EXPERIMENTAL WORKS

UDC 577.352.4+544.147+544.176+544.168 doi: http://dx.doi.org/10.15407/ubj88.02.005

THE INHIBITORY INFLUENCE OF CALIX[4]ARENE OF C-90 ON THE ACTIVITY OF Ca^{2+},Mg^{2+}-ATPases IN PLASMA MEMBRANE AND SARCOPLASMIC RETICULUM IN MYOMETRIUM CELLS

T. O. VEKLICH

Palladin Institute of Biochemistry,National Academy of Sciences of Ukraine, Kyiv;
e-mail: veklich@biochem.kiev.ua

Our study on the plasma membrane vesicles and myometrium cells treated with 0.1% digitonin showed that inhibitory effect of calix[4]arene C-90 (5,11,17,23-tetra(trifluoro)methyl(phenylsulphonylimino)-methylamino-25,26,27,28-tetrapropoxy-calix[4]arene) on the plasma membrane Ca^{2+},Mg^{2+}-ATPase was more significant than on the Ca^{2+},Mg^{2+}-ATPase in sarcoplasmic reticulum (the inhibition coefficient $I_{0.5}$ values were 20.2 ± 0.5 μM and 57.0 ± 1.4 μM for the plasma membrane Ca^{2+},Mg^{2+}-ATPase and Ca^{2+},Mg^{2+}-ATPase in sarcoplasmic reticulum, respectively (n = 5)). Inhibition kinetics of calix[4]arene C-90 effect on the Ca^{2+},Mg^{2+}-ATPase activities in plasma membrane and sarcoplasmic reticulum were studied. This substance inhibited both pumps as complete noncompetitive inhibitor. Calix[4]arene C-90 caused the increase of intracellular Ca^{2+} concentration and decrease of hydrodynamic diameter in smooth muscle cells similar to the action of uterotonic drug oxytocin.

K e y w o r d s: Ca^{2+},Mg^{2+}-ATPase, plasma membrane, sarcoplasmic reticulum, smooth muscle cells, myometrium, enzymatic hydrolysis of ATP, calix[4]arenes.

Calcium transporters, which include, for example, the calcium pumps of plasma membrane (PM) and sarcoplasmic reticulum (SR), play an essential role in controlling Ca ion concentration in the smooth muscle (SM) cytoplasm [1-6] The PM Ca^{2+},Mg^{2+}-ATPase maintains low Ca ion concentration in the relaxed myocytes and reduces Ca^{2+} concentration in myoplasm after muscle contraction and hence contributes to muscle relaxation [7, 8]. The SR Ca^{2+},Mg^{2+}-ATPase reduces intracellular Ca^{2+} concentration owing to cation accumulation in reticular pool [6, 9]. Recent literature data have indicated that SR is one of the largest cellular calcium depots [10].

Disorder in contractile function of myometrium in women often causes various pathologies, namely poor uterine contraction strength, spontaneous abortion, pre-term birth, miscarriage, atony, hypo- or hypertonic uterus [11, 12]. Typically, these pathologies are caused by abnormalities in the functioning of membrane-mediated cation transport systems. Therefore, the search for compounds which capable modifying the contractile function of myometrium upon these pathological conditions is a current point of interest.

In this context it would be of interest to study calixarenes, macrocyclic compounds synthesized by cyclocondensation of para-substituted phenols and formaldehyde. These compounds exhibit antiviral, antibacterial, antitumor and antithrombotic properties and may serve as effective inhibitors or activators of enzymatic, receptor and transport membrane-bound proteins [13, 14]. It should be noted that the majority of calixarenes are low toxic [14, 15], that along with their ability to bind to molecules of various ligands [16, 17] makes these compounds highly promising agents in biotechnology and medicine.

In our previous studies we showed that calix[4]arene C-90 (the code name) at concentration of 100 μM inhibited significantly (by 75% compared to control) the PM Ca^{2+},Mg^{2+}-ATPase activity in uterus

myocytes and practically did not affect the activities of Mg^{2+}-independent Ca^{2+}-dependent ATPase, Na^+,K^+-ATPase and Mg^{2+}-ATPase localized in the same membrane structure [18]. Ca ion accumulation in myometrium mitochondria, sensitive to the action of CCCP protonophore, was practically resistant to the calixarene action.

The aim of our study was to compare the effect of calix[4]arene C-90 on the activities of the PM and SR Ca^{2+},Mg^{2+}-ATPases in myometrium myocytes and to study kinetics of this effect, as well as influence of calix[4]arene C-90 on the intracellular Ca^{2+} concentration and hydrodynamic diameter of smooth muscle cells (SMC).

Materials and Methods

Synthesis and structure of calix[4]arene C-90. Calix[4]arene C-90 (5,11,17,23-tetra(trifluoro) methyl-(phenylsulphonylimino)-methylamino-25, 26,27,28-tetrapropoxy-calix[4]arene) (for structural formula, see below) was synthesized and characterized using NMR and infrared spectroscopy in the Phosphoranes Chemistry Department of the Institute of Organic Chemistry of NAS of Ukraine (Head of the Department – Corresponding Member of NAS of Ukraine, Prof. V. I. Kalchenko) [19].

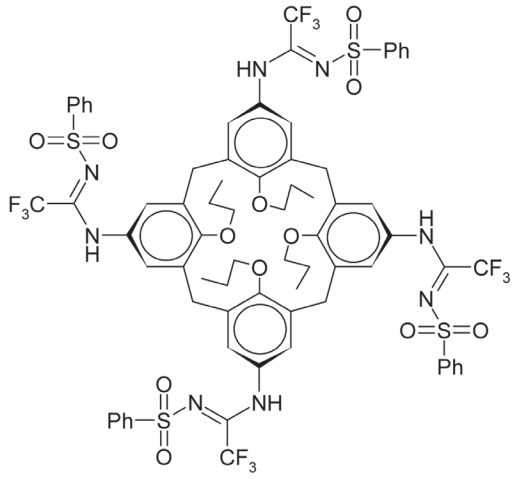
Biochemical studies. Biochemical studies have been carried out in the Department of Muscle Biochemistry of Palladin Institute of Biochemistry of NAS of Ukraine (Head of the Department – Prof. S. O. Kosterin, Member of NAS of Ukraine).

Preparative biochemistry. Fraction of uterine PM SMC was isolated from pig myometrium as previously described [20, 21].

Protein concentration in the membrane fraction was determined by Bradford assay with Coomassie G-250 [22].

Myocytes were isolated from the uterus of non-pregnant rats using collagenase and soybean trypsin inhibitor as described [23].

