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The Radioactive ^{45}Ca Cannot Be Used for Adequate Estimation of the Functional Activity of ^{40}Ca Ions in Cells and Organisms

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Abstract

Previously we have shown that nM ouabain-induced activation of cAMP-dependent Na/Ca exchange in reverse (R) mode in cell membrane has age-dependent weakening hydration effect on heart muscle and brain tissues and such Na/Ca exchange is characterized by quantum mechanical sensitivity. As in biological experiments radioactive ^{45}Ca is used for the study of cold ^{40}Ca exchange in cells and organisms, in the present work, the age-dependent effect of physiological solution (PS) containing either ^{40}Ca or ^{45}Ca on tissue hydration in different experimental conditions was studied in order to evaluate the bioequivalence of these two forms of Ca. The obtained data indicate that the intraperitoneal injections of ^{40}Ca PS and ^{45}Ca PS leading to activation of RNa/^{40}Ca and RNa/^{45}Ca exchanges, respectively, have different age-dependent effects on heart muscle and brain tissue hydration. As in myocyte membrane, the Na/Ca exchange is more expressed than in neuronal membrane, the age-dependent heart muscle hydration is more sensitive to quantum properties of Ca than brain tissue hydration. The $[^{45}Ca]_i$, in contrary to $[^{40}Ca]_i$, has age-dependent weakening and stabilizing effect on tissue hydration and makes the latter insensitive to ouabain. The obtained data bring us to a strong conclusion that RNa/Ca exchange has quantum mechanical properties and in biological experiments radioactive ^{45}Ca cannot be used for adequate estimation of the functional activity of ^{40}Ca ions in cells and organisms.

Keywords

Rat, Brain, Heart Muscle, ^{45}Ca, Na/Ca Exchange, Ouabain

1. Introduction

Metabolic control of cell hydration is a fundamental parameter determining its

functional activity. Our previous study has shown that the metabolically driven water efflux from the cell is a key mechanism controlling low membrane permeability for Na ions and membrane excitability [1]. Traditionally, the age-dependent increase of intracellular Ca ($[Ca]_i$) contents is considered as a result of activation of Na/Ca exchange in reverse (R) mode in response to Na/K pump dysfunction-induced increase of intracellular Na ($[Na]_i$) [2] [3]. However, we have shown that the activation of RNa/Ca exchange, occurring also upon the impact of extremely weak chemical and physical factors, is unable to change the Na/K pump and ionic channel activities in membrane, which are due to the increase of intracellular cAMP contents [4] [5] [6] [7] [8]. It is notable that, in spite of the fact that RNa/Ca exchange functions in stoichiometry of 3Na:1Ca, its activation, as a result of Na gradient decrease, only leads to cell dehydration, while in case of activation of cAMP-dependent decrease of intracellular Ca ($[Ca]_i$) contents, it has age-dependent weakening hydration effect on heart and brain tissues [9] [10].

Thus, on the basis of the above presented and literature data on the key role of intracellular messengers in regulation of $[Ca]_i$, the cGMP/cAMP-dependent Na/Ca exchange has been suggested as a universal membrane sensor through which the biological effects of weak signals on excitable cells are realized [8] [11] [12].

Our recent studies show that cAMP-dependent RNa/Ca exchange-induced cell hydration has quantum mechanical sensitivity: pM and nM radioactive [^{3}H]-ouabain modulate brain tissue hydration more effectively than the same doses of non-labeled (cold) ouabain [13]. Considering the fact that radioactive ^{45}Ca is widely used for the study of cold ^{40}Ca exchange in cells and organisms, it seems extremely important to evaluate the diversity of their functional activities. It is suggested that the comparative study of age-dependent effects of RNa/Ca exchange on heart muscle hydration (contraction) and brain tissue hydration after intraperitoneal (i/p) injections of physiological solution (PS) containing cold ^{40}Ca and radioactive ^{45}Ca could help to reveal the mechanism(s) through which the ^{45}Ca modulates heart muscle and brain tissue hydration. For this purpose, in the present work, the comparative study of age-dependent effects of RNa/^{40}Ca and RNa/^{45}Ca exchange on heart muscle and brain tissue hydration and ^{45}Ca uptake in different experimental conditions were performed.

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 young (6 weeks old) and old (18 months old) mail 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 was used. PS with radioactive ^{45}Ca (PerkinElmer, Massachusetts, USA) was received by substituting 0.0115 mM of $CaCl_2$ from 1.8 mM$CaCl_2$ with the radioactive one (with 11.2 mCi/l activity). The animals were i/p injected with PS containing ^{40}Ca (named as ^{40}Ca PS) and ^{45}Ca (named as ^{45}Ca PS). The volume of injected solutions was adjusted according to the weight of animals (0.02 ml/g). The ouabain solutions at 10^{-9} M and 10^{-4} M were used for incubation of tissue samples. PS with 50% of NaCl was received by replacing 68.5 mM of NaCl from 137 mM NaCl with 2 M mannitol dissolved in PS for maintaining the osmolarity of the solution. These two types of PS in corresponding figures are named as 100% Na PS and 50% Na PS. All chemicals were obtained from "Medisar" Industrial Chemical Importation Company (Yerevan, Armenia).

