

## CALIX[4]ARENE C-956 SELECTIVELY INHIBITS PLASMA MEMBRANE $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ -ATPase IN MYOMETRIAL CELLS

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Using enzymatic assay and kinetic analysis, we demonstrated that 100  $\mu\text{M}$  calix[4]arene C-956 (5,11,17,23-tetra(trifluoro)methyl-(phenylsulfonylimino) methylamino-25,27-dioctyloxy-26,28-dipropoxy-calix[4]arene) had the most significant inhibitory effect on the plasma membrane  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase activity compared to effects of other calix[4]arenes, and had no effect on specific activities of other membrane ATPases. Using confocal microscopy and fluorescent probe fluo-4, we observed an increase of the intracellular level of  $\text{Ca}^{2+}$  after application of calix[4]arene C-956 to immobilized myocytes. Analysis of the effect of calix[4]arene C-956 on the hydrodynamic diameter of myocytes demonstrated that application of calix[4]arene C-956 solution decreased this parameter by  $45.5 \pm 9.4\%$  compared to control value that was similar to the action of uterotonic drug oxytocin.

**Key words:**  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase, plasma membrane, smooth muscle cells, myometrium, enzymatic hydrolysis of ATP, calix[4]arenes.

**R**egulation of the intracellular calcium ions concentration  $[\text{Ca}^{2+}]_i$  plays a key role in the mechanisms of contraction and relaxation of muscle cells including uterus smooth muscle (SM) cells [1-4]. The  $\text{Ca}^{2+}$  entry into the cytoplasm of SM cells is vital for the muscle contraction and provided by a number of calcium channels which are localized in the plasma membrane (PM) (voltage-, ligand-, depot-gated  $\text{Ca}^{2+}$  channels) and sarcoplasmic reticulum (SR) (ryanodine,  $\text{IP}_3$  receptors) [5, 6]. After SM contraction, this secondary messenger is removed from the cytosol by the systems localized in PM ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase,  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger), SR ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase) and mitochondria [7-9].

Since the main source of  $\text{Ca}^{2+}$  for myometrium contraction is the extracellular space, the PM  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase is a leading “player” in the  $\text{Ca}^{2+}$  release from the myometrial SM cells after contraction [10]. In addition, this enzyme maintains a strict control of the  $[\text{Ca}^{2+}]_i$  level in the myometrial cells in a rest state by compensating the passive  $\text{Ca}^{2+}$  entry via PM [11]. During one catalytic cycle, the  $\text{Mg}^{2+}$ -dependent  $\text{Ca}^{2+}$  pump transports one Ca ion against

the concentration gradient using the energy of the hydrolysis of one ATP molecule [11, 12]. The catalytic function of PM  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase, as a fine regulator of the cytosolic  $\text{Ca}^{2+}$  concentration, is described with such enzyme properties - high affinity to  $\text{Ca}^{2+}$ , but the reaction catalyzed by this enzyme has much smaller turnover number compare to  $\text{Na}^+/\text{Ca}^{2+}$  exchanger [7, 11].

Since the obvious regulatory role of this transport enzyme in uterine SM cells, modulation of its activity may be one of the approaches to normalize the myometrium contractility and tone during some pathologies, such as atony, hypo-, hypertonic uterus, and weakness of labor [13, 14]. PM  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase has variety of regulatory sites at the cytoplasmic domain and is regulated by a wide range of biochemical mechanisms *in vivo*. In particular, calmodulin, PDZ domain-containing proteins and PM acidic phospholipids are considered as the pump modulator [15, 16]. Several artificial enzyme inhibitors (eosin Y, orthovanadate, calmodulin antagonists), though they are low-molecular compounds, have limited use due to their low selectivity

[16, 17]. Novel peptide inhibitors - caloxins 1, 2, 3 - despite their selective inhibitory effect on  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase, have also drawbacks: an increase of their affinity to the enzyme leads to a loss of selectivity [16, 18].

In previous studies, it was shown that the synthetic compound calix[4]arene C-90 (5,11,17,23-tetra(trifluoro)methyl(phenylsulfonylimino)-methylamino-25,26,27,28-tetrapropoxy-calix[4]-arene) in concentration 100  $\mu\text{M}$  efficiently (by 75% relative to control) and selectively inhibited the activity of PM  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase of the uterine myocytes, without affecting the activity of other PM ATPases [19, 20]. Sensitive to protonophore CCCP,  $\text{Ca}^{2+}$  accumulation in the myometrial mitochondria was found to be resistant to the action of this calixarene [21].