The study was performed in accordance with the Declaration of Helsinki (World Medical Assembly, 1964), the international principles of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the Declaration of Principles on Tolerance (UNESCO, 1995), Universal Declaration on Bioethics and Human Rights (UNESCO, 2005), the Convention for the Protection of Human Rights and Dignity of the Human Being with Regard to the Application of Biology and Medicine (Oviedo, Spain, 1997) and the Law of Ukraine On the Protection of Animals against Cruel Treatment approved by Verkhovna Rada of Ukraine in 2002.



Calix[4]arene C-90

Enzyme assays. Total ATPase activity was determined in fractions of myometrial PM at 37 °C in a standard medium (0.4 ml) containing (mM): 3 ATP, 3 $MgCl_2$, 0.95 $CaCl_2$, 25 NaCl, 125 KCl, 1 EGTA, 20 Hepes-tris-buffer (pH 7.4), 1 NaN_3 (mitochondrial ATPase inhibitor [24]), 1 ouabain (selective Na^+,K^+-ATPase inhibitor [25, 26]), 0.1 μM thapsigargin (selective inhibitor of Ca^{2+},Mg^{2+}-ATPase of endo(sarco)-plasmic reticulum [24]) and 0.1% digitonin (factor of PM perforation [27]). Concentration of free Ca^{2+} was calculated using Maxchel software and found to be 1 μM upon the given physical-chemical and concentration conditions of incubation medium. When studying the effect of various Ca ion concentrations on the Ca^{2+},Mg^{2+}-ATPase activity, required cation concentrations were calculated using mentioned above software. The protein concentration in the sample was 20-30 μg. Incubation time was 5 min. The enzyme reaction was initiated by adding aliquots (50 μl) of the PM suspension to the incubation medium and terminated by adding 1 ml of "stop"-solution comprising of 1.5 M sodium acetate, 3.7% formaldehyde, 14% ethanol, 5% TCA (pH 4.3 at 8 °C). The amount of the reaction product (P_i) was determined by the method of W. Rathbun et V. Betlach [28].

Plasma membrane Ca^{2+},Mg^{2+}-ATPase activity was calculated as the difference between the values of ATPase activity in the presence and absence of exogenous Ca ions in the incubation medium (in the

presence of 1 mM EGTA, a specific chelator of Ca ions).

The specific Ca^{2+},Mg^{2+}-ATPase activity in the fraction of myometrium PM was found to be 3.63 ± 0.21 μM P_i/mg protein per 1 h ($M \pm m$; $n = 7$).

For the assessment of the ATPase activity in SR, cells (protein concentration in a sample of 20 μg) were permeabilized by digitonin (0.1%). Total ATPase activity was measured in a standard medium (0.4 ml) containing (mM): 3 ATP, 3 $MgCl_2$, 0.95 $CaCl_2$, 25 NaCl, 125 KCl, 1 EGTA, 20 Hepes-tris-buffer (pH 7.4), 1 NaN_3, 1 ouabain. Ca^{2+},Mg^{2+}-ATPase activity in SR was calculated as the difference between the values of ATPase activity in the presence and absence of 0.1 μM thapsigargin in the incubation medium.

The Ca^{2+},Mg^{2+}-ATPase specific activity in rat myometrial SR was found to be 2.45 ± 0.14 μM P_i/mg protein per 1 h ($M \pm m$; $n = 7$).

In the experiments on the effect of various concentrations of calix[4]arene C-90 (1-100 μM) on Ca^{2+},Mg^{2+}-ATPase activity we used a standard incubation medium (as described above) to which aliquot of calix[4]arene solution at the corresponding concentration was added. Concentrated (20 mM) calix[4]arene C-90 solution in DMSO (diluted with water for further experiments) was used.

Kinetics study. For the study of the effect of different concentrations of calix[4]arene C-90 on the enzyme activities, inhibition coefficients $I_{0.5}$ and Hill coefficients nH were calculated using linear Hill plots according to equation $\lg[(A_0\text{-}A)/A] = -n_H \lg I_{0.5} + n_H \lg[I]$, where A_0 and A – specific enzyme activities in the absence of calix[4]arene C-90 ("zero point") and in the presence of calix[4]arene C-90 at a concentration [I] in the incubation medium.

The values of the activation constant for Ca^{2+} and Mg^{2+} (K_{Ca}, K_{Mg}), the activation constant for ATP (K_{ATP}) and the corresponding Hill coefficient n_H (n_H,Ca, $n_{H,Mg}$, $n_{H,ATP}$) were calculated using linear Hill plots according to equation $\lg[(A_0\text{-}A)/A] = n_H \lg K - n_H \lg[S]$, where A_0 ("zero point") and A are specific enzyme activities at actual concentration of compound S (Ca^{2+}, Mg^{2+} and ATP) in the incubation medium.

Dynamic light scattering. The characteristic size of myocytes (hydrodynamic diameter) was measured by DLS spectrometer "ZetaSizer-3" equipped with a computing correlator type 7032 (Malvern Instruments, UK). Helium-neon laser LGN-111 (P = 25 mW, λ = 633 nm) was used. The measure range was from 1 nm to 20 μm. Registration and statistical processing of the autocorrelation function of light scattering in cell suspension was carried out for 1 min, 5 times at a scattering angle of 90 °. Autocorrelation function was processed using a standard software PCS-Size mode v1.61.

Confocal microscopy. The SMC suspension (100 μl) was immobilized on a microscope slide treated with poly-L-lysine (200 μl) for 2 h at 25 °C. Unattached myocytes were washed with solution B ((mM): 136.9 NaCl, 5.36 KCl, 0.44 KH_2PO_4, 0.26 $NaHCO_3$, 0.26 Na_2HPO_4, 0.03 $CaCl_2$, 5.5 glucose), and then 100 μl of solution B was added to the immobilized cells. To determine the changes in $[Ca^{2+}]_i$, myocytes were treated with probes Hoechst 33342 (specific to cell nucleus) and fluo-4 AM (specific to changes in the intracellular Ca^{2+} concentration), 0.2% Pluronic was also added to the solution to facilitate the loading. Measurements were carried out in the Multi Track mode using laser scanning confocal microscope LSM 510 META (Carl Zeiss, Germany).

Cells images (time series) were captured for 5 min at 15-20 s each, fluorescence was induced by diode laser at 405 nm for Hoechst 33342, recorded using filter BP 420-480 nm, the fluorescence of fluo-4 AM was induced by an argon laser at 488 nm and recorded in the range of 505-530 nm (filter BP 505-530). For quantitative analysis, ROI (Region of Interest) function was used. It enabled to obtain plots of the fluorescence intensity dependence on time averaged in a selected area.

Spindle-shaped cells with well-defined nucleus stained by DNA-sensitive fluorescent probe Hoechst were selected for analysis. Changes in the cytoplasm Ca^{2+} concentration were registered by a series of consecutive images during which 2 μl of 20 μM C-90 (sample) or 2 μl of 20 μM C-150 (control) were added.