2.3. Tissue Preparation

The experimental data were received in *in vivo* and in *in vitro* conditions. The tissue samples from each experiment were investigated after decapitation. Since anesthetics with different chemical and pharmacological profiles have significantly effects on the metabolic processes in tissues [14] [15], in our experiments the animals were sharply immobilized by liquid nitrogen [16] and decapitated. After this procedure full absence of somatic reflexes was recorded. The heart muscle, brain cortex, subcortex and cerebellum tissues were isolated and dissected according to the corresponding experiments.

2.4. Experimental Design

The determination of tissue hydration and Ca uptake was carried out in *in vivo* conditions on young and old rats of intact and i/p injected groups. In each young and old animal groups 3 rats were taken. The animals of intact group were immobilized and decapitated at once and 5 samples from each animal's heart muscle, brain cortex, subcortex and cerebellum tissues were taken. The animals of the next groups were i/p injected with ^{40}Ca PS or ^{45}Ca PS, respectively. After 30 min they were immobilized and decapitated. From each animal, as in case of intact ones, the same number of tissue samples were taken. Thus, from each tissue 15 samples were received, where the water contents and ^{45}Ca uptake were defined. All our experiments were repeated three times.

The comparative effects on tissue hydration after their incubation in ouabain-free and 10^{-9} M, 10^{-4} M ouabain mediums were provided on nine young and old animals in control (preliminarily injected with ^{40}Ca PS) and experimen-

tal (preliminarily injected with ^{45}Ca PS) groups. From each group of animals 45 samples of heart muscle tissue and the same number of brain cortex samples were received. They were divided into 3 parts and incubated separately for 15 min in ouabain free PS (15 samples), 10^{-9} M ouabain solution (15 samples) and 10^{-4} M ouabain solution (15 samples).

The comparative effects on tissue hydration after their incubation in 100% Na PS and 50% Na PS were carried out on two parallel groups of animals. The control group of animals (6 young and 6 old rats) was preliminarily i/p injected with ^{40}Ca PS and from each animal 5 samples of heart muscle and brain cortex tissue were received. After that 15 samples of heart muscle (or brain cortex) tissue were incubated in 100% Na PS for 15 min, while the next 15 samples in 50% Na PS. The identical procedure was repeated on experimental group of young and old animals preliminarily i/p injected with ^{45}Ca PS.

2.5. Definition of Water Content

The water contents of heart muscle, brain cortex, subcortex and cerebellum tissues was determined by traditional "tissue drying" method [17]. After measuring the wet weight (w.w.) of tissue samples they were 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.

2.6. Measurement of ^{45}Ca Uptake

The measurement of ^{45}Ca uptake in tissue samples was carried out after the determination of their dry weights. Tissue samples were homogenized in 50 µl of 68% HNO_3 solution. Then 2 ml of Bray's scintillation fluid was added and chemo luminescence of samples were quantified with 1450-MicroBeta liquid scintillation counter (Wallac, Turku, Finland). The quantity of ^{45}Ca in tissue samples was expressed by cpm/mg d. w.

2.7. 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

Figure 1 shows the results of the experiments where the effects of i/p injections of ^{40}Ca PS and ^{45}Ca PS on tissue hydration are compared with those received from intact animals.

As can be seen, ^{40}Ca PS and ^{45}Ca PS have different effects on heart muscle and brain tissues hydration. The injection of ^{40}Ca PS leads to dehydration in all samples of heart muscle and brain tissues (except in cerebellum tissue of old animals),

