# EXPERIMENTAL WORKS

UDC 577.352.4+544.147+544.176+544.168 doi: https://doi.org/10.15407/ubj95.06.005

## THIACALIX[4]ARENE C-1087 IS THE SELECTIVE INHIBITOR OF THE CALCIUM PUMP OF SMOOTH MUSCLE CELLS PLASMA MEMBRANE

*T. O. VEKLICH[1]✉, R. V. RODIK[2], O. V. TSYMBALYUK[3], O. V. SHKRABAK[1], O. V. MALIUK[1], S. O. KARAKHIM[1], S. H. VYSHNEVSKYI[3], V. I. KALCHENKO[3], S. O. KOSTERIN[1]*

*[1]Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv;*
*✉e-mail: veklich@biochem.kiev.ua*
*[2]Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Kyiv;*
*[3]Educational and Scientific Institute of High Technologies, Taras Shevchenko National University of Kyiv, Kyiv, Ukraine;*

**Received:** 01 September 2023; **Revised:** 23 October 2023; **Accepted:** 01 December 2023

*The enzymatic and kinetic analyses were used to demonstrate that 5,11,17,23-tetra(trifluoro) methyl(phenylsulfonylimino)methylamino-25,27-dihexyloxy-26,28-dihydroxythiacalix[4]arene C-1087 effectively inhibited the $Ca^{2+}$,$Mg^{2+}$-ATPase activity of the rat myometrium cells plasma membrane ($I_{0.5}$ = 9.4 ± 0.6 μM) with no effect on the relative activity of other membrane ATPases. With the use of confocal microscopy and $Ca^{2+}$-sensitive fluorescent probe fluo-4, it was shown that the application of thiacalix[4]arene C-1087 to the immobilized uterus myocytes increased the cytosolic concentration of $Ca^{2+}$. Tenzometric studies of rat uterus smooth muscles with the subsequent mechanokinetic analysis revealed that thiacalix[4]arene C-1087 considerably decreased the maximal velocity of the relaxation of both spontaneous contractile response and contraction induced by hyperpotassium solution.*

*K e y w o r d s: plasma membrane $Ca^{2+}$,$Mg^{2+}$-ATPase, cytosolic $Ca^{2+}$, smooth muscle cell, myometrium, contraction-relaxation mechanokinetics, thiacalix[4]arene.*

Calcium ions ($Ca^{2+}$) are vital for the implementation of a broad spectrum of cellular functions. To realize the processes of smooth muscle (SM) contraction/relaxation, including myometrium, it is necessary for the level of cytoplasmic $Ca^{2+}$ in the myoplasm to change dynamically and reversibly in the concentration range from approximately 100 nM (the state of dormancy, relaxation of mechanic tension) to 1 μM (contraction) [1, 2]. $Ca^{2+}$ penetrates SM myocytes from the extracellular space via the plasma membrane (PM) using different calcium channels or is released from the intracellular $Ca^{2+}$ depots due to its passive transportation by the chemical gradient.

The main systems of energy-dependent removal of $Ca^{2+}$ from the cytosol are calcium pumps of PM and sarcoplasmic reticulum (SR), $Na^+$/$Ca^{2+}$-exchanger of PM, and $Ca^{2+}$-uniporter of mitochondria [3-5].

A relevant PM structure regulating the basal tone and contractility of the myometrium is a calcium pump ($Mg^{2+}$-ATP-dependent calcium pump) [6]. Four isoforms of this enzyme are expressed in the organism of humans and higher vertebrates and form about 30 additional kinds due to alternative splicing. Two isoforms of the calcium pump in the plasma membrane are expressed in non-pregnant myometrium: 1 (splice-variant b) and 4 (splice-variants a and b) [7, 8].

At present, there is a clear understanding of the fact that the calcium pump is one of the key structures in PM which plays a $Ca^{2+}$-transporting and signaling role in the cells, the disruption in the functioning of which is related to the development

of many pathologies (including arterial hypertension and myocardial hypertrophy, nervous disorders, male sterility, etc.). It demonstrates the powerful potential of the PM calcium pump; however, there are no satisfactory pharmacological means with the targeted effect on this enzyme [9-12]. Thus, there is an urgent need to develop, synthesize and study the biological effects of substances with the targeted effect on the PM calcium pump.

The disorder in the contractile myometrium function in women often becomes a cause for various pathologies: slow labor, spontaneous abortions, early labor, miscarriages, atony, hypo- and hypertonicity of the uterus [13, 14]. Usually, these pathologies are caused by disorders in the functioning of membrane-related systems of cation transfer. Thus, it would be advantageous to search for substances capable of modifying the contractile function of the myometrium in the case of the mentioned pathological states.

Calix[n]arenes – are nano-sized cup-shaped macrocyclic compounds [15, 16]. They are actively studied as nanovectors for targeted API delivery, enzymes modulators, molecular platforms for the design of bio-active compounds etc [17-20], due to the practically unlimited possibilities of chemical modification with various functional groups, regulation of the hydrophilic-hydrophobic balance of the molecule within wide ranges, low toxicity [21], lack of immunogenicity [18, 21].

Our previous investigation of the effect of calixarenes on the energy-dependent transportation of Ca ions via PM of SM cells demonstrated that some of these substances might be inhibitors of the mentioned process [22].

This article presents a description of the regularities of the inhibitory effect of thiacalix[4]arene C-1087 on the PM calcium pump of the uterine smooth muscle cells.

## Materials and Methods

### A. Synthesis and structure of thiacalixarenes

Schemes of the thiacalixarenes C-1087 and C-1145 (model thiacalixarene platform) synthesis (Fig. 1 and 2) are described in the section Results and Discussions. NMR spectra were recorded on Varian VXR 400 (400 MHz) spectrometer. $^{13}$C NMR spectra were recorded on Bruker Advance DRX 500 (125 MHz) spectrometer. Chemical shifts (δ) are expressed in ppm and referred to the residual peak of the solvent or TMS as an internal standard; coupling constants (J) are in Hz.

*5,11,17,23-tetranitro-25,27-dihexyloxi-26,28-dihydroxythiacalixarene **2**.* To suspension of tetranitrothiacalixarene **1** (1.7 g, 2.51 mmol) and potassium carbonate (2.75 g, 19.9 mmol) in DMF (25 ml) the hexyliodide (4.15 g, 3 ml, 19.6 mmol) was added under stirring. The reaction was stirred at 75°C for 48 h. The 1 M solution of sulfuric acid (30 ml) was added after cooling. After 10-15 min light suspension was decanted off, the sticky residue was suspended in chloroform (30 ml) and washed with 1 M hydrochloric acid (30 ml), brine (30 ml) and evaporated on rotor evaporator (15 mm Hg, 50°C). Dark-yellow solid was washed with diethyl ether (20 ml) and was filtrated. Obtained yellow crude thiacalixarene **2** was purified by column chromatography ($SiO_2$, 40-60 μm, $CHCl_3$-$CH_3OH$, 100:1 by volume, $R_f$ 0.5). Slightly yellow crystalline compound, yield 1.8 g (84.8%) M.P. 242-244°C. $^1$H NMR (399.98 MHz, $CDCl_3$) δ: 8.67 (s, 4H, ArH(OH)), 8.12 (s, 2H, OH), 7.99 (s, 4H, ArH (OHexyl)), 4.39 (t, J = 6.86 Hz, 4H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 2.03 (m, 4H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 1.58 (m, 4H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 1.40 (m, 8H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 0.93 (t, J = 6.86 Hz, 6H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), $^{13}$C NMR (125.70 MHz, $CDCl_3$) δ: 164.26, 162.87, 143.96, 140.19, 132.62, 131.69, 129.29, 121.89, 79.11, 31.45, 29.79, 25.28, 22.51, 14.02.