Statistical analysis. Statistical processing of the obtained data was performed by standard methods using Student t-test. Kinetic and statistical calculations were performed using software MS Excel.

Reagents. In the study the following reagents were used: ATP, Hepes, ouabain, thapsigargin (Sigma, USA), Tris-hydroxymethyl-aminomethane (Reanal, Hungary), digitonin (Merck, Germany), EGTA (Fluka, Switzerland) and other chemicals were of chemically pure grade (Ukraine).

Results and Discussion

Studying the concentration dependences of the inhibitory effect of calix[4]arene C-90 (10^{-8}-10^{-4} M) on the Ca^{2+},Mg^{2+}-ATPases activity in PM and SR (Fig. 1), it was found that calix[4]arene C-90 in concentration range from 0.1 to 100 μM inhibited the activity of these enzymes effectively and in dose-dependent manner. The inhibition coefficient values ($I_{0.5}$) of Ca^{2+},Mg^{2+}-ATPase activities in PM and SR were 20.2 ± 0.5 μM and 57.0 ± 1.4 μM, respectively (n = 5). The values of Hill coefficient (n_H) were 0.55 ± 0.02 and 0.60 ± 0.02 of Ca^{2+},Mg^{2+}-ATPase activities in PM and SR, respectively (n = 5).

It was observed that calix[4]arene C-90 inhibited the Ca^{2+} Mg^{2+}-ATPase activity in SR less compared to PM (Fig. 1).

Slight difference in the calix[4]arene C-90 inhibitory effect on the PM and SR Ca^{2+},Mg^{2+}-ATPase activities may be caused by the different structure of Ca^{2+},Mg^{2+}-ATPase in PM and SR, namely the presence of the regulatory COOH-terminal tail in PM Ca^{2+},Mg^{2+}-ATPase [29].

To understand the kinetic mechanism of calix[4]arene C-90 inhibitory effect, it was examined whether the affinity of Ca^{2+},Mg^{2+}-ATPases in PM and SR for their substrates would change under the influence of inhibitor.

Increasing the ATP concentration in the incubation medium from 0.01 to 3 μM (Fig. 2, *a* and *b*, control) resulted in an increase in the Ca^{2+},Mg^{2+}-ATPase activities in PM (Fig. 2, *a*) and SR (Fig. 2, *b*) at fixed $MgCl_2$ concentration (3 mM) in the incubation medium. Apparent half-activation constant K_{ATP} and Hill coefficient $n_{H,ATP}$ were calculated and found to be 56.3 ± 4.3 μM and 132.1 ± 5.1 μM in the case of Ca^{2+},Mg^{2+}-ATPase activity in PM and 1.32 ± 0.14 and 0.96 ± 0.01 in the case of Ca^{2+},Mg^{2+}-ATPase activity in SR (n = 5).

In further experiments, the effect of calix[4]-arene C-90 on the enzyme affinity for ATP was studied. The influence of calix[4]arene C-90 (50 μM) on the concentration dependence of both ATPases activities on ATP was examined. In both cases of the C-90 action, the decrease in the Ca^{2+},Mg^{2+}-ATPases activities in PM and SR was observed, but the dependences of the enzyme activity on ATP were similar to the corresponding dependences in control (in the absence of calix[4]arene C-90), however a decrease in V_{max} occurred (Fig. 2, *a*, *b*).

It was shown that calix[4]arene C-90 reduces the rate of ATP hydrolysis by both the PM and SR

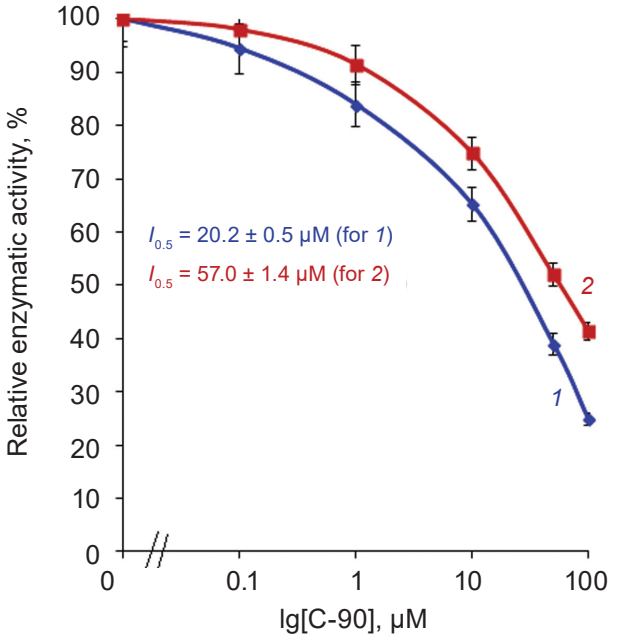


Fig. 1 Concentration dependence of calix[4]arene C-90 inhibitory effect on the Ca^{2+},Mg^{2+}-ATPase activity in PM (1) and SR (2) (n = 5). Specific enzyme activity in the absence of calix[4]arene in the incubation medium is taken as 100%

Ca^{2+},Mg^{2+}-ATPases that indicates the reducing of enzyme turnover number during its action. Apparent half-activation constant K_{ATP} and the Hill coefficient $n_{H,ATP}$ were also calculated for both ATPases under the influence of calix[4]arene C-90 and found to be 39.5 ± 9.4 μM and 141.4 ± 8.6 μM for PM Ca^{2+},Mg^{2+}-ATPase and 1.5 ± 0.3 and 0.9 ± 0.1 for SR Ca^{2+},Mg^{2+}-ATPase (n = 5). The obtained results could be interpreted as an absence of C-90 effect on the indicated parameters. That is, the influence of calix[4]arene C-90 on Ca^{2+}, Mg^{2+}-ATPases in PM and SR is non-competitive with respect to ATP.

Thus, affinity of both ATPases for ATP almost does not depend on the presence of calix[4]arene C-90 in the incubation medium, indicating an absence of competition between the inhibitor and ATP. Therefore, it may be assumed that the substrate sites of Ca^{2+},Mg^{2+}-ATPases in PM and SR and hypothetical interaction site of calix[4]arene C-90 do not overlap on the enzyme surface.