Calixarenes are cyclic oligomers formed from para-substituted phenols and formaldehyde, low-molecular, low-toxic supramolecular compounds capable of forming host-guest complexes with metal ions and organic compounds [22]. A lot of publications shows the potential use of these macrocyclic compounds as enzyme effectors, receptor simulators, organic polymer stabilizers, DNA microarray platform, etc. [23-25]. Knowing the effect of some the structural features (hydrophobic "bowl" with substituents on upper and lower rims) on specific biochemical properties (lipophilicity, low toxicity and immunogenicity) [26], it is possible to synthesize compounds with new properties and appropriate selectivity, changing a bowl size and substituents on its rims.

We investigated the effect of a number of calix[4]arenes (C-715, C-716, C-772, C-956, C-957, C-975) - the structural analogs of calix[4]arene

C-90 - on the activity of PM  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase, and found that calix[4]arene C-956 exhibits an even greater inhibitory effect than calix[4]arene C-90. Thus, the aim of this work was to study the inhibitory effect of calix[4]arene C-956 on the PM  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase activity and  $\text{Ca}^{2+}$  level of the uterine SM cells.

## Materials and Methods

**Structure and synthesis of calix[4]arene C-956.** Calix[4]arene C-956 (5,11,17,23-tetra(trifluoro)methyl(phenylsulfonylimino)methylamino-25,27-dioctyloxy-26,28-dipropoxycalix[4]arene) and calyx[4]arene "bowl" C-150 (Fig. 1) were synthesized and characterized using NMR and infrared spectroscopy in the Phosphoranes Chemistry Department of the Institute of Organic Chemistry, NASU.

**Biochemical study.** Biochemical studies were carried out in the Department of Muscle Biochemistry of Palladin Institute of Biochemistry, NASU. The PM fraction of uterine SM cells was obtained from the porcine myometrium as it was described earlier [27, 28]. The protein concentration in the membrane fraction was determined by Bradford method [29] using Kumasi-G250 reagent.

**Enzymatic studies.** Total ATPase activity was determined in the fraction of myometrium PMs at 37 °C in a standard medium (0.4 ml) containing (mM): 3 ATP, 3  $\text{MgCl}_2$ , 0.95  $\text{CaCl}_2$ , 25 NaCl, 125 KCl, 1 EGTA, 20 HEPES-Tris buffer (pH 7.4), 1  $\text{NaN}_3$  (inhibitor of mitochondrial ATPase [30]), 1 ouabain (selective inhibitor of  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase [31, 32]), 0.1  $\mu\text{M}$  thapsigargin (selective inhibitor of the sarco/endoplasmic reticulum  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase [30]) and 0.1% digitonin (for PM permeabilization [33]). Calculations made using MAXCHEL

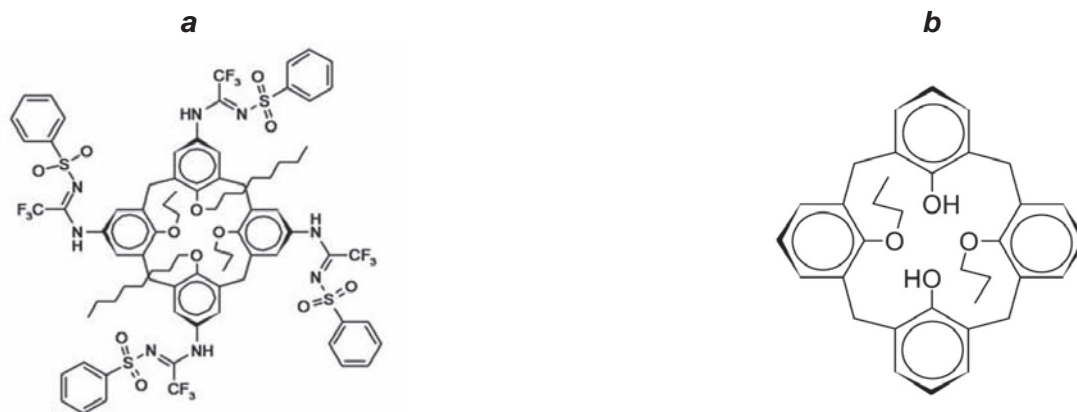


Fig. 1. Structural formulas of calix[4]arene C-956 (a) and calyx[4]arene "bowl" C-150 (b)

program indicated that under the mentioned physicochemical and concentration conditions of the incubation medium, the free  $\text{Ca}^{2+}$  concentration was 1  $\mu\text{M}$ . During the study of the effect of various  $\text{Ca}^{2+}$  concentrations on the  $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity, the required concentrations of  $\text{Ca}^{2+}$  were also determined using MAXCHEL calculations. The content of membrane fraction protein in the sample was 20–30  $\mu\text{g}$ , incubation time – 5 min. The enzymatic reaction was initiated by addition the aliquot (50  $\mu\text{l}$ ) of the PM suspension to the medium, and terminated by addition to the incubation mixture 1 ml of the “stop” solution composed 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  $\text{P}_i$  was determined by method [34].

