ is by 80% lower than that of sham animal group, i.e. the sensitivity of these animals has been greatly increased. LPPS of animal group injected with PS containing $^{45}Ca^{2+}$ is also low of LPPS of sham animal group by 57.6%. These data show that under the influence of $10^{-9}$M ouabain animals become more sensitive to pain than in case of the influence of $10^{-4}$M ouabain. Variation of water content in the investigated tissues is different. As can be seen in Fig. 6A, C in heart muscle and cerebellum tissues the identical effect is obtained in both types of injections. After injection with PS containing $^{40}Ca^{2+}$ the water content in heart muscle tissue has been increased by 7% and in cerebellum tissue by 6%. Meanwhile, the opposite effect has

been shown after injection with PS containing $^{45}Ca^{2+}$, the water content in heart muscle tissue has been decreased by 8%, in cerebellum tissue by 17%. As for brain cortex tissue there has been an extensive dehydration on both types of injections.

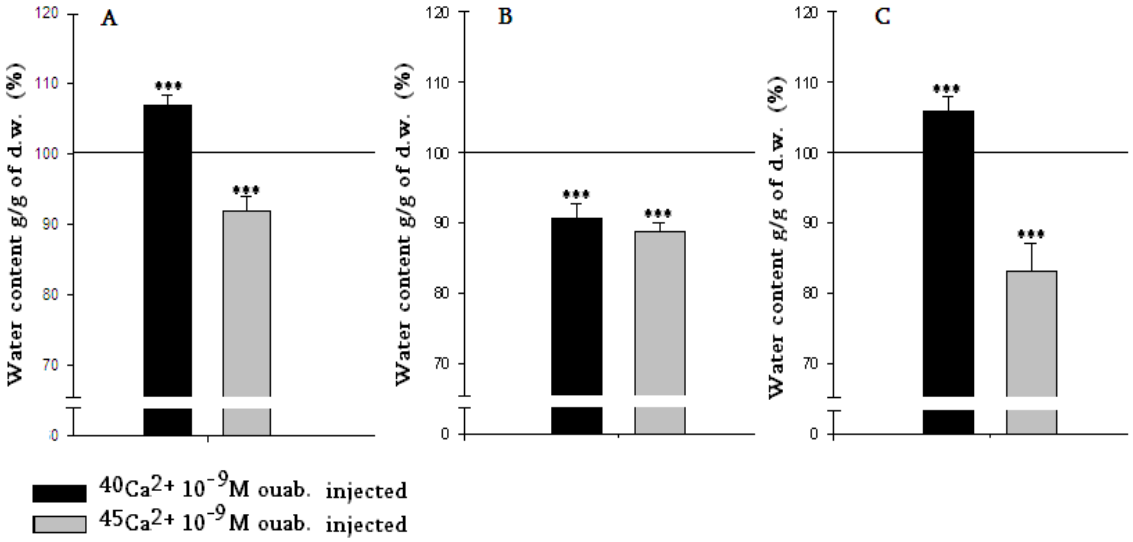


**Fig. 6: The effect of i/p injection with $^{40}Ca^{2+}$ containing PS (with addition of ouabain at $10^{-9}$M, black column) and $^{45}Ca^{2+}$ containing PS (with addition of ouabain at $10^{-9}$M, gray column) on water content in heart muscle (A), brain cortex (B) and cerebellum (C) tissues. Each bar represents the mean ±SEM (n=90). Horizontal line indicates the water content in corresponding data of sham animals i/p injected with PS. All data were expressed in %. Data of injected animals compared to that of sham group. The symbols (**) and (***) indicate p< 0.01 and p<0.001, respectively. All data were obtained from three independent experiments.**

The water content in this tissue after injection with PS containing $^{40}Ca^{2+}$ has been decreased by 10% and after injection with PS containing $^{45}Ca^{2+}$ it has been diminished to 12%. The obtained data show the different effects of PS containing $^{45}Ca^{2+}$ injections on LPPS as well as on tissue hydration, especially when in these solutions there are ouabain at $10^{-4}$M or at $10^{-9}$M. As PS containing $^{45}Ca^{2+}$ with $10^{-4}$M and $10^{-9}$M ouabain has pain relief and painful effects, respectively, in the next series of experiments the effects of both concentrations of ouabain on $^{45}Ca^{2+}$ uptake have been studied.