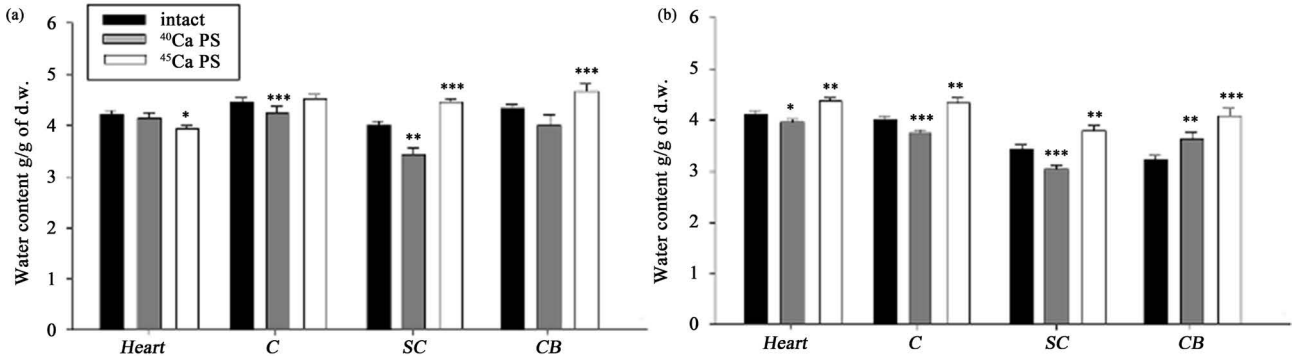

Figure 1. The effects of ^{40}Ca PS and ^{45}Ca PS on hydration of heart muscle (Heart), brain cortex (C), subcortex (SC), cerebellum (CB) tissues of intact and i/p-injected young and old rats. Black bars indicate the mean value of water contents in tissues of intact young (A) and old (b) animals. Gray and white bars indicate the mean value of water contents in tissues of young (a) and old (b) rats injected with ^{40}Ca PS and ^{45}Ca PS, respectively. Each bar represents the mean ± SEM (n = 45). The symbols (*), (**) and (***) indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. All data were obtained from three independent experiments.

while the injection of ^{45}Ca PS has dehydration effect on heart muscle tissue of young animals. Meanwhile, in brain tissues of young as well as in heart muscle and brain tissues of old rats the injection of ^{45}Ca PS brings to tissue hydration. Thus, the differences between the effects of ^{40}Ca PS and ^{45}Ca PS on tissue hydration indicate the distinctive nature of hydration mechanisms in heart muscle and brain tissues. In addition, the differences between the effects of ^{40}Ca PS and ^{45}Ca PS in heart muscle and brain cortex tissues have age-dependent increasing character, while in subcortex and cerebellum tissues age-dependent decreasing character was observed (**Figure 1(a)**, **Figure 1(b)**). Our previous study has shown that the high-affinity ouabain receptors (a_3) in the membrane with RNa/Ca exchange function, have more pronounced age-dependent increasing character in brain cortex tissue than in subcortex and cerebellum tissues [9]. Therefore, in the following experiments, brain cortex tissue has been chosen as a subject for the present investigation.

As can be seen in **Figure 2**, the level of ^{45}Ca uptake in heart muscle tissue is much higher than in brain tissues.

However, the age-dependent decrease of ^{45}Ca uptake by brain tissue is more pronounced than in case of heart muscle tissue.

As Ca uptake by RNa/Ca exchange leads to more effective changes of $[Ca]_i$ than by potential-dependent ionic channels [3], we have considered Ca uptake as a result of RNa/^{45}Ca exchange.

It is known that $[Ca]_i$ has multisided effects on intracellular metabolism [18] through which it can cause cell hydration, including the oxidative phosphorylation-induced endogenous water formation [19], the stimulation of Ca-Calmoduline-NO-cGMP pathway-induced activation of Na/Ca exchange in forward (F) mode [11] [20] and inhibition of Na/K-pump activity [21]. Therefore, in the next series of experiments the individual role of each above-mentioned pathway in determination of differences between the effects of $[^{40}Ca]_i$ and $[^{45}Ca]_i$ on heart muscle and brain cortex tissue hydration was studied.

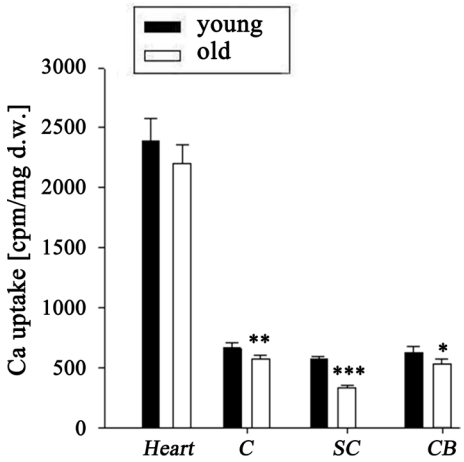

Figure 2. The age-dependent effects of ^{45}Ca uptake in heart muscle (Heart), brain cortex (C), subcortex (SC), cerebellum (CB) tissues. Black and white bars indicate the mean value of ^{45}Ca uptake in tissues of young and old animals, respectively. Each bar represents the mean ± SEM (n = 45). The symbols (*), (**) and (***) indicate $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. All data were obtained from three independent experiments.