$\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity was calculated as the difference between the ATPase activities in the presence and absence of exogenous  $\text{Ca}^{2+}$  in the incubation medium (on the background of 1 mM EGTA - specific  $\text{Ca}^{2+}$  chelating agent). In the porcine myometrium sarcolemma, the specific  $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase activity was  $3.4 \pm 0.3 \mu\text{M P}_i/\text{mg}$  of protein per hour ( $M \pm m$ ;  $n = 7$ ).

The “basal”  $\text{Mg}^{2+}$ -ATPase activity was determined in the fraction of PM of myometrium cells at 37 °C in a medium (volume – 0.4 ml) containing (mM): 1 ATP, 3  $\text{MgCl}_2$ , 125 NaCl, 25 KCl, 1 EGTA, 20 Hepes-tris buffer (pH 7.4), 1  $\text{NaN}_3$ , 1 ouabain, 0.1  $\mu\text{M}$  thapsigargin and 0.1% digitonin. The “basal”  $\text{Mg}^{2+}$ -ATPase activity was calculated as the difference between the amounts of  $\text{P}_i$  produced in the incubation medium in the presence and absence of the PM fraction with correction for the content of endogenous  $\text{P}_i$  in the membrane fraction.

$\text{Na}^+, \text{K}^+$ -ATPase activity was determined in the same medium and was calculated as the difference between the ATPase activities in the absence and presence of 1 mM ouabain.

The average values of the specific activities of  $\text{Na}^+, \text{K}^+$ -ATPase,  $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase and the “basal” PM  $\text{Mg}^{2+}$ -ATPase were  $10.2 \pm 0.7$ ,  $3.4 \pm 0.3$  and  $18.1 \pm 1.2 \mu\text{M P}_i/\text{mg}$  of protein per hour ( $M \pm m$ ;  $n = 7$ ), respectively.

It should be noted that  $\text{Ca}^{2+}$ -ATPase, which differ by its properties from  $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase, was also found in the PM of the uterine myocytes. Its activity determined in the presence of mM concentrations of  $\text{Ca}^{2+}$  and ATP in the incubation medium and the absence of  $\text{Mg}^{2+}$  [35, 36].  $\text{Ca}^{2+}$ -ATPase has a low affinity to activating cation (the activation con-

stant by  $\text{Ca}^{2+}$ ,  $K_{\text{Ca}^{2+}}$  is 1 mM) [36]. Low-affine  $\text{Mg}^{2+}$ -independent  $\text{Ca}^{2+}$ -ATPase activity was determined in the PM fraction of myometrium cells at 37 °C in a medium (0.4 ml) containing (mM): 1 ATP, 3  $\text{CaCl}_2$ , 125 NaCl, 25 KCl, 1 EGTA, 20 HEPES-tris buffer (pH 7.4), 1  $\text{NaN}_3$ , 1 ouabain, 0.1  $\mu\text{M}$  thapsigargin and 0.1% digitonin.  $\text{Ca}^{2+}$ -ATPase activity was calculated as the difference between the amount of  $\text{P}_i$  produced in the incubation medium in the presence and absence of the PM fraction with correction for the content of endogenous  $\text{P}_i$  in the membrane fraction. In porcine myometrium sarcolemma, the specific activity of low-affine PM  $\text{Mg}^{2+}$ -independent  $\text{Ca}^{2+}$ -ATPase was  $12.7 \pm 2.0 \mu\text{M P}_i/\text{mg}$  of protein per hour ( $M \pm m$ ;  $n = 7$ ).

The effect of calix[4]arene C-956 at various concentrations (1–100  $\mu\text{M}$ ) on  $\text{Ca}^{2+}, \text{Mg}^{2+}$ -ATPase was investigated using the standard incubation medium (as described above) to which the solution of calix[4]arene at the relevant concentration was added. A concentrated (20 mM) calix[4]arene C-956 solution in DMSO was diluted with water to required concentrations.