In further experiments, the dependence of the specific activities of Ca^{2+},Mg^{2+}-ATPases in PM and SR on the Ca^{2+} concentration in the incubation medium in the presence and absence of calix[4]arene C-90 was studied. The Ca ion concentration was calculated given the ATP and EGTA concentrations and

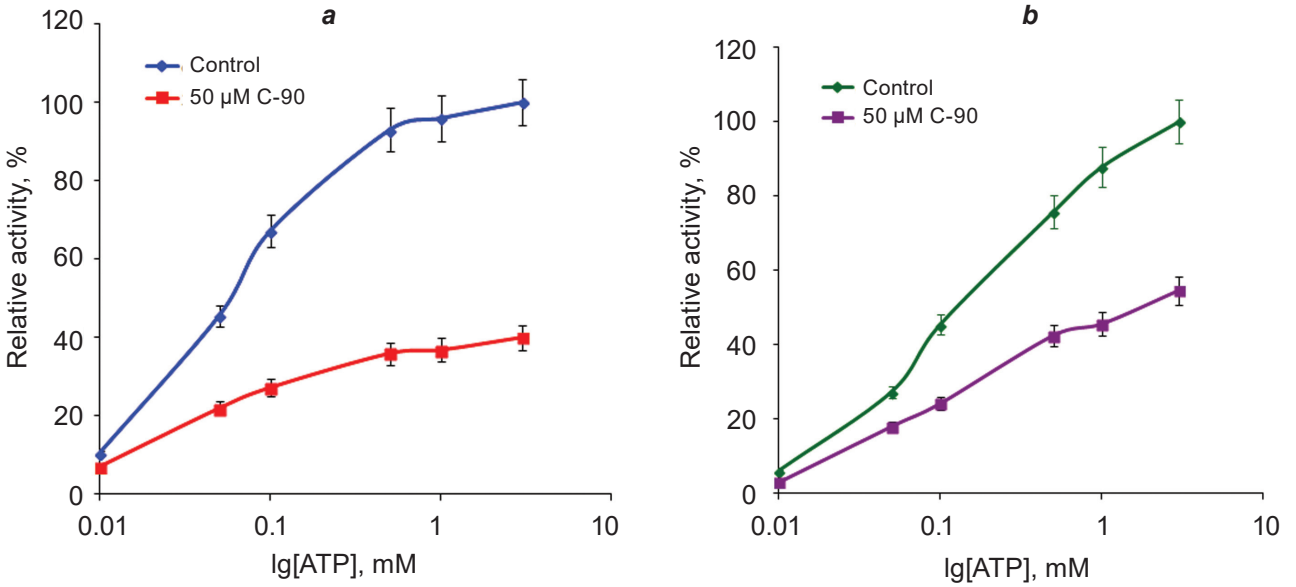


Fig. 2. Influence of calix[4]arene C-90 on the dependence of Ca^{2+},Mg^{2+}-ATPase activity in PM (a) and SR (b) of myometrium cells on the ATP concentration (n = 5)

their affinity for Ca^{2+} (mentioned in the Materials and Methods). Ca^{2+},Mg^{2+}-ATPase activity in PM and SR of myometrium increased with increasing Ca ion concentration from 100 to 1000 nM (Fig. 3, *a*, *b*, control). Apparent activation constant K_{Ca} and Hill coefficient $n_{H,Ca}$ were calculated using Hill method and found to be 190 ± 1 nM and 376 ± 11 nM for PM Ca^{2+},Mg^{2+}-ATPase activity and 2.1 ± 0.1 and 1.90 ± 0.06 for SR Ca^{2+},Mg^{2+}-ATPase activity (n = 5).

The influence of calix[4]arene C-90 (50 μM) on the affinity of Ca^{2+},Mg^{2+}-ATPases of PM and SR for Ca^{2+} was also studied. Activity of both studied ATPases decreased, but the dependence of enzyme activity on the Ca^{2+} concentration was similar to the corresponding control (without calix[4]arene C-90), though a decrease in V_{max} in the presence of calix[4]-arene C-90 was observed (Fig. 3, *a*, *b*). Apparent activation constant K_{Ca} and Hill coefficient $n_{H,Ca}$ in

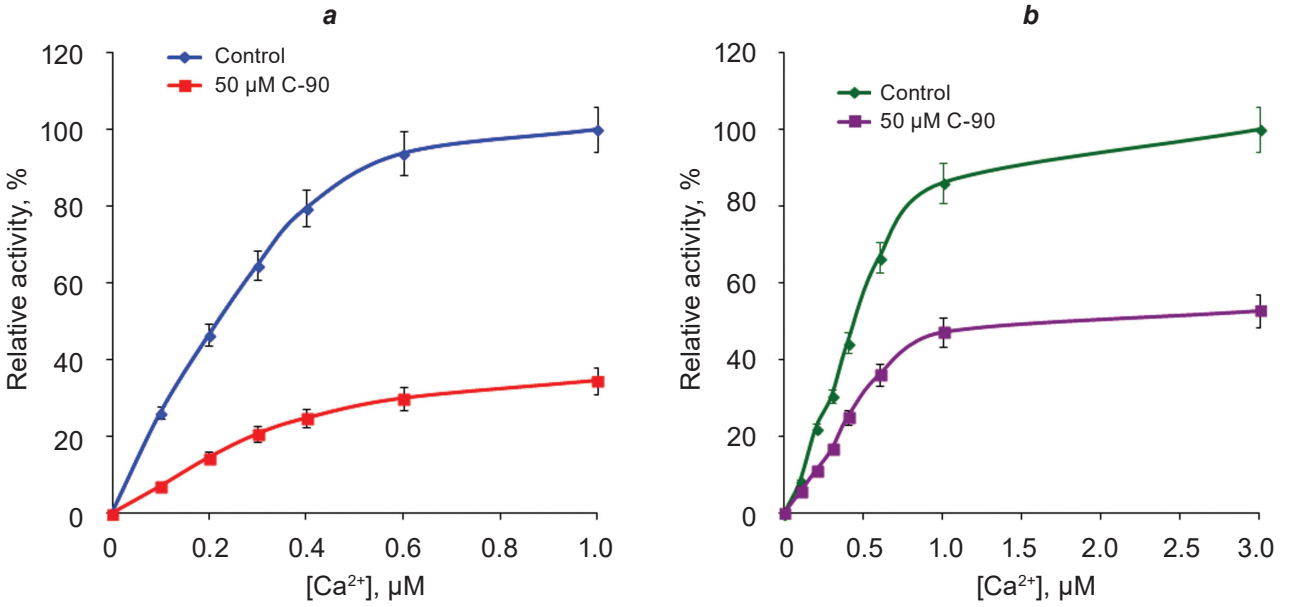


Fig. 3. Influence of calix[4]arene C-90 on the dependence of Ca^{2+},Mg^{2+}-ATPase activity in PM (a) and SR (b) of myometrium on the Ca ion concentration (n = 5)

the presence of calix[4]arene C-90 were 206 ± 3 nM and 394 ± 29 nM for Ca^{2+},Mg^{2+}-ATPase activity in PM and 1.97 ± 0.08 and 1.90 ± 0.15 for Ca^{2+},Mg^{2+}-ATPase activity in SR (n = 5).