Considering the high expression of RNa/Ca exchange in heart muscle tissue compared with brain cortex one, it was predicted that in heart muscle tissue the differences between the effects of ^{40}Ca and ^{45}Ca on cell hydration would be more pronounced than in brain tissue. Therefore, to evaluate the nature of the mechanisms through which the effects of ^{40}Ca PS and ^{45}Ca PS on tissues hydration are realized, their effects on tissue hydration in various experimental conditions were studied.

The effects of ^{40}Ca PS and ^{45}Ca PS on heart muscle tissue hydration

The results presented in **Figure 3** show that in ouabain-free PS the hydration of heart muscle samples of young animals injected with ^{40}Ca PS is more pronounced than that of young animals injected with ^{45}Ca PS.

The hydration of heart muscle samples from old animals injected with ^{40}Ca PS is less than the hydration of samples from old animals injected with ^{45}Ca PS (**Figure 3(b)**).

As mentioned in the introduction part of the present study, 10^{-9} M and 10^{-4} M ouabain activate the RNa/Ca exchange by both the decrease of $[Ca]_i$ [7] [11] and the increase of $[Na]_i$ [22], respectively.

The incubation of heart muscle tissue samples of young animals preliminarily injected with ^{40}Ca PS (**Figure 3(a)**) in 10^{-9} M ouabain solution, having cAMP-dependent activation effect on RNa/^{40}Ca exchange [7], causes pronounced dehydration effect, while in heart muscle tissue samples of young animals injected with ^{45}Ca PS, only slight dehydration effect can be recorded. The same study in old animals injected with ^{40}Ca PS shows more pronounced hydration effects as compared with the injection of ^{45}Ca PS (**Figure 3(b)**).

The incubation of heart muscle tissue samples of young animals preliminarily injected with ^{40}Ca PS in 10^{-4} M ouabain solution leads to more pronounced dehydration effect (**Figure 3(a)**) than in case of 10^{-9} M ouabain. However, in heart

muscle tissue samples of young animals injected with ^{45}Ca PS, 10^{-4} M ouabain brings to the same level of dehydration as in the case of 10^{-9} M ouabain (**Figure 3(a)**).

The same procedures in old animals preliminarily injected with ^{40}Ca PS show that the incubation of their heart muscle tissue samples in 10^{-4} M ouabain leads to the decrease of hydration in contrast to those incubated in 10^{-9} M ouabain (**Figure 3(b)**). On the other hand, in animals preliminarily injected with ^{45}Ca PS the incubation of heart muscle tissue samples in 10^{-4}M ouabain brings to sharp dehydration (**Figure 3(b)**). The age-dependent reverse character of hydration, in case when heart muscle tissue samples are incubated in ouabain solutions (compare the continuous lines with the dotted ones in **Figure 3(a)**, **Figure 3(b)**), is also worth mentioning.

It is known that both 10^{-9} M and 10^{-4} M ouabain-induced activations of RNa/Ca exchange are accompanied with the increase of intracellular cAMP contents [12], having an important role in muscle contractility (hydration). Therefore, to exclude the role of cAMP contents in determination of differences between the effects of activation of RNa/^{40}Ca and RNa/^{45}Ca exchange on heart muscle tissue hydration, in the next series of experiments the mentioned differences are studied by the decrease of Na gradient on the membrane. For this purpose, two various ages of animals were preliminarily injected with ^{40}Na PS (^{40}Ca or with ^{45}Ca) and 30 min later their heart muscle tissue samples were separately incubated in 100% Na PS and 50% Na PS.

As can be seen in **Figure 4**, the decrease of Na ions ($[Na]_o$) concentration by 50% in cell bathing medium leads to more pronounced dehydration in heart