*Determination of the intracellular  $\text{Ca}^{2+}$  concentration by confocal microscopy.* To measure the  $\text{Ca}^{2+}$  concentration in SM cells, the suspension of myocytes obtained by the modified Mollard method [37, 38] was being loaded with a  $\text{Ca}^{2+}$ -sensitive probe fluo-4 AM for 20 min at room temperature, the suspension then was centrifuged for 15 min at 1000 g, the pellet was diluted with isotonic storage medium (containing 25 mM HEPES-KOH (pH 7.4, 8 °C), 150 mM NaCl) and put on poly-L-lysine-coated glass surface. The samples of immobilized cells were analyzed using a laser confocal scanning microscope LSM 510 META. For the analysis, spindle-shaped cells with clearly defined nucleus dyed with DNA-sensitive fluorescent Hoechst probe (added 10 min prior to registration) were selected. The relative changes in  $\text{Ca}^{2+}$  concentration in the cytoplasm were registered by taking a series of photographic images. During analysis, C-956 solution (test) or C-150 solution (control) at a concentration of 20  $\mu\text{M}$  (5  $\mu\text{l}$ ) were applied.

*Determination of the hydrodynamic diameter of myocytes by dynamic light scattering.* The hydrodynamic diameter of myocytes was determined using ZetaSizer-3 (Malvern Instruments, UK), equipped with a He-Ne laser LGN-111 ( $P = 25 \text{ mW}$ ,  $\lambda = 633 \text{ nm}$ ). The size measuring ranges from 1 nm to 20  $\mu\text{m}$ . The registration and statistical processing

of laser light scattered from the aqueous suspension of particles ( $n = 1.33$ ) were carried out 5 times for 60 sec at 22 °C at a scattering angle of 90 °.

First, 100  $\mu$ l of cell suspension and 900  $\mu$ l of Hanks A solution composed of, mM: 136.9 NaCl, 5.36 KCl, 0.44  $\text{KH}_2\text{PO}_4$ , 0.26  $\text{NaHCO}_3$ , 0.26  $\text{Na}_2\text{HPO}_4$ , 1.26  $\text{CaCl}_2$ , 0.4  $\text{MgCl}_2$ , 5.5 glucose, 10 HEPES (pH 7.4 at 37 °C) were placed into a cuvette. After registration of control values, 10  $\mu$ l of the calix[4]arene solution in DMSO (final concentration 50  $\mu$ M) was introduced into the cuvette and measurements were made, as described above. The measurement data were processed using PCS-Size mode v1.61 program.

**Kinetic calculations.** For quantifying of the calix[4]arenes effect on enzymatic activity, the inhibition coefficients  $I_{0.5}$  and Hill coefficients  $n_H$  were calculated using linearized Hill plots of the equation  $\lg[(A_{\max} - A)/A] = -n_H \lg I_{0.5} + n_H \lg [\text{C-956}]$ , where  $A_{\max}$  and  $A$  are specific enzymatic activities in the absence (“zero point”) or in the presence of calix[4]arene at the concentration [C-956] in the incubation medium.

**Statistical analysis.** Statistical analysis of the obtained data was performed using Student’s *t*-test. Kinetic and statistical parameters were calculated using MS Excel software.

**Reagents.** The following reagents were used in the study: ATP, Hepes, ouabain, thapsigargin, Hoechst, Fluo-4 AM, collagenase, poly-L-lysine (Sigma, USA), Tris-hydroxymethyl-aminomethane (Reanal, Hungary), digitonin (Merck, Germany), EGTA (Fluka, Switzerland), oxytocin (Gedeon Richter, Hungary). Other reagents of domestic production were of reagent grade or laboratory grade.

## Results and Discussion

The obtained results of the studying the effect of calix[4]arenes on  $\text{Mg}^{2+}$ -dependent ATP-hydrolase activities in the fraction of PM of myometrium cells indicate that calix[4]arene C-956 (5,11,17,23-tetra(trifluoro)methyl(phenylsulfonylimino)methylamino-25,27-dioctyloxy-26,28-dipropoxycalix[4]arene) at a concentration of 100  $\mu$ M efficiently inhibited the  $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ -ATPase activity of PM of myometrium cells to a level of  $20.8 \pm 0.4\%$  relative to control value taken as 100% ( $M \pm m$ ;  $n = 5$ ) (Fig. 2). In addition, this compound at the same concentration did not affect the activities of  $\text{Na}^+$ , $\text{K}^+$ -ATPase, “basal”  $\text{Mg}^{2+}$ -ATPase and  $\text{Ca}^{2+}$ -ATPase of PM: the respective values were  $95.2 \pm 0.6$ ;  $108.4 \pm 1.0$  and