*5,11,17,23-tetraamino-25,27-dihexyloxi-26,28-dihydroxythiacalixarene **3**.* To the suspension of tetranitrothiacalixarene 2 (1.3 g, 1.54 mmol) in 2-propanol (40 ml) the hydrazine hydrate (4 ml) was added. Obtained reaction mass was heated under stirring to full dissolution. To the obtained solution the Raney nickel suspension (10% by weight in water, 3 ml) was added. The reaction mass was refluxed till its yellow color disappearance. In the case of the end of gas evolution, the hydrazine hydrate (2 ml) was added and the reaction continued. After cooling, reaction mixture was filtrated through silica gel. Silica gel was washed by warm (40-50°C) DMF (20 ml). Filtrate volumes were combined and evaporated on rotor evaporator (15 mm Hg, 65°C). A toluene (20 ml) was added to light brown oily residue and resulted mixture was again evaporated on rotor evaporator (15 mm Hg, 65°C). Tetraaminothiacalixarene 3 was dried in vacuum (12 mm Hg, 80°C) for 4 h. Gray-green solid compound, yield 0.92 g (82.6%) M.P. 205-208 °C. 1H NMR (399.98 MHz, DMSO-$d_6$) δ: 7.06 (s, 2H, OH), 6.88 (s, 4H, ArH(OH)), 6.28 (s, 4H, ArH(OHexyl)), 4.71 (br.

s 8H, $NH_2$), 4.24 (br. t 4H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 1.82 (br. m 4H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 1.53 (br. m 4H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 1.35 (br. s 8H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 0.87 (br. t 6H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$). $^{13}$C NMR (125.70 MHz, DMSO-$d_6$) δ: 148.88, 148.39, 145.63, 140.79, 128.15, 121.79, 121.77, 120.72, 74.99, 31.17, 29.53, 25.08, 22.12, 13.98.

*5,11,17,23-tetrakis-N-phenylsulfonyltrifluoro-acetamidino-25,27-dihexyloxi-26,28-dihydroxythiacalixarene* ***C-1087***. To suspension of tetraaminothiacalixarene 3 (0.435 g, 0.6 mmol) and triethylamine (0.265 g, 2.61 mmol) in THF (30 ml) a solution of N-phenylsulfonyltrifluoroacetylimidoylchloride [23] (0.815 g, 3 mmol) in THF (15 ml) was added dropwise. The reaction mixture is stirred for 8 h at 70°C in bath. After cooling, a precipitate was filtered off, the filtrate was evaporated on rotor evaporator (15 mm Hg, 50°C). To residue light orange hard foam ethanol (9 ml) and water (2 ml) were added. Obtained precipitate was filtrated, dried in vacuum (12 mm Hg) for 3 h, dissolved in hot benzene (5 ml) and precipitated by heptanes (15 ml). Obtained solid C-1087 was purified by column chromatography ($SiO_2$, 40-60 μm, $CHCl_3$-$CH_3OH$, 100:1 by volume, $R_f$ 0.6). Pale-yellow solid compound, yield 0.24 g (24%) M.P. 185-187°C. $^1$H NMR (400.13 MHz, $CD_3OD$) δ: 7.91 (s, 4H, ArH(OH)), 7.85 (d, J = 7.28 Hz, 4H $SO_2$PhH-*orto*), 7.65 (t, J = 7.03 Hz, 4H $SO_2$PhH-*meta*), 7.54 (m, 12H, $SO_2$PhH-*para* and *meta*), 6.95-7.4 (br. s, 4H, ArH(OHexyl)), 4.37 (br. t, 4H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 1.93 (m, 4H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 1.60 (m, 4H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 1.41 (m, 8H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 0.93 (t, J = 6.78 Hz, 6H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$). $^{19}$F NMR (470.26 MHz, DMSO-$d_6$) δ: -65.55, -65.67. $^{13}$C NMR (125.70 MHz, DMSO-$d_6$) δ: 163.81, 156.44, 142.75, 142.63, 132.58, 132.40, 129.18, 127.64, 125.93, 125.80, 120.76, 117.73, 115.45, 73.63, 31.38, 29.05, 24.81, 22.06, 13.90.

*25,27-dihexyloxi-26,28-dihydroxythiacalix-arene C-114***5**. To suspension of thiacalixarene **4** (0.495 g, 1 mmol) in THF (10 ml) triphenylphopshine (0.655 g, 2.5 mmol) and n-hexanol (1.02 g, 10 mmol) were added and resulting mixture was stirred for 15 min. Then diethylazodicarboxylate (DEAD 0.435 g, 2.5 mmol) was added dropwise. The reaction mixture was stirred for 12 h at room temperature. Light precipitate was filtered off and filtrate was evaporated on rotor evaporator (15 mm Hg, 50°C). Oily brown-orange residue was washed by methanol (15 ml). Precipitate of crude C-1145 was filtrated, dried on air and purified by column chromatography ($SiO_2$, 40-60 μm, Ethylacetate-Hexane, 4:1 by volume, $R_f$ 0.62). Colorless crystalline compound, yield 0.51 g (77%) M.P. 202-204°C. $^1$H NMR (399.98 MHz, $CDCl_3$) δ: 7.61 (t, J = 7.38 Hz, 4H, ArH(OH)), 7.35 (s, 2H, OH), 6.82 (d, J = 7.77 Hz, 4H, ArH(OHexyl)), 6.81 (t, J = 7.77 Hz, 2H, ArH(OH)), 6.48 (t, J = 7.77 Hz, 2H, ArH(OHexyl)), 4.35 (t, J = 6.86 Hz, 4H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 1.98 (m, 4H, O-$CH_2$-$CH_2$-CH2-$(CH_2)_2$-$CH_3$), 1.55 (br. m, 4H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 1.38 (br. s, 8H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$), 0.91 (t, J = 6.86 Hz, 6H, O-$CH_2$-$CH_2$-$CH_2$-$(CH_2)_2$-$CH_3$).

**B. Biochemical studies**

*Preparative biochemistry.* The PM fraction of uterine SM cells was isolated from the porcine myometrium as described before [24, 25].

The protein content in the membrane fraction was determined by the method of M. Bradford [26] using the reaction with Coomassie reagent – G250.

The myocytes were isolated from the uterus of non-pregnant laboratory rats using collagenase and soy inhibitor trypsin by Mollard's method [27].

The work was conducted 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 (28th UNESCO Assembly, 1995), the Universal Declaration on Bioethics and Human Rights (UNO, 1997), the norms of the Convention for the Protection of Human Rights, adopted in 1997 due to the introduction of new biomedical technologies, in Oviedo (Spain) and ratified by the Verkhovna Rada of Ukraine in 2002, the Law of Ukraine No. 3447 IV "On Protection of Animals from Cruelty".