As can be seen in Fig. 7 the $^{45}Ca^{2+}$ uptake in tissues is different at $10^{-4}$M and $10^{-9}$M. For example, in heart muscle tissue it is lower of that in sham animal group (i/p injected with PS containing $^{45}Ca^{2+}$ (Fig. 7A)). In case of injection of ouabain at $10^{-4}$M the $^{45}Ca^{2+}$ uptake is

decreased by 17%, while the injection of ouabain at $10^{-9}$M the $^{45}Ca^{2+}$ uptake is decreased by 16%. Another feature of $^{45}Ca^{2+}$ uptake is demonstrated in brain cortex and cerebellum tissues (Fig. 7B, C). In both cases the $^{45}Ca^{2+}$ uptake is increased being higher of that in sham animal group. In brain cortex tissue the $^{45}Ca^{2+}$ uptake is increased by 51% (when rats are injected in ouabain at $10^{-4}$M) and by 94% (when they are injected in ouabain at $10^{-9}$M). As for cerebellum tissue the $^{45}Ca^{2+}$ uptake is increased by 20% (when animals are injected in ouabain at $10^{-4}$M) and by 67% (when they are injected in ouabain at $10^{-9}$M).

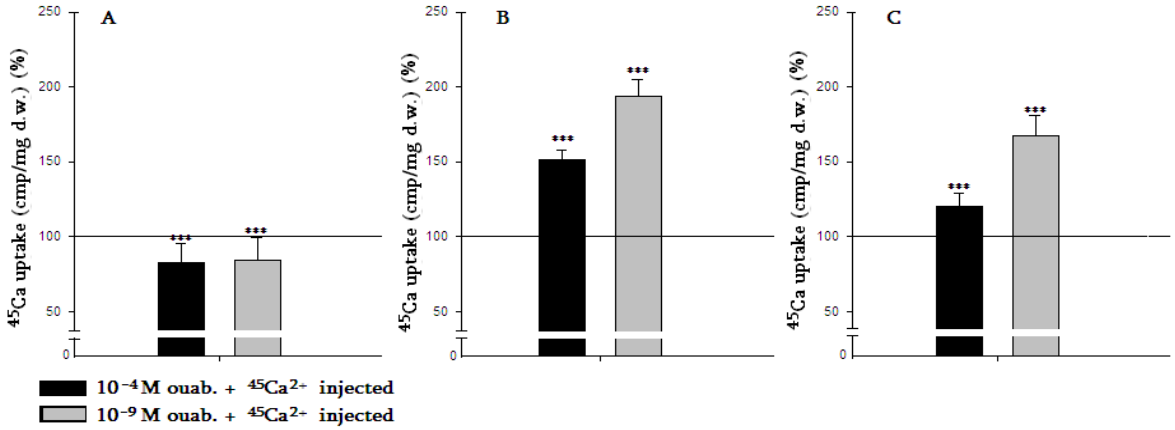


**Fig. 7: Comparative data of the $^{45}Ca^{2+}$ uptake in different concentrations of ouabain solution in heart muscle (A), brain cortex (B) and cerebellum (C). Black bars indicate the $^{45}Ca^{2+}$ uptake in animal tissues after i/p injection with PS containing $^{45}Ca^{2+}$ (with addition of ouabain at $10^{-4}$M). Gray bars indicate the $^{45}Ca^{2+}$ uptake after i/p injection with PS containing $^{45}Ca^{2+}$ (with addition of ouabain at $10^{-9}$M). Each bar represents the mean $\pm$SEM (n=90). Horizontal line indicates the water content in corresponding data of sham animals i/p injected with PS containing the $^{45}Ca^{2+}$. All data were expressed in %. Data of injected animals compared to that of sham group. The symbol (***) indicates p<0.001. All data were obtained from three independent experiments.**

It must be noticed that in both types of tissues the $^{45}Ca^{2+}$ uptake is higher in incubation of tissues in ouabain at $10^{-9}$M than in incubation of tissues in ouabain at $10^{-4}$M.