Thus, calix[4]arene C-90 (concentration in the incubation medium was 50 μM) had almost no effect on the affinity of Ca^{2+},Mg^{2+}-ATPases of PM and SR for Ca^{2+} as well as on the cooperative effect of enzyme activation by this ion.

Mg^{2+} plays an essential role in metabolism owing to its ability to modulate macromolecule structure, bind substrate and carry electrons. There are many Mg^{2+}-dependent enzymes, where Mg^{2+} is involved not only in substrate activation, but also in the formation of active (catalytic) sites. Though, the most significant and well known role of Mg^{2+} is the formation of a chelate complex with ATP (substrate in adenosine triphosphate reaction). It is believed that Mg^{2+} ions react with ATP phosphate charged groups, polarize them and increase the system's reactivity, facilitating the nucleophilic attack the ATP terminal phosphate residue [30].

The obtained result showed that the enzyme activities of both Ca^{2+},Mg^{2+}-ATPases (PM and SR) increased with the increasing of $MgCl_2$ concentration from 0.1 to 3 mM at a fixed concentration of ATP (3 mM) in the incubation medium (Fig. 4, *a*, *b*, control).

The values of the apparent activation constant K_{Mg} of Ca^{2+},Mg^{2+}-ATPases in PM and SR were 0.70 ± 0.08 and 0.26 ± 0.02 mM, respectively ($M \pm m$; n = 5). The values of the Hill coefficient $n_{H,Mg}$ were 1.00 ± 0.11 and 1.10 ± 0.03 for Ca^{2+},Mg^{2+}-ATPases in PM and SR, respectively (n = 5).

Studying the effect of calix[4]arene C-90 on the enzyme affinity to Mg^{2+}, its influence (concentration 50 μM) on dependence of the ATP hydrolase activity on the Mg ion concentration was evaluated. As in previous experiments, a decrease in the Ca^{2+},Mg^{2+}-ATPase activities in PM and SR (regarding control) was observed, however the character of the dependence of enzyme activity on the Mg ion concentration remained the same, as for control in the absence of calix[4]arene C-90. That is, V_{max} for the Ca^{2+},Mg^{2+}-ATPase activities in PM and SR in the presence of calix[4]arene C-90 decreased (Fig. 4, *a*, *b*). It was found that in the presence of calix[4]-arene C-90 (50 μM), a slight increase in the value of activation coefficient K_{Mg} to 1.05 ± 0.07 mM and 0.31 ± 0.02 mM for Ca^{2+},Mg^{2+}-ATPases in PM and SR respectively ($M \pm m$; n = 5) occurred. Although the values of Hill coefficient of both enzymes (1.08 ± 0.06 and 1.00 ± 0.04 in the presence of C-90 for Ca^{2+},Mg^{2+}-ATPases in PM and SR, respectively ($M \pm m$; n = 5)) remained almost unchanged.

Thus, the affinity of both ATPases to Mg ions does not depend on the presence of calix[4]arene C-90 in the incubation medium, indicating an absence of competition between Mg ion and C-90.

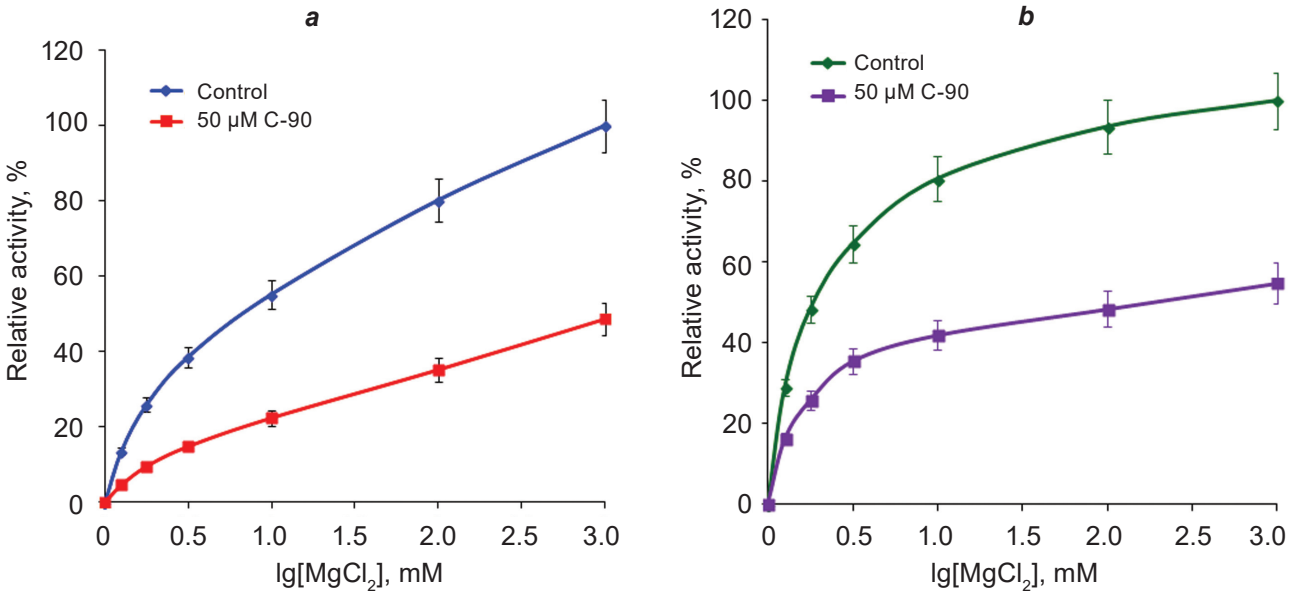


Fig. 4. Influence of calix[4]arene C-90 on the dependence of Ca^{2+},Mg^{2+}-ATPase activity in PM (a) and SR (b) of myometrium cells on the Mg ion concentration (n = 5)