*Enzymological studies.* The total ATPase activity was determined in the PM fraction of myometrium cells at 37°C in the standard medium (volume – 0.4 ml), containing (mM): 3 ATP, 3 $MgCl_2$, 0.95 $CaCl_2$, 25 NaCl, 125 KCl, 1 EDTA, 20 Hepes-tris-buffer (pH 7.4), 1 $NaN_3$, 1 ouabain, 0.1 μM thapsigargin, and 0.1 % digitonin. The calculations, made in the MAXCHEL program, demonstrated that under these physical, chemical, and concentration conditions of the incubation medium, the concentration of free Ca (proper $Ca^{2+}$) was 1 μM. While studying the impact of different concentrations of Ca ions on the activity of $Ca^{2+}Mg^{2+}$-ATPase, the required concen-

trations of the mentioned cation were also preset by computerized estimates using the abovementioned program. The amount of membrane fraction protein in the probe was 20–30 μg. The incubation time was 5 min. The enzymatic reaction was initiated by the introduction of the aliquot (50 μl) of PM suspension to the incubation medium, and terminated by the introduction of 1 ml of "stop"-solution to the incubation mixture as follows: 1.5 M acid sodium acetate, 3.7% formaldehyde, 14% ethanol, 5% TCA, pH 4.3 (at 8°C). The amount of Pi reaction product was determined by the method of W. Rathbun et V. Betlach [28].

$Ca^{2+}$,$Mg^{2+}$-ATPase activity was estimated by the difference between the values of ATPase activities in the presence and the absence of exogenous Ca ions (against the background of 1 mM EDTA – specific chelating agent for Ca ions) in the incubation medium. In the sarcolemma of the porcine myometrium, the relative enzymatic activity of $Ca^{2+}$,$Mg^{2+}$-ATPase was 3.4 ± 0.3 μmol $P_i$/mg of protein per one hour, respectively ($M \pm m$; $n = 7$).

The basal $Mg^{2+}$-ATPase activity was determined in the PM fraction of myometrium cells at 37°C in the medium (volume – 0.4 ml), containing (mM): 1 ATP, 3 $MgCl_2$, 125 NaCl, 25 KCl, 1 EDTA, 20 Hepes-tris-buffer (pH 7.4), 1 $NaN_3$, 1 ouabain, 0.1 μM thapsigargin, and 0.1% digitonin. The "basal" $Mg^{2+}$-ATPase activity was estimated as a difference between the amount of $P_i$, which was formed in the incubation medium in the presence and the absence of PM fraction with the consideration of the amendment for the content of endogenous $P_i$ in the membrane preparation.

$Na^+$,$K^+$-ATPase activity was determined in the same medium and estimated by the difference between the values of ATPase activities in the absence and presence of 1 mM ouabain.

In our experiments, the average value of relative activities of $Na^+$,$K^+$-ATPase and "basal" $Mg^{2+}$-ATPase of PM was 10.2 ± 0.7 and 18.1 ± 1.2 μmol $P_i$/mg of protein per one hour, respectively ($M \pm m$; $n = 7$).

It should be noted that the PM of uterine myocytes was found to also contain $Ca^{2+}$-ATPase, the properties of which differed from $Ca^{2+}$,$Mg^{2+}$-ATPase, since its activity was manifested in the presence of millimolar concentrations of $Ca^{2+}$ and ATP in the incubation medium against the background of Mg ions [29, 30]. $Ca^{2+}$-ATPase was of low affinity to the activating cation, the constant of activation with Ca ions ($K_{Ca}$) was 1 mM [30]. The low-affinity $Mg^{2+}$-independent $Ca^{2+}$–ATPase activity was determined in the PM fraction of myometrium cells at 37°C in the medium (volume – 0.4 ml), containing (mM): 1 ATP, 3 $CaCl_2$, 125 NaCl, 25 KCl, 1 EDTA, 20 Hepes-tris-buffer (pH 7.4), 1 $NaN_3$, 1 ouabain, 0.1 μM thapsigargin, and 0.1% digitonin. The mentioned $Ca^{2+}$-ATPase activity was estimated as a difference between the amount of $P_i$, which was formed in the incubation medium in the presence and the absence of PM fraction with the consideration of the amendment for the content of endogenous $P_i$ in the membrane preparation. In the sarcolemma of the porcine myometrium, the relative enzymatic activity of low-affinity $Mg^{2+}$-independent $Ca^{2+}$-ATPase of PM was 12.7 ± 2.0 μmol $P_i$/mg of protein per one hour, respectively ($M \pm m$; $n = 7$).

To determine ATPase activity in SR, the cell suspension (the protein concentration – 20 μg in the sample) was permeabilized by digitonin (0.1%). The total ATPase activity was determined in the standard medium (volume – 0.4 ml), containing (mM): 3 ATP, 3 $MgCl_2$, 0.95 $CaCl_2$ , 25 NaCl, 125 KCl, 1 EDTA, 20 Hepes-tris-buffer (pH 7.4), 1 $NaN_3$, 1 ouabain. $Ca^{2+}$,$Mg^{2+}$-ATPase activity in SR was estimated by the difference between the values of ATPase activities in the presence and in the absence of 0.1 μM thapsigargin in the incubation medium.

It was found in our experiments that in the sarcolemma of the rat myometrium, the relative enzymatic activity of $Ca^{2+}$,$Mg^{2+}$-ATPase was 2–3 μmol $P_i$/mg of protein per one hour.

In the experiments on the impact of different concentrations of C-1087 (1–100 μM) on $Ca^{2+}$,$Mg^{2+}$-ATPase activity, the above-described standard incubation medium was used with the addition of the aliquot of the solution of C-1087 in the relevant concentration. The experiments involved the use of the concentrated (20 mM) solution of C-1087 in DMSO, which was further diluted with water.

*Determining the changes in the concentration of intracellular $Ca^{2+}$ with the addition of confocal microscopy.* To register the changes in the concentration of $Ca^{2+}$ in SM cells, the suspension of myocytes, obtained by the modified method of Mollard [27, 31], was loaded with $Ca^{2+}$-sensitive probe fluo-4 AM at room temperature for 20 min, then the cells were precipitated by centrifugation for 15 min at 1,000 g, diluted in the isotonic storage medium (containing 25 mM HEPES-KOH (pH=7.4; 8°C), 150 mM NaCl) and applied to a glass surface with poly-L-lysine.

The obtained preparation of the immobilized cells was investigated with the laser scanning confocal microscope LSM 510 "META". For analysis, we selected the spindle-shaped cells with a clearly defined nucleus, stained with DNA-sensitive fluorescent probe Hoechst, (applied 10 min before the registration). To register relative changes in the concentration of $Ca^{2+}$ in the cytoplasm, a series of consecutive photographs was taken, during which the aliquot of the C-1087 solution (in case of the probe) or C-1145 (in case of the control) was introduced in the concentration of 20 μM (5 μl).

*Kinetic estimates.* While studying the concentration dependence of the effect of thiacalix[4]arene on the enzymatic activity, the values of inhibition coefficients $I_{0.5}$ and Hill coefficients $n_H$ were estimated using the linearized charts of Hill according to the equation $\lg[(A_{max}-A)/A] = -n_H \lg I_{0.5} + n_H \lg[\text{C-1087}]$, where $A_{max}$ and $A$ – relative enzymatic activities in the absence ("zero point") and the presence of thiacalix[4]arene in the medium at the concentration of [C-1087].

**C. Tenzometric and mechanokinetic studies.**

*Tenzometric studies.* The experiments were conducted using outbred white rats (the average weight of animals was 200–250 g). All the manipulations with the animals were conducted according to the International Convention for the Protection of Animals and the Law of Ukraine "On Protection of Animals from Cruelty". The animals were killed by the displacement of cervical vertebrae after the administration of ether anesthesia.