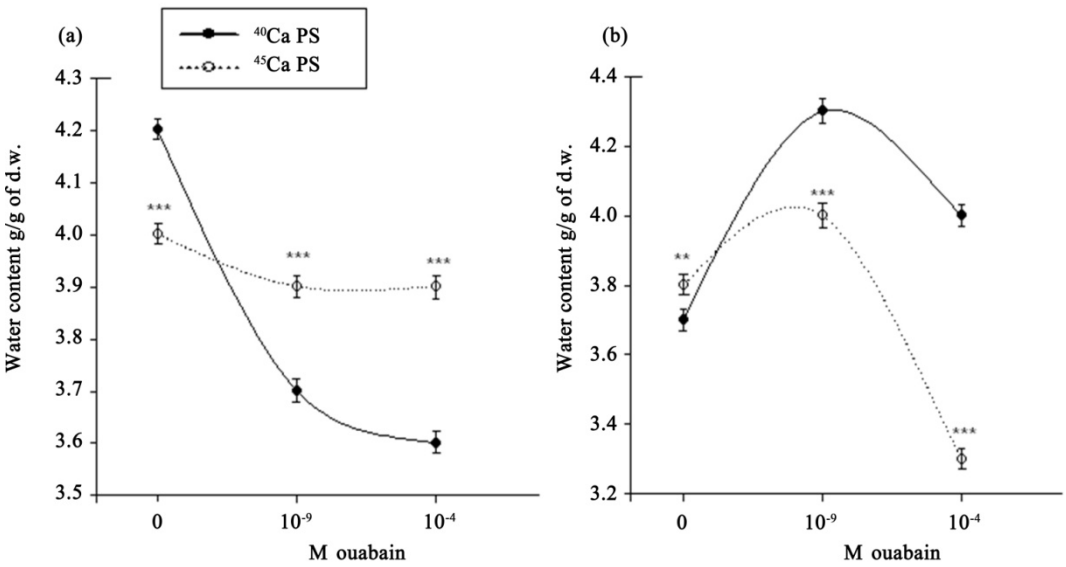

Figure 3. The effects of ouabain-free PS, 10^{-9} M and 10^{-4} M ouabain solutions on water contents variation in heart muscle tissue samples of young (a) and old (b) animals preliminarily injected with ^{40}Ca PS (continuous lines) and ^{45}Ca PS (dotted lines). Each point in line represents the mean ± SEM (n = 45). The symbols (**) and (***) indicate $p < 0.01$ and $p < 0.001$, respectively. All data were obtained from three independent experiments.

muscle tissue samples than in heart muscle tissue samples incubated in normal 100% Na PS.

However, the dehydration effect induced by 50% Na PS is more expressed in heart muscle tissue samples of animals injected with ^{45}Ca PS than in those injected with ^{40}Ca PS (Figure 4(a), Figure 4(b)).

The effects of ^{40}Ca PS and ^{45}Ca PS on brain cortex tissue hydration

The same protocols of experiments performed on heart muscle tissue were repeated with brain cortex tissue. The data presented in Figure 5 indicate that in ouabain-free PS brain cortex tissue samples of young as well as of old animals preliminarily injected with ^{40}Ca in ouabain-free PS are more dehydrated than those animals injected with ^{45}Ca, while the incubation of brain cortex tissue samples of young rats injected with ^{40}Ca in 10^{-9} M ouabain shows significantly higher level of hydration as compared with the samples of animals injected with ^{45}Ca (Figure 5(a)).

The incubation of brain cortex tissue samples of young rats injected with ^{40}Ca PS in 10^{-4} M ouabain leads to dehydration, while the same procedure in young rats injected with ^{45}Ca appears to have less pronounced hydration effect: *i.e.* there is a slight dose-dependent increase of tissue hydration at ouabain (Figure 5(a)).

As can be seen in Figure 5(b), the incubation of brain cortex tissue samples of old animals preliminarily injected with ^{40}Ca PS in 10^{-9} M and 10^{-4} M ouabain medium brings to dose-dependent increase of hydration level, while in case of old animals injected with ^{45}Ca PS brain cortex tissue hydration is slightly increased in 10^{-9} M ouabain and decreased in 10^{-4} M ouabain medium.

The effects of 100% Na PS and 50% Na PS on brain cortex tissue hydration are

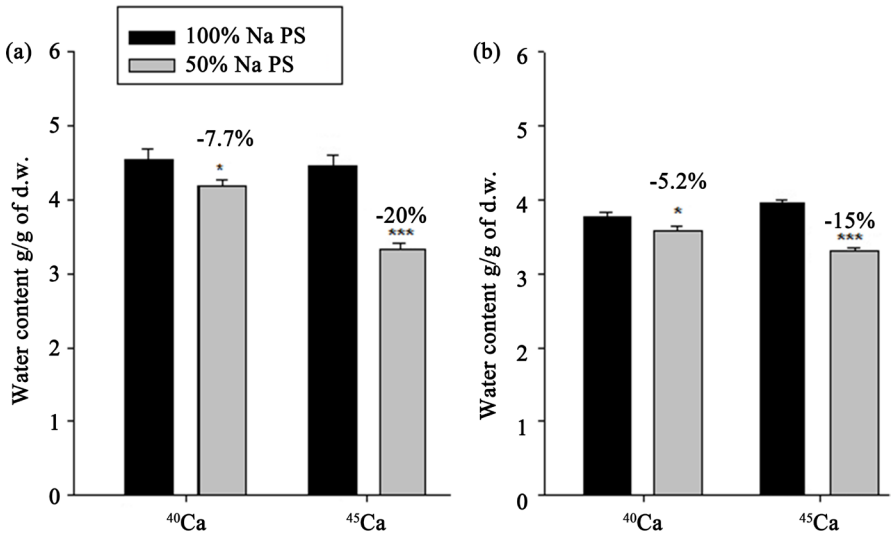

Figure 4. The effects of 100% Na PS (black bars) and 50% Na PS (white bars) on water contents variation in heart muscle tissue samples of young (a) and old (b) rats preliminary injected with ^{40}Ca PS and ^{45}Ca PS. The numbers in % indicate the difference between levels of hydration. Each bar represents the mean ± SEM (n = 45). The symbols (*) and (***) indicate $p < 0.05$ and $p < 0.001$, respectively. All data were obtained from three independent experiments.

the same as in the identical case of heart muscle tissue hydration (Figure 4). As is shown in Figure 6(a), Figure 6(b).