$104.3 \pm 0.9\%$ , relative to the control (Fig. 2). It should be noted that the calixarene “bowl” (calix[4]arene C-150) (25,27-dipropoxycalix[4]arene) had practically no effect on the PM  $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ -ATPase activity (data are not presented). Thus, calix[4]arene C-956 at a concentration of 100  $\mu$ M selectively (at the PM level) inhibited the PM  $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ -ATPase activity without affecting the activity of  $\text{Na}^+$ , $\text{K}^+$ -ATPase,  $\text{Mg}^{2+}$ -ATPase and  $\text{Ca}^{2+}$ -ATPase of PM.

It was previously shown by researchers of our department that calix[4]arene C-956 had also an inhibitory effect on  $\text{H}^+$ - $\text{Ca}^{2+}$ -exchanger of the inner mitochondrial membrane of rat uterine myocytes ( $I_{0.5} = 35.1 \pm 7.9 \mu\text{M}$ ) and did not affect the voltage-dependent accumulation of  $\text{Ca}^{2+}$  in mitochondria [39].

In our further research, we studied a concentration-dependence of the inhibitory effect of calix[4]arene C-956 ( $10^{-8}$ - $10^{-4}$  M) on the activity of  $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ -ATPase of PM.

Calix[4]arene C-956 inhibited  $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ -ATPase activity in a dose-dependent manner (Fig. 3). The calculated inhibition coefficient  $I_{0.5}$  was  $15.0 \pm 0.5 \mu\text{M}$ , the Hill coefficient  $n_H$  was  $0.55 \pm 0.01$  ( $M \pm m$ ;  $n = 5$ ). Therefore, the inhibition coefficient  $I_{0.5}$  for calix[4]arene C-956 was found to be less than the same parameter of calix[4]arene C-90 ( $I_{0.5} = 20.2 \pm 0.5 \mu\text{M}$ ) [19], and the Hill coefficient  $n_H$  (0.55 for C-90) was the same for both calixarenes.

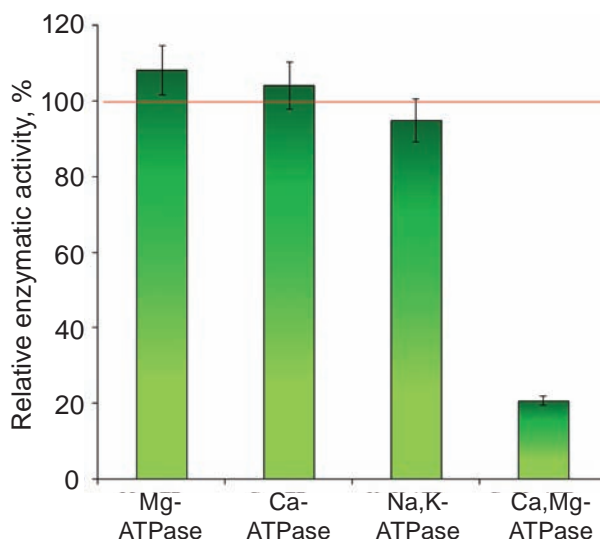


Fig. 2. The effect of calix[4]arene C-956 (100  $\mu$ M) on ATP-hydrolase activities of PMs of myometrium cells. 100% is enzymatic activity in the absence of calix[4]arene C-956 in the incubation medium



Affecting ion transport systems of myocytes, calix[4]arenes can influence both the contraction of SM cells and water-salt metabolism, and can also change the shape and volume of these cells that can be detected by dynamic light scattering. This method allows measuring the effective hydrodynamic diameter of SM cells and its changes under various conditions. The literature data indicate that the factors which increase the contractile response of SM also change the effective hydrodynamic diameter of SM cells. It was reported in [40] that the addition of  $\text{Ca}^{2+}$  (3 mM), treatment of a suspension of cells with A-23187 (10  $\mu\text{M}$ ), tetraethyl ammonium (1 mM) or 4-aminopyridine (1 mM) led to a decrease of the cells' effective hydrodynamic diameter, which correlates with the SM contraction.