The tenzometric experiments were conducted using the preparations of longitudinal smooth muscles of uterine horns with preserved endothelium. Muscle stripes (the average size – 2×10 mm) were placed into the working chamber (the volume of 2 ml) with the flowing Krebs solution (the flow rate of 5 ml/min), and thermostated at 37°C. The contractile activity was registered in the isometric and isotonic modes. In the isometric registration mode, the muscle preparation was subjected to passive tension at the rate of 10 mN; in the isotonic registration mode, the free end of the muscle preparation was loaded with 10 mN. In both cases, the preparation was left for 1 h until stable reproduction of contractions was achieved. The signals were registered with analogue-to-digital transformer.

The Krebs solution was used in the experiments (mM): 120.4 NaCl; 5.9 KCl; 15.5 $NaHCO_3$; 1.2 $NaH_2PO_4$; 1.2 $MgCl_2$; 2.5 $CaCl_2$; 11.5 glucose; pH of the solution was 7.4. The hyperkalemic solution, containing $K^+$ ions in the concentration of 80 mM, was prepared by isotonic replacement of the required amount of $Na^+$ ions in the initial Krebs with the equimolar amount of $K^+$ ions.

Thiacalix[4]arene C-1087 was preliminarily dissolved in DMSO and added to Krebs solution or HKS in the concentration of 10 μM (the final aliquot of the organic solvent solution was 0.1% from the total volume of this solution). The control contractions were studied in solutions, containing 0.1% DMSO. It was previously found that the application of 0.1% DMSO did not affect the basal tension or spontaneous and induced contractions in the myometrium preparations.

*Mechanokinetic analysis.* The kinetics of spontaneous contractile activity (isometric contraction-relaxation and isotonic contraction-relaxation) of the muscle preparations was studied according to the empirical multiparameter method of the complex mechanokinetic analysis, separately at the levels of contraction phase and relaxation phase [32]. The contraction phase was defined as a fragment of a contractile response from the beginning of force variation up to its maximal value (the amplitude of the phase contraction). The relaxation phase started with the maximum phase contraction and lasted till a stable level of the tonic contraction was achieved. According to this method, the experimental mechanokinetic curves of isometric contraction-relaxation were linearized within the coordinates $[ln(f_R/f_C); ln(1+\Delta t/t)]$, where $f$ and $t$ – instant values of force and time at the level of the contraction cycle, $F_C$ and $F_R$ – respective values of the force at the inflexion points of the mechanogram at the level of contraction and relaxation phases, $\Delta t$ – an arbitrarily fixed time interval (in these studies it varied within 35–70 s). The isotonic curves (separate at the levels of the contraction phase and the relaxation phase) were linearized within the coordinates $[ln(\Delta L_R/\Delta L_C); ln(1+\Delta t/t)]$, where $\Delta L$ and $t$ – instant values of the preparation shortening (compared to the initial value of the preparation length, $L_0$) and time at the level of the contractile cycle, $\Delta L_C$ and $\Delta L_R$ – respective values of the preparation shortening at the inflexion points of the mechanogram at the level of contraction and relaxation phases, $\Delta t$ – an arbitrarily fixed time interval (in this study it varied within 20–29 s).

The linearization charts were used to determine the constants $k$ and $n$, which were further used to calculate the parameters: time ($\tau_0$, $\tau_C$, and $\tau_R$), force

($F_{max}$, $F_C$, and $F_R$ for isometric contractions, or $\Delta L_{max}$, $\Delta L_C$, and $\Delta L_R$ for isotonic contractions), velocity ($V_C$ and $V_R$), and relative parameters ($\tau_0/\tau'_0$, $\tau_C/\tau'_C$, $\tau_R/\tau'_R$, $F_{max}/F'_{max}$, $F_C/F'_C$, $F_R/F'_R$, $\Delta L_{max}/\Delta L'_{max}$, $\Delta L_C/\Delta L'_C$, $\Delta L_R/\Delta L'_R$, $V_C/V'_C$ and $V_R/V'_R$).

The mechanokinetics of the induced and some spontaneous contractions was studied with the estimation of normalized maximal velocities of the contraction and relaxation phases ($V_{nc}$ and $V_{nr}$, respectively) according to the method described in [33]. It is founded on the linearization of the relaxation phase of the mechanokinetic curve within the coordinates [$ln((f_{max}–f)/f)$; $ln(t)$], where $f_{max}$ – the value of the maximal contraction force. The time of reaching $f_{max}$ is accepted as the starting point for the relaxation phase, $t = 0$; the current time value, $t$, corresponds to the instant force value, $f$.

*Statistical analysis.* The statistical analysis of the data obtained was conducted by the methods of variation statistics. The kinetic and statistical calculations were done using the programs MS Excel and Origin 2018.

The samples were checked in terms of belonging to normally distributed general populations according to the Shapiro-Wilk criterion. The Student's *t*-test was used to determine the reliable differences between the mean values of samplings. In all cases, the results were considered reliable on condition of $P < 0.05$. The validation analysis of data approximation by the linear function was performed using Fisher's F-criterion; determination coefficients ($R^2$) were at least 0.96. The results were presented as the arithmetic mean ± standard error of the mean value, $n$ – number of experiments.

*Reagents.* The following reagents were used in the experiments: ATP, Hepes, ouabain, thapsigargin, Hoechst, fluo-4 AM, collagenase, poly-L-lysine (Sigma, USA), tris-hydroxymethyl-aminomethane (Reanal, Hungary), digitonin (Merck, Germany), EDTA (Fluka, Switzerland), oxytocin (Gideon Richter, Hungary). Other reagents were analytically and chemically pure, produced in Ukraine.

## Results and Discussion

Thiacalixarene C-1087 was obtained via three-step procedure starting from tetranitrothiacalixarene **1** (Fig. 1). First stage is regioselective distal bis-alkylation of 1 with hexyliodide in DMF in the presence of potash. The next step is reduction of tetranitrothiacalixarene **2** with Hydrazine hydrate in the presence of Raney nickel. The reaction of tetraaminothiacalixarene **3** with N-sulfonylimidoyl-chloride in the presence of triethylamine leads to desired thiacalixarene C-1087 on the third step.

Thiacalixarene C-1145 was synthesized by Mitzunobu's reaction of tetrahydroxythiacalixarene **4** and n-hexanol in the presence of triphenylphosphine and diethylazadicarboxylate (Fig. 2).

Dihexyloxythiacalixarenes **2**, **3**, C-1087 and C-1145 have adopted in solutions cone conformation which characterized *syn*-orientation of four macrocyclic phenyl rings. This is proved by chemical shifts $OCH_2$ hydrogen atoms of hexyl moieties which located at 4.24-4.39 ppm in $^1$H NMR spectra. In case 1,3-alternate conformation which characterized *syn-anti*-orientation of adjacent phenyl rings pairs of the macrocycle corresponding chemical shifts must be shifted to strong field δ 3.81-3.86 ppm as a result their shielding by phenyl rings of macrocyclic skeleton (Fig. 3) [34-36].

In our previous studies, we demonstrated that a synthetic substance, 5,11,17,23-tetra(trifluoro)-methyl(phenylsulfonylimino)-methylamino-25,26, 27,28-tetrapropoxy-calix[4]arene C-90 in the concentration of 100 μM effectively (by 75% as compared to the control) inhibited the activity of $Ca^{2+}$,$Mg^{2+}$-ATPase of PM in uterine myocytes (Fig. 4). At the same time, it was found that this calix[4]arene also inhibited the enzymatic activity of $Ca^{2+}$,$Mg^{2+}$-ATPase of SR (by 58% as compared to the control) (Fig. 4).