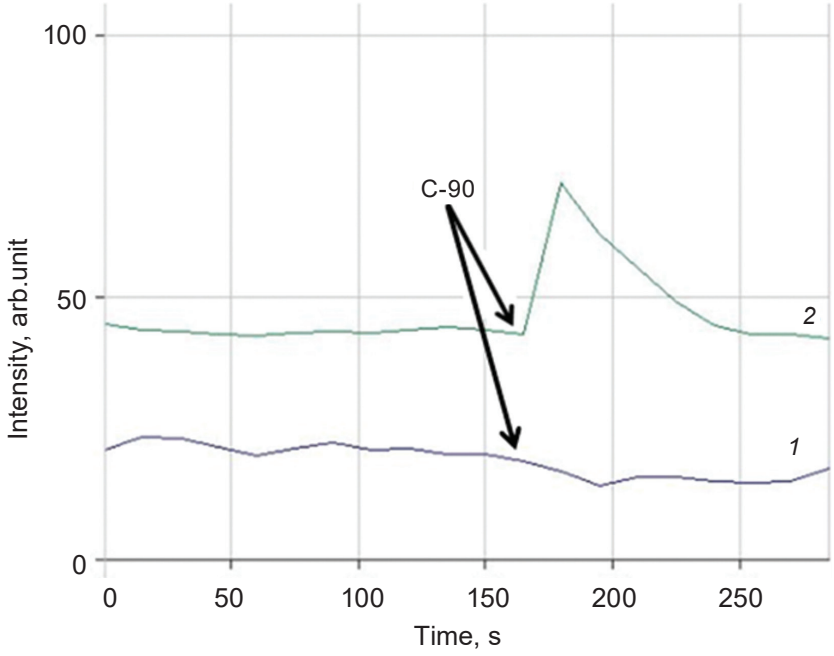


Fig. 5. The probe fluorescence intensity in the uterus myocytes has been recorded by confocal microscopy: DNA-sensitive Hoechst (1) and Ca^{2+}-sensitive fluo-4 AM (2). The C-90 solution (final concentration 20 μM) was introduced at 160 s. The result of a typical experiment is presented

The obtained data indicate that calix[4]arene C-90 inhibited the PM and SR pumps through complete noncompetitive mechanism.

Considering that PM and SR pumps play an essential role in control of the Ca ion concentration in the smooth muscle cytoplasm, it is important to find out whether calix[4]arene C-90 would affect intracellular Ca^{2+} concentration in the SMC. Therefore our further experiments were performed using confocal microscopy. It was found that in the presence of C-90 (20 μM), a sharp increase in the fluorescent response of Ca^{2+}-sensitive probe fluo-4 AM in cell occurred (Fig. 5), then a decrease in the probe quantum yield and restoring of the fluorescence intensity to the initial level were observed. In the control, where calix[4]arene "cup" C-150 (20 μM) was used (according to our previous result [18] calix[4]arene "cup" C-150 did not exhibit pronounced effect on the PM Ca^{2+},Mg^{2+}-ATPase activity), such an increase was not observed (the results have not been presented). Fluorescence of Hoechst which was localized mainly in the SMC nucleus as well as background fluorescence also did not chang in the presence of calix[4]arene C-150. These results indicate that calix[4]arene C-90 (20 μM) causes a sharp increase in the intracellular Ca^{2+} concentration, which is associated with a decrease in activity of Ca^{2+},Mg^{2+}-ATPase in PM and SR. However, during the next 100 s, the Ca^{2+} concentration declined to initial level that may be a result of the involvement of myocytes compensatory Ca^{2+}-transporting systems, which have a low affinity for Ca^{2+} and react to its high intracellular concentration (the mitochondrial Ca^{2+} uniporter, the PM Na^{+}-Ca^{2+}-exchanger).

Influence of calix[4]arenes on myocytes ion transport systems can determine their impact on the cell shape. Changes in the SMC shape owing to changes in water and osmotic balance can be detected by dynamic light scattering, which records alterations in the SMC effective hydrodynamic diameter.

According to the literature, factors that increase the contractile response of the SM also alter evidently the SMC effective hydrodynamic diameter. As shown in [31], introduction of Ca^{2+} (3 mM), treatment with A-23187 (10 μM), tetraethylammonium (1 mM) and 4-aminopyridine (1 mM) decrease the effective hydrodynamic diameter that correlates with the state of SM contraction. According to our results, the introduction of uterotonic drug oxytocin (100 nM) led to a decrease in effective hydrodynamic diameter of the SMC by 23.3 ± 3.2% ($M \pm m$, $n = 6$) relative to control (Fig. 6, blue bar).

The average value of effective hydrodynamic diameter of myocytes (control) was 15183 ± 574 nm (n = 5). Hydrodynamic diameter was measured during 1 min 5 times and then the average value was calculated. Adding DMSO did not lead to significant changes in the studied parameter, namely hydrodynamic diameter altered by 3.5 ± 1.6% compared to control (Fig. 6, green bar). Whereas calix[4]arene C-90 (50 μM) led, similar to the effect of oxytocin (100 nM), to reduction of the hydrodynamic diameter by 27.8 ± 3.8% and 23.3 ± 2.6%, respectively, relative to control (Fig. 6 red and blue bars).

Changes in the SMC hydrodynamic diameter were confirmed by tensometric study of SM [32]. In the myocytes suspension cells have a rounded shape, therefore changes in the hydrodynamic diameter cannot be interpreted as a shortening/lengthening, but can be explained by the rearrangement of the cytoskeleton elements [33, 35] that occurs during contraction, which in turn leads to a change in the SMC morphology. Furthermore, changes in the ion active transport may lead to a modification of the water-osmotic balance between cell and incubation medium that may also affect cell volume.

Thus, calix[4]arene C-90 reduces the SMC effective hydrodynamic diameter identically to the effect of uterotonic drug oxytocin. Such change in the hydrodynamic diameter could be interpreted as a combination of events that accompany the SMC contraction/relaxation processes, namely changes in osmotic-water balance, rearrangement of the cytoskeleton elements. Since it has been previously shown that a decrease in the SMC hydrodynamic diameter in the presence of contractile agents correlates with the state of SM contraction, these results suggest a promising use of calix[4]arene C-90 as a regulator of the contractile activity of the uterus SM.

Thus, the obtained experimental data allow us to conclude that calix[4]arene C-90 inhibits Ca^{2+},Mg^{2+}-ATPase activity in PM more significant than the same enzyme in SR. This compound inhibits the PM and SR pumps through complete non-competitive mechanism. Calix[4]arene C-90 causes the increase in the intracellular Ca^{2+} concentration and reduction of the SMC effective hydrodynamic diameter (similar to the effect of uterotonic drug oxytocin).