The average magnitude of the effective hydrodynamic diameter of myocytes was found to be about 9  $\mu\text{M}$  (Fig. 4). The addition of uterotonic hormone oxytocin (100 nM) led to a decrease of the effective hydrodynamic diameter of SM cells by  $28.7 \pm 4.5\%$  ( $M \pm m$ ;  $n = 6$ ), relative to the control value (Fig. 4) that might indicate a trigger for mechanisms of SM cell contraction. The application of calix[4]arene C-956 (50  $\mu\text{M}$ ) inhibitor of  $\text{Mg}^{2+}$ , ATP-dependent  $\text{Ca}^{2+}$  pump of the PM of SM cells, led to a decrease of the hydrodynamic diameter by  $45.5 \pm 9.4\%$  ( $M \pm m$ ,  $n = 6$ ), relative to the control value, therefore, oxytocin and calix[4]arene C-956 have a similar effect (Fig. 4). We also analyzed the effect of DMSO to exclude a potential impact of this solvent of the calix[4]arene C-956 solution and found that DMSO solution (0.25%) did not affect the hydrodynamic diameter of cells (Fig. 4).

Thus, the effective hydrodynamic diameter of the SM cells was decreased by calix[4]arene C-956 similarly to the uterotonic oxytocin. Such alterations in the hydrodynamic diameter can be interpreted as a result of the events that accompany the processes of SM cell contraction/relaxation, namely changes in the water-osmotic balance, the rearrangement of the cytoskeleton elements. Since the change in the hydrodynamic diameter of SM cells in the presence of contractile agents correlates with the SM contraction, the obtained results show the promising potential of calix[4]arene C-956 as a regulator of the contractile activity of the uterine SMs.

Taking into consideration an important role of PM  $\text{Ca}^{2+}$  pump in controlling the  $\text{Ca}^{2+}$  concentration in the cytoplasm of SM, it is important to find out whether calix[4]arene C-956 affects the intracel-

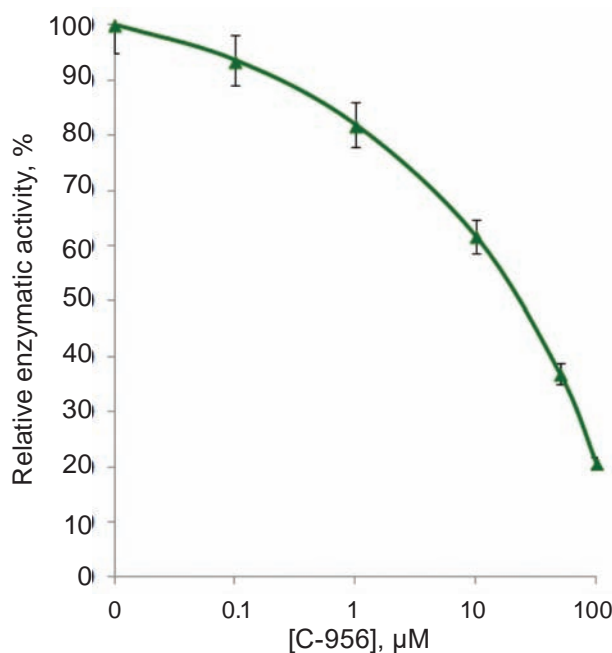


Fig. 3. The effect of calix[4]arene C-956 on the activity of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -ATPase of myometrium cell PMs ( $M \pm m$ ,  $n = 5$ ). 100% is the relative enzymatic activity in the absence of calix[4]arene C-956 in the incubation medium

lular  $\text{Ca}^{2+}$  concentration in SM cells. Therefore, in our further experiments, we detected changes of the  $\text{Ca}^{2+}$  concentrations in myoplasm in the presence of calix[4]arene C-956 by confocal microscopy using  $\text{Ca}^{2+}$ -sensitive fluo-4 probe. It was observed that, af-

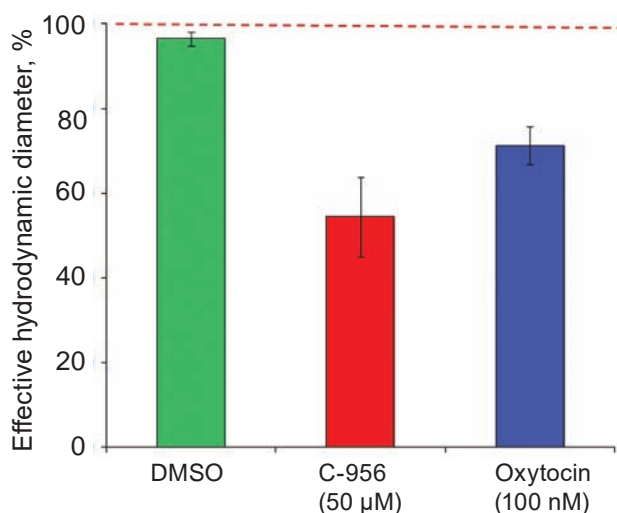


Fig. 4. Effect of calix[4]arene and oxytocin on hydrodynamic diameter of SM cells ( $M \pm m$ ,  $n = 6$ ). 100% is the control value of the hydrodynamic diameter of the SM cells in the absence of any effectors