## 4. DISCUSSION

By previous study we have shown that rats' painful heating (at 52.2$^{o}$C) leads to dehydration effect on heart muscle and brain cortex tissues compared to that of painless heating (at 38$^{o}$C), while in cerebellum tissue the opposite feature is observed, the painful heating brings to hydration effect. On the basis of the fact that the difference between effects of painful and

painless heating on tissue hydration becomes more pronounced upon the rats' injection with nM ouabain, having activation effect on cAMP-dependent $RNa^{+}/Ca^{2+}$ exchange we have suggested on involvement of $RNa^{+}/Ca^{2+}$ exchange in generation of pain signal.[13] The data that the cerebellum regulating body motor function is characterized by tissue hydration to the painful heating is suggested as a marker for pain sensation.[13]

The obtained data have demonstrated that i/p injection with PS containing the $^{40}Ca^{2+}$ leads to the increase of LPPS (Fig. 1). This effect is accompanied by the increase of hydration in brain cortex and cerebellum tissues and dehydration of heart muscle tissue (Fig. 2), which indicates that brain tissue hydration is due to the release of hormonal factors in blood having contraction effect on heart muscle. As heart muscle hydration has a key role in the regulation of contractility depending on $[Ca^{2+}]i$, the mentioned hormonal-factor induces the dehydration of heart muscle tissue, which brings to the increase of $[Ca^{2+}]i$.

The data that PS containing the $^{45}Ca^{2+}$ injections have even slight shortening effect on LPPS (Fig. 1), and tissue dehydration (Fig. 2), i.e. injection with PS containing the $^{45}Ca^{2+}$ has stress release effect on rats (induced by punching) clearly indicate that the $^{45}Ca^{2+}$ uptake having quantum mechanical sensitivity has different metabolic effect on LPPS and tissues hydration than $^{40}Ca^{2+}$.

Our study has shown that the activation of water efflux from the cells by i/p injection with hypertonic solutions leads to brain cortex tissue dehydration accompanied by the increase of LPPS.[9] These data predict that the injection with PS containing $^{40}Ca^{2+}$ could have strong dehydration effect on brain cortex tissue, while the injection with PS containing $^{45}Ca^{2+}$ has dehydration effect. However, the injection with PS containing $^{40}Ca^{2+}$ having hydration effect on brain cortex tissue and dehydration effect on heart muscle tissue allows to suggest that the increase of LPPS probably can be explained by stress-hormone-induced contraction in muscle, which is absent in case of PS containing $^{45}Ca^{2+}$ injection. The data that the heart muscle contraction is accompanied by dehydration of brain cortex tissue in case of injection with PS containing $^{45}Ca^{2+}$ leading to stress release effect on LPPS indicate on different metabolic effects on brain cortex in case of injections with PS containing $^{40}Ca^{2+}$ and PS containing $^{45}Ca^{2+}$ (Fig. 2).

The injection with PS containing the $^{40}Ca^{2+}$ with ouabain at $10^{-4}M$ leads to the decrease of LPPS (Fig. 3) can be explained by inactivation of electrogenic $Na^{+}/K^{+}$ leading to membrane

depolarization as well as by hydration-induced increase of functionally active ionic channels in the membrane.[4,2] It is known that the $Na^+/K^+$ pump inactivation leads to the activation of $RNa^+/Ca^{2+}$ exchange leading to membrane hyperpolarization and to the increase of endogen water formation through the activation of mitochondrial function. The fact that $Na^+/K^+$ pump inactivation causes more pronounced dehydration effect on heart muscle (Fig. 4) than in ouabain-free medium (Fig. 2) indicates on the activation of $RNa^+/Ca^{2+}$ exchange leading to $[Ca^{2+}]$i,-dependent muscle contraction. Therefore, the brain cortex tissue dehydration can be explained by the activation of $RNa^+/Ca^{2+}$ exchange, while cerebellum tissue and cerebellum tissue hydration as a result of activation of defense pathways.