We determined that thiacalix[4]arene C-1087 (5,11,17,23-tetra(trifluoro)methyl(phenylsulfonylimino)methylamino-25,27-dihexyloxy-26,28-dihydroxy-thiacalix[4]arene) in the concentration of 100 μM effectively inhibited $Ca^{2+}$,$Mg^{2+}$-ATPase activity of PM in myometrium cells to the level of 14.9 ± 0.5% as compared to the control value (accepted as 100%) ($M \pm m$; $n = 5$) (Fig. 4). It inhibited the enzymatic activity of $Ca^{2+}$,$Mg^{2+}$-ATPase of SR much less effectively (by 34% as compared to the control) (Fig. 4).

In our further studies, we investigated the concentration dependence of the inhibitory effect of C-1087 ($10^{-8}$–$10^{-4}$ M) on the activity of $Ca^{2+}$,$Mg^{2+}$-ATPase in PM and SR (Fig. 5).

As seen in Fig. 5, thiacalix[4]arene C-1087 inhibits $Ca^{2+}$,$Mg^{2+}$-ATPase activity of PM in a dose-dependent way. The estimated value of the inhibition coefficient $I_{0.5}$ is 9.4 ± 0.6 μM, and the value of Hill coefficient $n_H$ is 0.58 ± 0.03 ($M \pm m$; $n = 5$). The value of the inhibition coefficient $I_{0.5}$ for thiacalix[4]arene C-1087 is lower than in the case of using calix[4]-

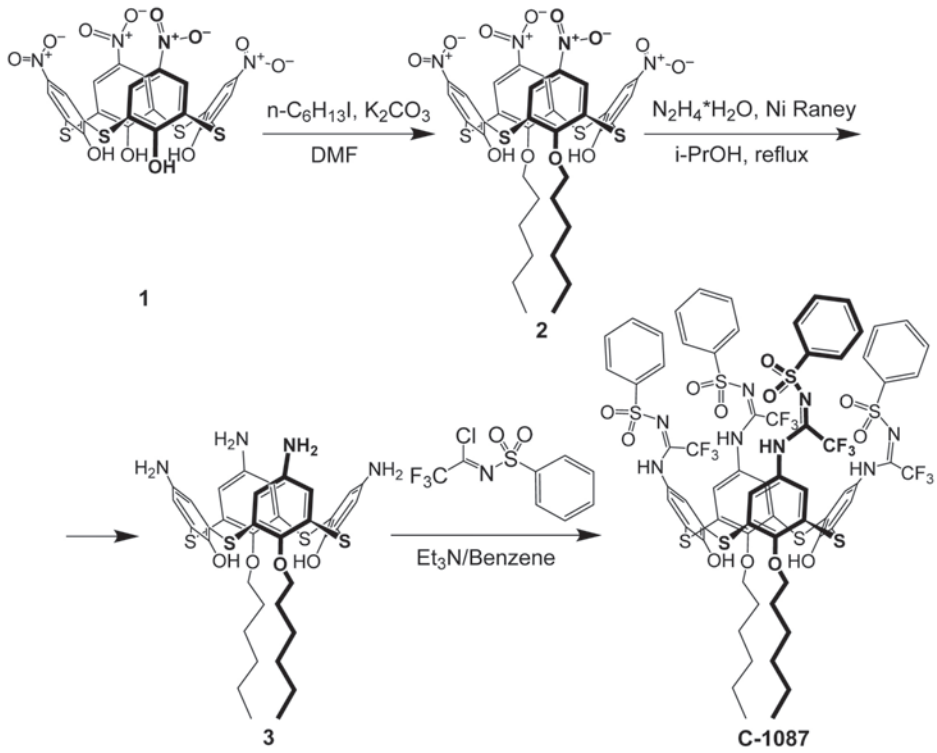



*Fig. 1. Scheme of synthesis of thiacalixarene C-1087*

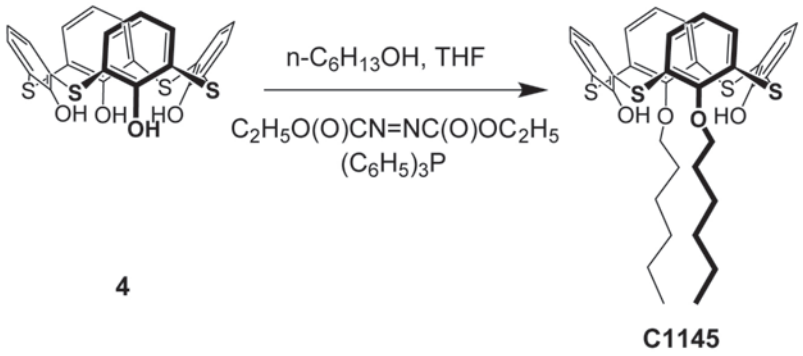



*Fig. 2. Scheme of synthesis of thiacalixarene C-1145*

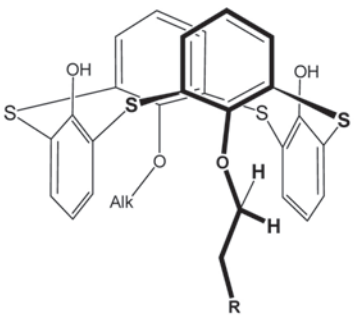
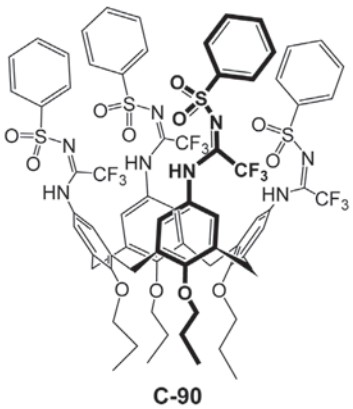



*Fig. 3. Electronic shielding of methylene protons by a phenyl ring in the conformation of 1,3-alternate of 1,3-dialkoxythiacalixarene*



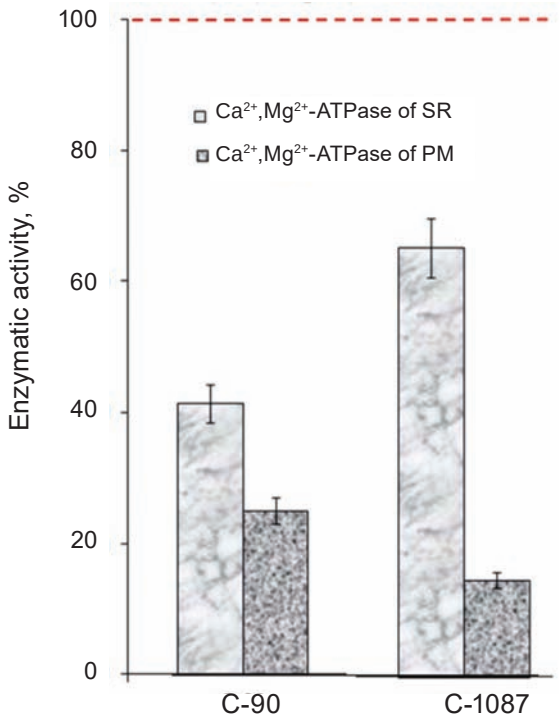



*Fig. 4. The effect of calixarene C-90 and thiacalixarene C-1087 (100 μM) on the activity of $Ca^{2+}$,$Mg^{2+}$-ATPase of sarcoplasmic reticulum (SR) and plasma membrane (PM) in myometrium myocytes (M ± m, n = 5). The values of enzymatic activities in the absence of calixarenes in the incubation medium are accepted as 100%*