The dehydration in brain cortex tissue samples incubated in 50% Na PS is more pronounced in animals of both ages, which are preliminarily injected with ^{45}Ca PS, than in tissue samples of animals preliminarily injected with ^{40}Ca PS.

4. Discussion

It is known that Ca uptake by cells is realized by potential-dependent ionic channels and RNa/Ca exchange. As the threshold of RNa/Ca exchange activation

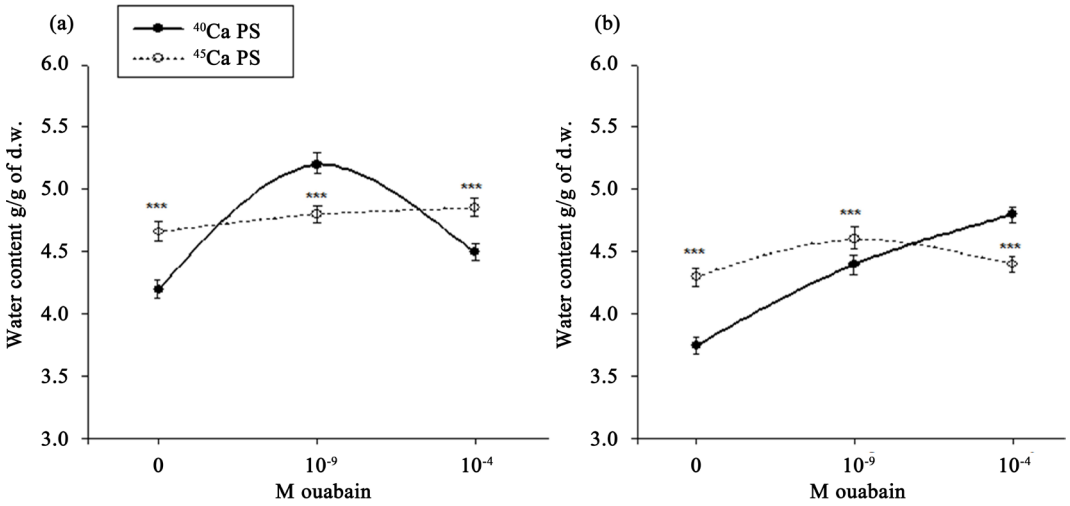

Figure 5. The effects of ouabain-free PS, 10^{-9} M and 10^{-4} M ouabain solutions on water content variation in brain cortex tissue samples of young (a) and old (b) animals preliminary injected with ^{40}Ca PS (continuous lines) and ^{45}Ca PS (dotted lines). Each point in line represents the mean ± SEM (n = 45). The symbol (***) indicate $p < 0.001$, respectively. All data were obtained from three independent experiments.

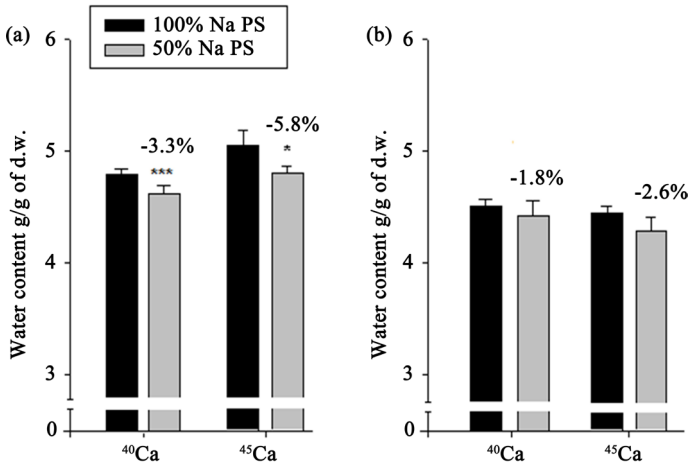

Figure 6. The effects of 100% Na PS (black bars) and 50% Na PS (white bars) on water contents variation in brain cortex tissue samples of young (a) and old (b) rats preliminarily injected with ^{40}Ca PS and ^{45}Ca PS. The numbers in % indicate the difference between levels of hydration. Each bar represents the mean ± SEM (n = 45). The symbols (*) and (***) indicate $p < 0.05$ and $p < 0.001$, respectively. All data were obtained from three independent experiments.

is incomparable less than of ionic channel activity, in the present experiments, the PS injection-induced stimulation of Ca uptake can mainly be considered as a result of RNa/Ca exchange activation [3]. As the energy source for RNa/Ca exchange is E_{ca} - E_{Na}, it is predicted that $E_{45Ca} > E_{40Ca,}$ because of $[^{45}Ca]_i$, is close to "0" mM. Therefore, it is predicted that the rate of RNa/^{45}Ca exchange must be higher than the rate of RNa/^{40}Ca exchange. However, it is not clear whether the physiological difference between the activations of RNa/^{40}Ca and RNa/^{45}Ca exchange is only due to their different rates or not.