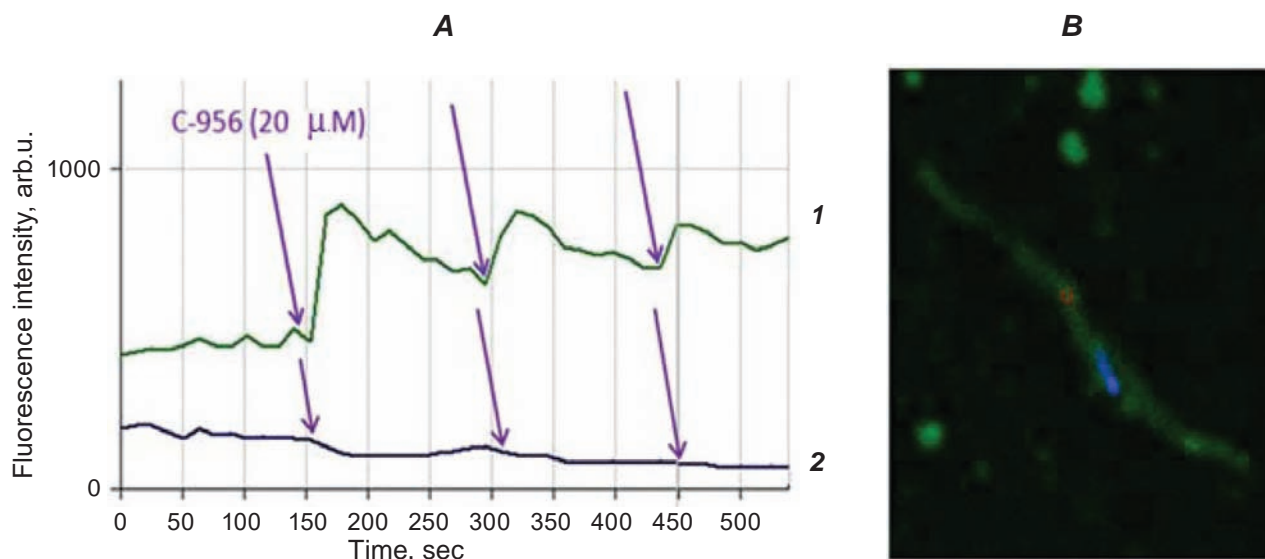


Fig. 5. **A.** Time course of fluorescence intensity of Ca<sup>2+</sup>-sensitive probe fluo-4 AM (1) and DNA-sensitive probe Hoechst (2) in SM cell after addition of calix[4]arene C-956. **B.** Image of the uterine SM cells obtained with a scanning laser confocal microscope. The arrows show the time points of adding of the calix[4]arene C-956 solution. The results of the typical experiment are presented

ter addition of calix[4]arene C-956 (20 μM), there was a sharp increase in the fluorescence response of Ca<sup>2+</sup>-sensitive fluo-4 AM probe in the cell (Fig. 5). Then the Ca<sup>2+</sup> concentration decreased within the next 2.5 min that indicates the engagement of other homeostatic mechanisms of Ca<sup>2+</sup> concentration regulation (mitochondrial Ca<sup>2+</sup>-uniporter, PM Na<sup>+</sup>/Ca<sup>2+</sup>-exchanger). Re-adding of calix[4]arene C-956 led to a less increase of the cell Ca<sup>2+</sup> concentration, compared to first addition, probably because the Ca<sup>2+</sup> gradient was not completely restored. Thus, calix[4]arene C-956, inhibitor of PM Ca<sup>2+</sup>,Mg<sup>2+</sup>-ATPase, increases the cytosolic Ca<sup>2+</sup> concentration in SM cells.

In the control experiments, where calix[4]arene “bowl” C-150 (20 μM) was used, which according to our results had no significant effect on the PM Ca<sup>2+</sup>,Mg<sup>2+</sup>-ATPase activity (see above), any increase in fluorescence intensity of fluo-4 was not observed (results are not presented). Hoechst fluorescence intensity also remained unchanged.