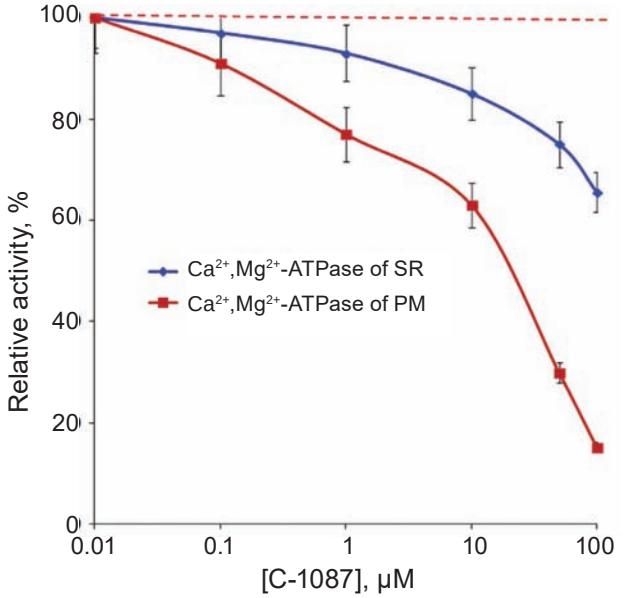



*Fig. 5. The concentration dependencies of the C-1087 effect on the $Ca^{2+}$,$Mg^{2+}$-ATPase activity of plasma membrane (PM) and sarcoplasmic reticulum (SR) of the myometrium cells (M ± m, n = 5). The values of relative enzymatic activities in the absence of thiacalix[4]arene C-1087 in the incubation medium are accepted as 100%*

arene C-90 ($I_{0.5}$ = 20.2 ± 0.5 μM) [37], and the value of Hill coefficient $n_H$ (0.55 for C-90) was practically the same for both calixarenes. In the entire range of concentrations ($10^{-8}$–$10^{-4}$ M), thiacalix[4]arene C-1087 also inhibited $Ca^{2+}$,$Mg^{2+}$-ATPase activity in SR in a dose-dependent way, but it was not possible to estimate the value of the inhibition coefficient $I_{0.5}$ since in the maximal concentration this substance had a 50% lower inhibitory effect than the control value. However, it is obvious that the value $I_{0.5}$ in the case of $Ca^{2+}$,$Mg^{2+}$-ATPase activity in SR cannot be < 100 μM.

The experiment results demonstrate that C-1087 effectively inhibits the enzymatic activity of $Ca^{2+}$,$Mg^{2+}$-ATPase of PM, and it has much less effect on the activity of $Ca^{2+}$,$Mg^{2+}$-ATPase of SR (Fig. 4, 5).

In our further studies, we tried to find out the selectivity of the effect of C-1087 on ATP-hydrolase activities in PM of myocytes. As shown above, thiacalix[4]arene C-1087 in the concentration of 100 μM effectively inhibits $Ca^{2+}$,$Mg^{2+}$-ATPase activity of PM of myometrium cells to the level of 14.9 ± 0.5% regarding the control value (accepted as 100%) (*M* ± *m*, *n* = 5) (Fig. 4, 6).

At the same time, this substance, used in the same concentration, practically did not impact the enzymatic properties of $Na^+$,$K^+$-ATPase, "basal" $Mg^{2+}$-ATPase, and $Ca^{2+}$-ATPase of PM: the corresponding activities were 90.1 ± 0.7; 95.3 ± 1.0, and 89.1 ± 0.8% regarding the control value (*M* ± *m*, *n* = 5) (Fig. 6).

It is noteworthy that the proper thiacalixarene "bowl" C-1145 practically does not impact the enzymatic activity of $Ca^{2+}$,$Mg^{2+}$-ATPase of PM (the data are not presented).

Therefore, thiacalix[4]arene C-1087, used in the concentration of 100 μM, effectively inhibited the activity of $Ca^{2+}$,$Mg^{2+}$-ATPase in the fraction of PM of uterine myocytes. At the same time, it practically did not impact the activity of other ATP-hydrolases in PM. Thus, C-1087 selectively (at the PM level) inhibits the activity of $Ca^{2+}$,$Mg^{2+}$-ATPase of PM, not affecting the activities of $Na^+$,$K^+$-ATPase, $Mg^{2+}$-ATPase and $Ca^{2+}$-ATPase in PM.

Considering that the $Ca^{2+}$-pump in PM plays a relevant role in the control over the concentration of

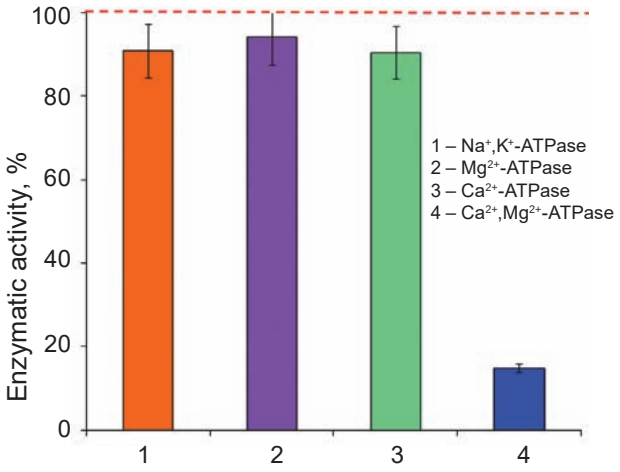



*Fig. 6. The effect of calixarene C-1087 (100 μM) on ATP-hydrolase activities of myometrium cell plasma membrane (M ± m, n = 5). The values of enzymatic activities in the absence of C-1087 in the incubation medium are accepted as 100%*

Ca ions in SM cytoplasm, it was important to find out whether C-1087 would impact the intracellular concentration of $Ca^{2+}$ in SM cells.

In our further experiments, we used the method of confocal microscopy and $Ca^{2+}$-sensitive probe fluo-4 to investigate the changes in the concentration of $Ca^{2+}$ in myocytes under the effect of thiacalix[4]-arene C-1087. It was demonstrated that under the effect of thiacalix[4]arene C-1087 (20 μM), there was a sharp increase in the fluorescent response of $Ca^{2+}$-sensitive probe fluo-4 AM in the cells (Fig. 7, 8). In the course of 2.5 min, the concentration of $Ca^{2+}$ decreased, which demonstrated the involvement of the compensatory mechanisms ($Ca^{2+}$-uniporter of mitochondria, $Na^+$-$Ca^{2+}$-exchanger of PM) until the relaxation of the calcium signal. Thus, thiacalix[4]-arene C-1087 – the inhibitor of $Ca^{2+}$,$Mg^{2+}$-ATPase of PM, induced the increase in $Ca^{2+}$ concentration in SMC.

When thiacalix[4]arene "bowl" C-1145 (25,27-dipropoxythiacalix[4]arene) (20 μM) was used in control and, according to our results it did not have any vivid effect on the activity of $Ca^{2+}$, $Mg^{2+}$-ATPase of PM (see above), this increase in the fluorescent signal of fluo-4 was not observed (the results are not presented). There was also a stable fluorescence level of Hoechst, mainly localized in the nucleus of smooth muscle cells and the background.