As was noted in introduction part, the activation of RNa/Ca exchange has double effects on cell hydration: passive-dehydration because of its electrogenic character and $[Ca]_i$-induced metabolic effects. The obtained data showing that the activation of RNa/^{40}Ca and RNa/^{45}Ca exchanges has different effects on heart muscle and brain tissue hydration with age-dependent character reveals the different metabolic effects of intracellular $[^{40}Ca]_i$ and $[^{45}Ca]_i$ on cell hydration (**Figure 1**).

By previous study it has been shown that the increase of $[Ca]_i$ leads to heart muscle hydration because of activation of Ca-Calmoduline-NO-cGMP-induced stimulation of FNa/Ca exchange [22]. The data that in young rats the activation of RNa/^{45}Ca exchange has more pronounced dehydration effect than the activation of RNa/^{40}Ca exchange, and in heart muscle tissue of old rats it leads to more hydration compared with the activation of RNa/^{40}Ca exchange in ouabain-free medium, can support the suggestion that the rate of RNa/^{45}Ca exchange is higher than the rate of RNa/^{40}Ca exchange. The RNa/^{45}Ca exchange-induced brain tissue hydration compared with the activation of RNa/^{40}Ca exchange (**Figure 1(a)** and **Figure 1(b)**) can be explained by the same mechanism.

The data on age-dependent decrease of ^{45}Ca uptake by tissues can be considered as a result of aging-induced increase of $[Ca]_i$, which is in harmony with literature data [2]. It is worth noting that in spite of the fact that the expression of RNa/Ca exchange in heart muscle tissue is much higher than in brain tissue, the age-dependent decrease of Ca uptake in brain tissue is more pronounced than in heart muscle tissues (**Figure 2**). Such a weak age-dependency of Ca uptake in heart muscle tissue probably can be explained by higher $[Ca]_i$-buffering properties of heart muscle tissue as compared with brain tissue. Therefore, we suggest that discussing the comparative results of the effects of ^{40}Ca PS and ^{45}Ca PS on heart muscle and brain tissues could help to evaluate the nature of different metabolic mechanisms of $[^{40}Ca]_i$ and $[^{45}Ca]_i$.

The effects of ^{40}Ca PS and ^{45}Ca PS on heart muscle tissue hydration

The obtained data that in ouabain-free medium heart muscle tissue samples from young and old rats injected with ^{45}Ca PS are dehydrated and hydrated, respectively, compared with heart muscle hydration of animals injected with ^{40}Ca PS (**Figure 3(a)**, **Figure 3(b)**), can be explained by the above mentioned suggestion that the rate of RNa/^{45}Ca exchange is higher than the rate of RNa/^{40}Ca exchange. The results showing that heart muscle tissue samples of ^{40}Ca PS-injected

young rats are sharply dehydrated upon the impact of 10^{-9} M and 10^{-4} M ouabain, while in the rats injected with ^{45}Ca PS both concentrations of ouabain have slight dehydration effects on muscle (**Figure 3(a)**), can probably be explained by ^{45}Ca-induced transition of cytoplasm from sol into gel state because of high Ca-dependent phosphorylation of myofibrils in cytosol or by compensation of RNa/^{45}Ca exchange-induced dehydration by hydration of FNa/Ca exchange activation in result of high $[^{45}Ca]_i$-induced activation of Ca-Calmoduline-NO-cGMP pathway [11].

The obtained result that in old rats injected with ^{45}Ca PS heart muscle tissue hydration becomes ouabain-sensitive is probably due to age-dependent weakening of heart muscle contractility leading to abnormal increase of $[Ca]_i$ as well as aging-induced dysfunction of intracellular cAMP controlling system.

The 10^{-9} M ouabain-activation of RNa/Ca exchange which leads to heart muscle hydration can be explained by $[Ca]_i$-induced activation of mitochondrial function leading to stimulation of endogenous water molecules' formation, which is based on our previous data [19]. The fact that the 10^{-9} M ouabain-induced activation of RNa/^{45}Ca exchange has less pronounced hydration effects on heart muscle of young animals than the activation of RNa/^{40}Ca exchange (**Figure 3**) can also be explained by high $[^{45}Ca]_i$ leading to depression of 10^{-9} M induced activation of RNa/Ca exchange.