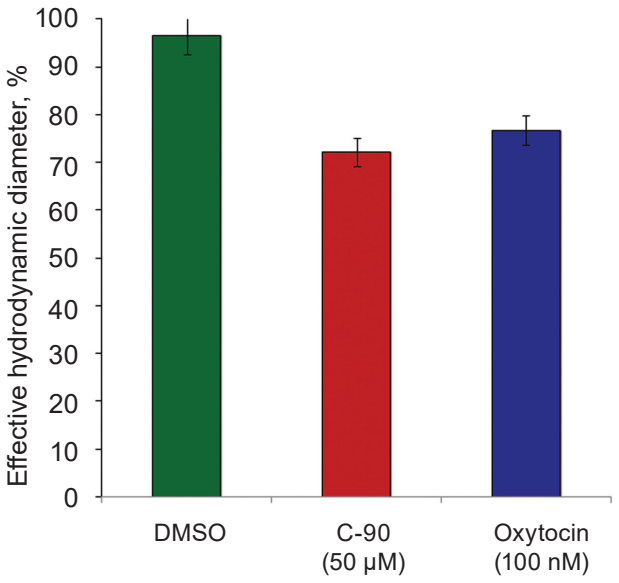


Fig. 6. Alteration in the SMC hydrodynamic diameter caused by the influence of various effectors (n = 6). 100% is the control value of the SMC hydrodynamic diameter in the absence of effectors

It is assumed that the obtained data on the effect of calix[4]arene C-90 might be valuable for further of ascertaining the ionic, molecular and membrane mechanisms of calcium metabolism in SM. The discovered phenomenon of increase in the SMC intracellular Ca^{2+} concentration in the presence of calix[4]arene may also be promising for development of new drugs on the basis of indicated supramolecular compound, namely a stimulator of uterine basal tone.

Acknowledgment

The author is thankful to S. O. Kosterin Member of NAS of Ukraine (Palladin Institute of Biochemistry of NAS of Ukraine) and V. I. Kalchenko Corresponding Member of NAS of Ukraine (IOCh of NAS of Ukraine) for substantive discussions and valuable comments, as well as Ph.D. O. A. Shkrabak and postgraduate Iu. Iu. Mazur for assistance during the experiments.

This work was supported by grants No.: 0114U003213, 0112U002624, 0115U003639, 0115U003638.

ІНГІБІТОРНИЙ ВПЛИВ КАЛІКС[4]АРЕНУ С-90 НА ЕНЗИМАТИЧНУ АКТИВНІСТЬ ТРАНСПОРТНИХ Ca^{2+},Mg^{2+}-АТРаз ПЛАЗМАТИЧНОЇ МЕМБРАНИ ТА САРКОПЛАЗМАТИЧНОГО РЕТИКУЛУМА МІОМЕТРІЯ

Т. О. Векліч

Інститут біохімії ім. О. В. Палладіна
НАН України, Київ;
e-mail: veklich@biochem.kiev.ua

В експериментах, виконаних на суспензії плазматичних мембран та клітинах міометрія, оброблених 0,1%-им розчином дигітоніну, показано, що калікс[4]-арен С-90 (5,11,17,23-тетра(трифтор)метил(фенілсульфоніліміно)метиламіно-25,26,27,28-тетрапропокси-калікс[4]арен) ефективніше інгібує ензиматичну активність Ca^{2+},Mg^{2+}-АТРази плазматичної мембрани, ніж відповідну активність у саркоплазматичному ретикулумі (величини коефіцієнта інгібування $I_{0,5}$ становлять 20,2 ± 0,5 та 57,0 ± 1,4 мкМ для Ca^{2+},Mg^{2+}-АТРази плазматичної мембрани та саркоплазматичного ретикулума відповідно (n = 5)). Дослідили кінетичні закономірності інгібувальної дії калікс[4]арену С-90 на Ca^{2+},Mg^{2+}-АТРазну активність плазматичної мембрани та саркоплазматичного ретикулума. Цей інгібітор діє на обидві помпи за механізмом повного неконкурентного інгібування. Під впливом калікс[4]арену С-90 відбувається підвищення концентрації Ca^{2+} в клітині та зменшується ефективний гідродинамічний діаметр гладеньком`язових клітин подібно до дії утеротоніка окситоцину.

Ключові слова: Ca^{2+},Mg^{2+}-АТРаза, плазматична мембрана, саркоплазматичний ретикулум, гладеньком’язові клітини, міометрій, ензиматичний гідроліз АТР, калікс[4]арени.

ИНГИБИТОРНОЕ ВЛИЯНИЕ КАЛИКС[4]АРЕНА С-90 НА ЭНЗИМАТИЧЕСКУЮ АКТИВНОСТЬ ТРАНСПОРТНЫХ Ca^{2+},Mg^{2+}-АТРаз ПЛАЗМАТИЧЕСКОЙ МЕМБРАНЫ И САРКОПЛАЗМАТИЧЕСКОГО РЕТИКУЛУМА МИОМЕТРИЯ

Т. А. Веклич

Институт биохимии им. А.В.Палладина
НАН Украины, Киев;
e-mail: veklich@biochem.kiev.ua

В экспериментах, проведенных на суспензии плазматических мембран и клетках миометрия, обработанных 0,1%-ым раствором дигитонина, показано, что каликс[4]арен С-90 (5,11,17,23-тетра(трифтор)метил(фенилсульфонилимино)-метиламино-25,26,27,28-тетрапропокси-каликс[4]арен) эффективнее угнетает энзиматическую активность Ca^{2+},Mg^{2+}-АТРазы плазматической мембраны, чем соответствующую активность в саркоплазматическом ретикулуме (величины коэффициента ингибирования $I_{0,5}$ составляют 20,2 ± 0,5 и 57,0 ± 1,4 мкМ для Ca^{2+},Mg^{2+}-АТРазы плазматической мембраны и саркоплазматического ретикулума соответственно (n = 5)). Исследовали кинетические закономерности ингибирующего действия каликс[4]арена С-90 на Ca^{2+},Mg^{2+}-АТРазную активность плазматической мембраны и саркоплазматического ретикулума. Этот ингибитор действует на оба насоса по механизму полного неконкурентного ингибирования. Под влиянием каликс[4]арена С-90 происходит повышение концентрации Ca^{2+} в клетке и уменьшается эффективный гидродинамический диаметр гладкомышечных клеток идентично действию утеротоника окситоцина.

Ключевые слова: Ca^{2+},Mg^{2+}-АТРаза, плазматическая мембрана, саркоплазматический ретикулум, гладкомышечные клетки, миометрий, энзиматический гидролиз АТР, каликс[4]арены.