These results indicate that after application of calix[4]arene C-956 (20 μM) the cell Ca<sup>2+</sup> concentration increased sharply that was probably related to a decrease in the basal activity of PM Ca<sup>2+</sup>,Mg<sup>2+</sup>-ATPase inhibited by this calixarene. Moreover, the Ca<sup>2+</sup> concentration decreased to the initial level

within next 100 sec, therefore, the compensatory Ca<sup>2+</sup>-transport systems, which have a low affinity for Ca<sup>2+</sup> and mainly respond to its high concentrations in cells (mitochondrial Ca<sup>2+</sup>-uniporter, PM Na<sup>+</sup>/Ca<sup>2+</sup>-exchanger), are employed for relaxation of the calcium signal.

Thus, the obtained data can provide the basis for using of calix[4]arene C-956 as a selective and effective inhibitor of PM Ca<sup>2+</sup>,Mg<sup>2+</sup>-ATPase that can be important for further investigation of the membrane mechanisms of Ca<sup>2+</sup> exchange in the SM, in particular, PM participation in electromechanical coupling in SMs. The obtained results may also be used in further studies aimed to determine a correlation between the calix[4]arenes' structure and their effect on various cation transport enzymatic systems, that can be helpful in enhancing selectivity and efficiency of the calixarene-based inhibitors. In addition, our data can provide a basis for the development of potential pharmacological agents for modulating uterine contractile function during pathologies of the SM contractile activity.

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**КАЛІКС[4]АРЕН С-956  
СЕЛЕКТИВНО ІНГІБУЄ  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -  
АТРазу ПЛАЗМАТИЧНОЇ  
МЕМБРАНИ КЛІТИН МІОМЕТРІЯ**

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Із використанням методів ензиматичного та кінетичного аналізу продемонстровано, що калікс[4]арен С-956 (5,11,17,23-тетра(трифтор)метил-(феніл-сульфоніліміно)метиламіно-25,27-діоктилокси-26,28-дипропоксикалікс[4]арен) у концентрації 100 мкМ чинив найбільшу інгібіторну дію на  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -АТРазу активність плазматичної мембрани порівняно з іншими аналогами цього калікс[4]арену і не впливав на питому активність інших мембранних АТРАЗ. За допомогою конфокальної мікроскопії з використанням кальційчутливого флуоресцентного зонда fluo-4 показано, що внесення калікс[4]арену С-956 до іммобілізованих міоцитів матки зумовлює зростання рівня внутрішньоклітинної концентрації  $\text{Ca}^{2+}$ . Аналіз впливу калікс[4]арену С-956 на ефективний гідродинамічний діаметр міоцитів міометрія продемонстрував, що додавання розчину цієї сполуки до суспензії міоцитів зменшує вказаний показник на  $45,5 \pm 9,4\%$  подібно до дії утеротоніка окситоцину.

**Ключові слова:**  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -АТРаза, плазматична мембрана, гладеньком'язові клітини, міометрій, ензиматичний гідроліз АТР, калікс[4]арени.

**КАЛИКС[4]АРЕН С-956  
СЕЛЕКТИВНО ИНГИБИРУЕТ  
 $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -АТРазу  
ПЛАЗМАТИЧЕСКОЙ МЕМБРАНЫ  
КЛЕТОК МИОМЕТРИЯ**

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С использованием методов энзиматического и кинетического анализа продемонстрировано, что калікс[4]арен С-956 (5,11,17,23-тетра(трифтор)метил-(феніл-сульфоніліміно)метиламіно-25,27-діоктилокси-26,28-дипропоксикалікс[4]арен) в концентрации 100 мкМ оказывал наибольшее ингибирующее действие на  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -АТРазную активность плазматической мембраны по сравнению с другими аналогами указанного калікс[4]арена и практически не влиял на удельную активность других мембранных АТРАЗ. Кроме того, при помощи конфокальной микроскопии с использованием кальцийчувствительного флуоресцентного зонда fluo-4 показано, что внесение калікс[4]арена С-956 в иммобилизованные миоциты матки вызывает повышение внутриклеточной концентрации  $\text{Ca}^{2+}$ . Анализ влияния калікс[4]арена С-956 на эффективный гидродинамический диаметр продемонстрировал, что добавление раствора данного соединения в суспензию миоцитов уменьшает этот показатель на  $45,5 \pm 9,4\%$  подобно действию утеротоника окситоцина.

**Ключевые слова:**  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ -АТРаза, плазматическая мембрана, гладкомышечные клетки, миометрий, энзиматический гидролиз АТР, кинетические свойства АТРАЗы, калікс[4]арены.

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