These results demonstrate that under the effect of C-1087 (20 μM), there is a sharp increase in the concentration of $Ca^{2+}$ in the cell, which may be related to the decrease in the activity of $Ca^{2+}$,$Mg^{2+}$-ATPase of PM, inhibited by this thiacalix[4]arene. However, taking into account the results, presented in Fig. 4, it is likely that the increase in the concentration of $Ca^{2+}$ in myocytes may partially be explained by the inhibited activity of the calcium pump in the reticulum, induced by thiacalix[4]arene. At the same time, during the other 100 s, the concentration of $Ca^{2+}$ decreases to the initial level, which demonstrates the involvement of compensatory $Ca^{2+}$-transporting systems in myocytes in the relaxation of the calcium signal, which have lower affinity to $Ca^{2+}$ and mainly react to its high concentrations in the cell ($Ca^{2+}$-uniporter of mitochondria, $Na^+$-$Ca^{2+}$-exchanger of PM).

The impact of calix[4]arenes on ion transportation systems of myocytes may determine their impact on the form of the cells. The change in the form of SMC due to the change in the osmotic balance can be determined by laser correlation spectroscopy, which can register the changes in the effective hydrodynamic diameter of SMC.

It was determined using the literature data that the factors, enhancing the contractile response of SM, also change the effective hydrodynamic diameter of SMC very vividly. As described in [38], the introduction of $Ca^{2+}$ (3 mM), the processing with A-23187 (10 μM), tetraethylammonium (1 mM) and 4-aminopyridine (1 mM) led to a decrease in the effective hydrodynamic diameter which correlated with the state of SM contraction. According to our results, the introduction of uterotonic oxytocin (100 nM) led to a decrease in the effective hydrodynamic diameter in the case of SMC suspension by 27.3 ± 3.2% ($M \pm m$, $n = 7$) regarding the control value (Fig. 9, red column).

The average value of the effective hydrodynamic diameter of myocytes is about 9 μm in control. The hydrodynamic diameter was measured five times in one minute, and the average value was calculated. Being the control for the solvent, the application of DMSO aliquot did not result in considerable changes in the investigated parameter – the hydrodynamic parameter changed by 3.5 ± 1.6% regarding the control (Fig. 8, green column). At the same time, the use of the inhibitor of $Mg^{2+}$-ATP-dependent $Ca^{2+}$-pump of PM in SMC, thiacalix[4]arene C-1087 (50 μM) leads to the decrease in the hydrodynamic diameter by 44.6 ± 4.2% regarding the control value, similar to the effect of oxytocin, and these changes correlate with similar ones, achieved for oxytocin (100 nM) – Fig. 9 (blue column).

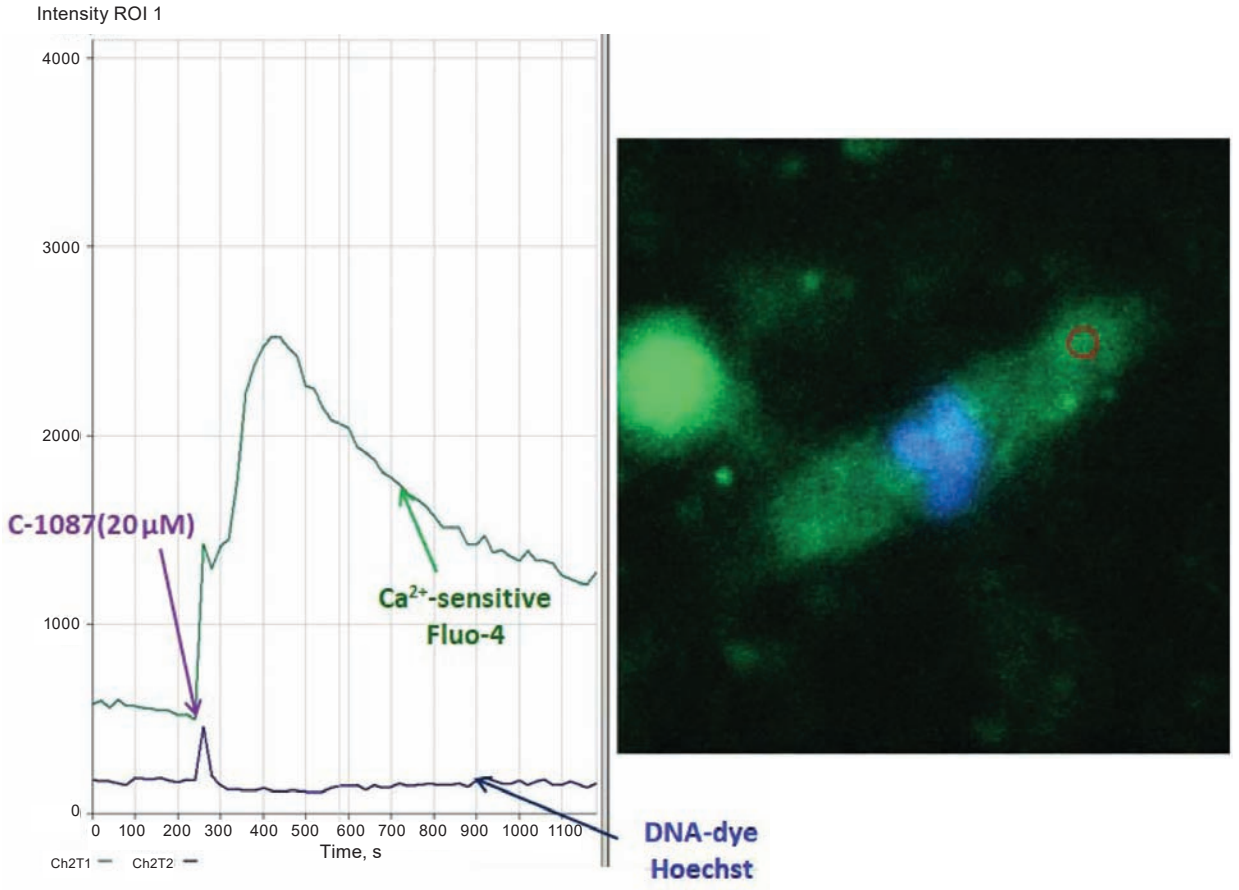



*Fig. 7. The change of the fluorescence of probes in the uterine myocyte under the effect of calixarene C-1087, detected using the confocal microscopy: $Ca^{2+}$-sensitive fluo-4 AM (green) and DNA-sensitive Hoechst (blue). The application of the thiacalix[4]arene C-1087 solution (the final concentration is 20 µM) is indicated with the asterisks. The results of the typical experiment are presented*

Thus, thiacalix[4]arene C-1087 decreases the effective hydrodynamic diameter of SMC identically to the effect of uterotonic oxytocin. A similar change in the hydrodynamic diameter may be interpreted as the combination of events, accompanying the processes of SMC contraction/relaxation, namely, the change in the osmotic balance and restructuring of cytoskeleton elements. Since it was previously shown that the change in the hydrodynamic diameter of SMC under the effect of contractile agents correlated with the state of SM contraction, such results demonstrated promising possibilities for the use of C-1087 as a regulator of contractile activity in uterine SM.

Since the changes in the intracellular concentration of $Ca^{2+}$ under the effect of thiacalix[4]arene C-1087 may cause changes in the basal tension and contractility of myometrium tissue, in our further experiments, we studied the effect of this substance on mechanokinetic parameters of the contractions of the longitudinal smooth muscles of uterine horns in non-pregnant rats.