Pain relief effect by injection with PS containing $^{45}Ca^{2+}$ at $10^{-4}$M ouabain, which is accompanied with more pronounced dehydration effects on heart muscle as well as on brain cortex and cerebellum tissues (Fig. 4) than in case of injection with PS containing $^{40}Ca^{2+}$ with ouabain at $10^{-4}$M can be explained by the $^{45}Ca^{2+}$ induced more pronounced activation of $RNa^+/Ca^{2+}$ exchange. These suggestions are supported by the data on comparative study of the effects of i/p injections with PS containing $^{40}Ca^{2+}$ and PS containing $^{45}Ca^{2+}$ with nM ouabain, which has activation effect on cAMP-dependent $RNa^+/Ca^{2+}$ exchange in brain cortex and heart muscle tissues where $Na^+/K^+$ pump activity does not have changes.[4,6]

The injection with PS containing $^{40}Ca^{2+}$ with nM ouabain, having activation of cAMP-dependent $RNa^+/Ca^{2+}$ exchange leads to the strong decrease of LPPS, which is accompanied by hydration of heart muscle tissue, pronounced dehydration of cortex brain tissue and expressed hydration of cerebellum tissue (Fig. 5). The heart muscle hydration can be explained by cAMP-dependent $RNa^+/Ca^{2+}$ exchange-induced sharp increase of heart muscle contraction, which stimulates the $[Ca^{2+}]_i$-calmodulin-NO-cGMP pathways activating $Na^+/Ca^{2+}$ exchange in the forward mode, which has hydration effect on heart muscle.[4] The dehydration in brain cortex tissue is due to the activation of cAMP dependent $RNa^+/Ca^{2+}$ exchange, which is the result of cAMP–induced activation of $Ca^{2+}$-pump in membrane ER, which through junction between ER and mitochondria stimulates the oxidative phosphorylation and releases water molecules in cytoplasm.[4]

The data that the injection with PS containing $^{45}Ca^{2+}$ at nM ouabain has less painful effect (Fig. 5) than the injection with PS containing $^{40}Ca^{2+}$ is accompanied by dehydration effect of heart muscle tissues as well as cortex and cerebellum (Fig. 6). Probably, this is due to more

pronounced activation effect of $^{45}Ca^{2+}$ on mitochondrial activity, which produces release of water molecules in cytoplasm than $^{40}Ca^{2+}$.

The injection with PS containing $^{45}Ca^{2+}$ at nM ouabain has less pain relief effect than the injection with PS containing $^{45}Ca^{2+}$ at $10^{-4}$M ouabain can be explained by $RNa^{+}/Ca^{2+}$ exchange induced more intensive water efflux leading depression membrane excitability because of $Na^{+}/K^{+}$ pump inactivation induced overhydrated initial state of brain cells.

The data that $10^{-9}$M ouabain has more pronounced activation effect on $RNa^{+}/Ca^{2+}$ exchange than $10^{-4}$M ouabain clearly indicate that $10^{-9}$M ouabain-induced activation cannot be explained by inactivation of $Na^{+}/K^{+}$ pump as traditionally suggested[10] and it is due to the activation of quantum mechanical sensitive cAMP–dependent decrease of $[Ca^{2+}]_i$ (Fig. 7). However, the question of the nature of quantum mechanical sensitive target through which the injection with PS containing $^{45}Ca^{2+}$ has more pronounced activation on $RNa^{+}/Ca^{2+}$ and pain relief effects than the injection with PS containing $^{40}Ca^{2+}$ stays open and needs more detailed investigation.

## CONCLUSION

**Thus, summary of the obtained results brings to the conclusion**

1. The activation of cAMP-dependent $RNa^{+}/Ca^{2+}$ exchange leads to the generation of water efflux from the cells and pain relief effects, which are more pronounced in case of injection with PS containing $^{45}Ca^{2+}$ than that containing $^{40}Ca^{2+}$,
2. The quantum mechanical sensitive cAMP dependent $Na^{+}/Ca^{2+}$ exchange-induced water efflux from the excitable cells has pain relief, while its impairments pain signal generation commands cellular mechanism, which is a novel target for pain therapy.

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