The strong dehydration effect of RNa/^{45}Ca exchange at 10^{-4} M ouabain in heart muscle of old animals supports the previous suggestion that the dehydration effect of RNa/^{45}Ca exchange on heart muscle becomes more effective at high $[Na]_i$, when Na/K pump is in inactive state.

The effect of ^{45}Ca-induced stabilization of muscle hydration in young rats and its absence in aged ones seems extremely interesting and the elucidation of its exact mechanism can serve as a subject for a special investigation.

The data revealing that 50% Na PS-induced activation of RNa/^{45}Ca exchange has stronger effects on muscle hydration than RNa/^{40}Caexchange activation (**Figure 4**) indicate that the rate of RNa/^{45}Ca exchange is higher than that rate of RNa/^{40}Ca exchange.

The effects of R Na/^{40}Ca and R Na/^{45}Ca exchange on brain cortex tissue hydration

As in the case of heart muscle study, the data showing that in ouabain-free PS brain cortex tissue samples of young as well as of old animals preliminarily injected with ^{40}Ca are more dehydrated than of those of animals injected with ^{45}Ca PS (**Figure 5**) can be explained by high rate of RNa/^{45}Ca exchange compared with rate of RNa/^{40}Ca exchange. The RNa/^{45}Ca exchange brings to hydration through elevation of $[^{45}Ca]_i$ than by activation of FNa/Ca exchange and passive dehydration effect on tissue, respectively. The data that in 10^{-9} M ouabain brain cortex tissue samples of young animals injected with ^{40}Ca PS demonstrate hydration effect through $[Ca]_i$-induced increase of endogenous water formation by mitochondria [19] and in the same conditions the absence of such effect in

young animals injected with ^{45}Ca PS and less sensitivity to 10^{-4} M ouabain (**Figure 5(a)**) allow us to suggest that $[^{45}Ca]_{i,}$ besides the activation of FNa/Ca exchange, which can balance RNa/Ca exchange-induced tissue dehydration by an unknown mechanism, also causes transformation of cytoplasm from sol into gel state in young animals. The data that in 10^{-9} M and 10^{-4} M ouabain mediums the hydration level of brain cortex samples from ^{40}Ca PS-injected old rats has dose-dependent increasing character, while in the case of ^{45}CaPS-injected animals brain cortex tissue hydration is slightly increased in 10^{-9} M ouabain and decreased in 10^{-4} M ouabain mediums indicate that $[^{45}Ca]_i$-induced stabilizing mechanism of brain cortex hydration in young animals has age-dependent weakening character.

The data that in both ages of animals the decrease of $[Na]_o$ leads to more pronounced dehydration in cortex samples of ^{45}Ca PS-injected animals compared to dehydration in cortex samples from ^{40}Ca PS-injected animals can be an additional support for the aforementioned suggestion that the rate of RNa/^{45}Ca exchange is higher than the rate of RNa/^{40}Ca exchange (6A and B).

5. Conclusions

Thus, the obtained data of the present work bring us to the following conclusions:

- The intraperitoneal injections of ^{40}Ca PS and ^{45}Ca PS which bring to activation of RNa/^{40}Ca and RNa/^{45}Ca exchange, respectively, have different effects on heart muscle and brain tissue hydration with different age-dependent characters.
- These differences between RNa/^{40}Ca and RNa/^{45}Ca exchange-induced tissue hydrations are much more pronounced in heart muscle tissues than in brain tissues as RNa/Ca exchange is expressed incomparably higher in heart muscle tissues than in brain tissues.
- The rate of RNa/^{45}Ca exchange is higher than the rate of RNa/^{40}Ca exchange, because of $E_{45Ca} > E_{40Ca}$.
- The $[^{45}Ca]_i$ and $[^{40}Ca]_i$ have different metabolic effects on heart muscle and brain cortex tissue hydration. In young animals tissue hydration in the case of $[^{40}Ca]_i$ has dose-dependent ouabain sensitivity, while in the case of $[^{45}Ca]_i$ tissue hydration becomes ouabain-insensitive. Upon the impact of $[^{45}Ca]_i$, the heart muscle tissue hydration of old rats becomes ouabain-sensitive, while brain cortex tissue hydration remains significantly less ouabain-sensitive than in the case of $[^{40}Ca]_i$.
- The main summary of this work is that radioactive ^{45}Ca is not bioequivalent to cold ^{40}Ca. Therefore, ^{45}Ca cannot be used in biological experiments for evaluation of the functional role of ^{40}Ca.

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Conflicts of Interest

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

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