References

1. Kosterin SO. Kinetics and energetics of Mg^{2+},ATP-dependent Ca^{2+} transport in the plasma membrane of smooth muscle cells. *Neurophysiology*. 2003; 35(3/4): 187-200.
2. Strehler EE. Plasma membrane calcium ATPases as novel candidates for therapeutic agent development. *J Pharm Pharm Sci.* 2013; 16(2): 190-206.
3. Burdyga T, Paul RJ. Calcium homeostasis and signaling in smooth muscle. *Muscle. Fundam BiolMechan Dis.* 2012; 2: 1155-1171.
4. Lehotsky J, Kaplán P, Murín R, Raeymaekers L. The role of plasma membrane Ca^{2+} pumps (PMCAs) in pathologies of mammalian cells. *Front Biosci.* 2002; 7: d53-84.
5. Prasad V, Okunade GW, Miller ML, Shull GE. Phenotypes of SERCA and PMCA knockout mice. *Biochem Biophys Res Commun.* 2004; 322(4): 1192-1203.
6. Wray S, Shmygol A. Role of the calcium store in uterine contractility. *Semin Cell Dev Biol.* 2007; 18(3): 315-320.
7. Holton ML, Wang W, Emerson M, Neyses L, Armesilla AL. Plasma membrane calcium ATPase proteins as novel regulators of signal transduction pathways. *World J Biol Chem.* 2010; 1(6): 201-208.
8. Kosterin SO. Calcium transport in smooth muscles. K.: Science opinion, 1990. 216 p.
9. Shmigol AV, Eisner DA, Wray S. The role of the sarcoplasmic reticulum as a Ca^{2+} sink in rat uterine smooth muscle cells. *J Physiol.* 1999; 520(Pt 1): 153-163.
10. Foskett JK, White C, Cheung KH, Mak DO. Inositol trisphosphate receptor Ca^{2+} release channels. *Physiol Rev.* 2007; 87(2): 593-658.
11. Uterine contractility. Ed. by R.E. Garfield. Serano Simposia, VSA, Norwill, Massachuses, 1990. 388 p.
12. Hertelendy F, Zakar T. Regulation of myometrial smooth muscle functions. *Curr Pharm Des.* 2004; 10(20): 2499-2517.
13. Luo ZG, Zhao Y, Maa C, Xu XM, Zhang XM, Huang NY, He HQ. Synthesis and anti-integrase evaluation of novel calix[4]arene derivatives containing the triazolyl 1,3-diketo moiety. *Chin Chem Letters.* 2014; 25(5): 737-740.
14. Xue Y, Guan Y, Zheng A, Xiao H. Amphoteric calix[8]arene-based complex for pH-triggered drug delivery. *Colloids Surf B Biointerfaces.* 2013; 101: 55-60.
15. Consoli GML, Galante E, Daquino C, Granata G, Cunsolo F, Geraci C. Hydroxycinnamic acid clustered by a calixareneplatform: radical scavenging and antioxidant activity. *Tetrahedron Lett.* 2006; 47(37): 6611-6614.
16. Rodik RV. Application of calixarenes for DNA transfection in cells. *Ukr Biokhim Zhurn.* 2012; 84(5): 5-15. (In Ukrainian).
17. Kalchenko VI, Rodik RV, Boyko VI. Calixarenes with bio-medical potential. *J Organic Pharmaceut Chem.* 2005; 3(4): 13-29. (In Ukrainian).
18. Veklich TO, Shkrabak OA, Mazur IuIu, Rodik RV, Boyko VI, Kalchenko VI, Kosterin SO. Kinetic properties of calixarene C-90 action on the myometrial plasma membrane Ca^{2+},Mg^{2+}-ATPase activity and on the Ca^{2+} concentration in unexcited cells of the myometrium. *Ukr Biokhim Zhurn.* 2013; 85(4): 20-29. (In Ukrainian).
19. Rodik R, Boiko V, Danylyuk O, Suwinska K, Tsymbal I, Slinchenko N, Babich L, Shlykov S, Kosterin S, Lipkowski J, Kalchenko V. Calix[4]-arenesulfonylamidines. Synthesis, structure and influence on Mg^{2+},ATP-dependent calcium pumps. *Tetrahedron Letters.* 2005; 46(43): 7459-7462.
20. Veklich TO, Kosterin SO. Comparative study of properties of Na^{+},K^{+}-ATPase and Mg^{2+}-ATPase of the myometrium plasma membrane. *Ukr Biokhim Zhurn*. 2005; 77(2): 66-75. (In Ukrainian).
21. Kondratiuk TP, Bychenok SF, Prishchepa LA, Babich LG, Kurskiy MD. Isolation and characteristics of the plasma membrane fraction from the swine myometrium *Ukr Biokhim Zhurn.* 1986; 58(4): 50-56. (In Russian).
22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976; 72(1-2): 248-254.
23. Mollard P, Mironneau J, Amedee T, Mironneau C. Electrophysiological characterization of single pregnant rat myometrial cells in short-term primary culture. *Am J Physiol.* 1986; 250(1 Pt 1): C47-54.
24. Flynn ER, Bradley KN, Muir TC, McCarron JG. Functionally separate intracellular Ca^{2+} stores in smooth muscle. *J Biol Chem.* 2001; 276(39): 36411-3648.

25. Valente RC, Capella LS, Monteiro RQ, Rumjanek VM, Lopes AG, Capella MA. Mechanisms of ouabain toxicity. *FASEB J.* 2003; 17(12): 1700-1702.
26. Wang H, Haas M, Liang M, Cai T, Tian J, Li S, Xie Z. Ouabain assembles signaling cascades through the caveolar Na^+/K^+-ATPase. *J Biol Chem.* 2004; 279(17): 17250-17259.
27. Veklich TO, Kosterin SO, Shynlova OP. Cationic specificity of a Ca^{2+}-accumulating system in smooth muscle cell mitochondria. *Ukr Biokhim Zhurn.* 2002; 74(1): 42-48. (In Ukrainian).
28. Rathbun WB, Betlach MV. Estimation of enzymically produced orthophosphate in the presence of cysteine and adenosine triphosphate. *Anal Biochem.* 1969; 28(1): 436-445.
29. Strehler EE, Zacharias DA. Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. *Physiol Rev.* 2001; 81(1): 21-50.
30. Boldyrev AA. Na/K^+-ATPase as an oligomeric ensemble. *Biochemistry (Mosc).* 2001; 66(8): 821-831.
31. Danylovych IuV, Chunikhin OIu, Danylovych HV. Investigation of the changes in uterine myocytes size depending on contractile activity modulators by photon correlation spectroscopy. *Fiziol Zh.* 2013; 59(1): 32-39. (In Ukrainian).
32. Danylovych HV, Danylovych IuV, Kolomiiets OV, Kosterin SO, Rodik RV, Cherenok SO, Kalchenko VI, Chunikhin OIu, Horchev VF, Karakhim SO. Changes in polarization of myometrial cells plasma and internal mitochondrial membranes under calixarenes action as inhibitors of plasma membrane Na^+,K^+-ATPase. *Ukr Biokhim Zhurn.* 2012; 84(6): 37-48. (In Ukrainian).
33. Crotty TP. Contraction in the smooth muscle cell. *Med Hypotheses.* 1999; 53(5): 432-446.
34. Stamenović D. Cytoskeletal mechanics in airway smooth muscle cells. *Respir Physiol Neurobiol.* 2008; 163(1-3): 25-32.

Received 19.11.2015