Under the conditions of the isometric mode of registering the contractile activity of uterine SM, the addition of thiacalix[4]arene C-1087 (10 µM) to the solution to wash SM preparations did not entail the changes in their basal tension (Fig. 10, *A*), but over time (after 20 min of pre-incubation) it led to the decrease in the amplitude of spontaneous contractions down to 78.4% on average regarding the control, accepted as 100% ($P < 0.01$, $n = 8$). In similar conditions in the isotonic mode of registration, thiacalix[4]arene C-1087 (10 µM) caused the relaxation of SM and the inhibition of the amplitude of their spontaneous contractions on average to 76.6% regarding the control, accepted as 100% ($P < 0.001$, $n = 7$) (Fig. 10, *B*).

The analysis of mechanokinetic parameters of single contraction-relaxation cycles (we analyzed the mechanogram fragment with contractions after

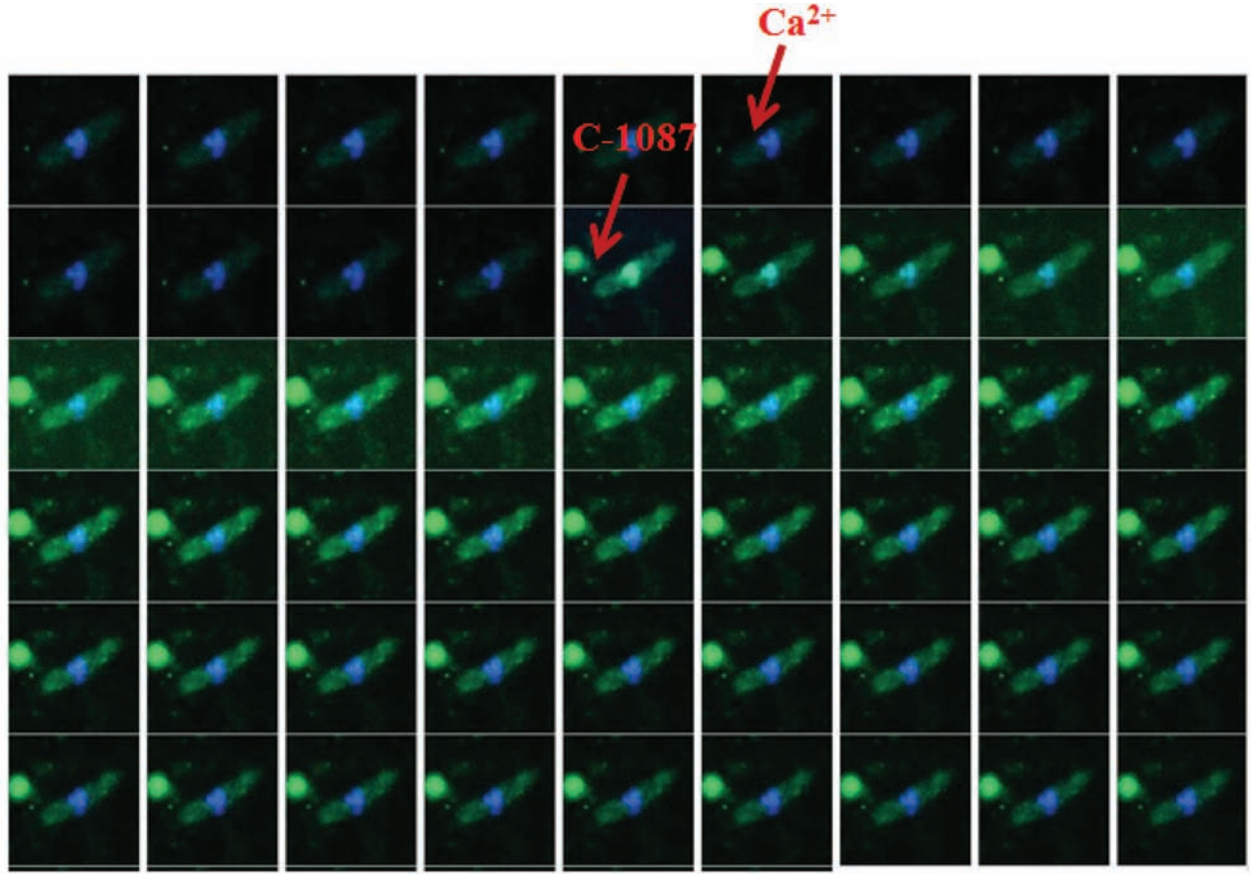



*Fig. 8. The series of consecutive photographs of SMC using the scanning confocal microscopy. $Ca^{2+}$-sensitive fluorescent probe fluo-4 AM (green) and DNA-sensitive Hoechst (blue). The asterisks indicate the moments of $Ca^{2+}$ (1 μM) and C-1087 (20 μM) application. The results of the typical experiment are presented*

20 min since the beginning of C-1087 effect) demonstrated that in the case of both isometric and isotonic modes of registration, under the effect of C-1087 there was a considerable decrease in the indices of force (for the isometric mode) and length (for the isotonic mode) at the inflexion points of the phases of contraction (respectively, $F_C$ and $\Delta L_C$) and relaxation (respectively, $F_R$ and $\Delta L_R$). For instance, under the effect of C-1087, the parameters $F_C$ and $F_R$ were 71.9 and 79.4% on average, as compared to the control value, accepted as 100% ($P < 0.05$, $n = 6$). Under the effect of C-1087, the parameters $\Delta L_C$ and $\Delta L_R$ were 73.8 and 77.1% on average as compared to the control values, accepted as 100% ($P < 0.001$, $n = 7$).

In the case of registering the contractile activity in the isometric mode under the effect of C-1087, there was a considerable inhibition of the maximal velocity of the relaxation phase: parameter $V_R$ was 70.5% on average regarding the control, accepted as 100% ($P < 0.01$, $n = 6$). A similar index of the isotonic relaxation did not have reliable differences regarding the control (85.1%, $P > 0.05$, $n = 6$).

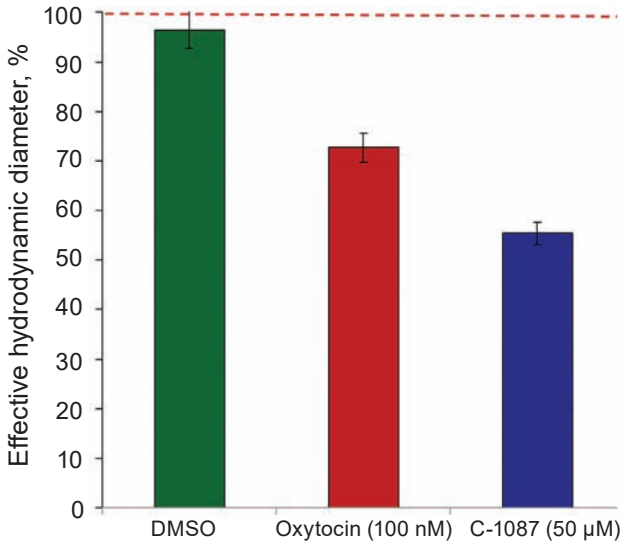



*Fig. 9. The change of the effective hydrodynamic diameter of SMC in the presence of different effectors ($M \pm m$, $n = 7$). The control value of the hydrodynamic SMC diameter in the absence of any effectors was accepted as 100%*

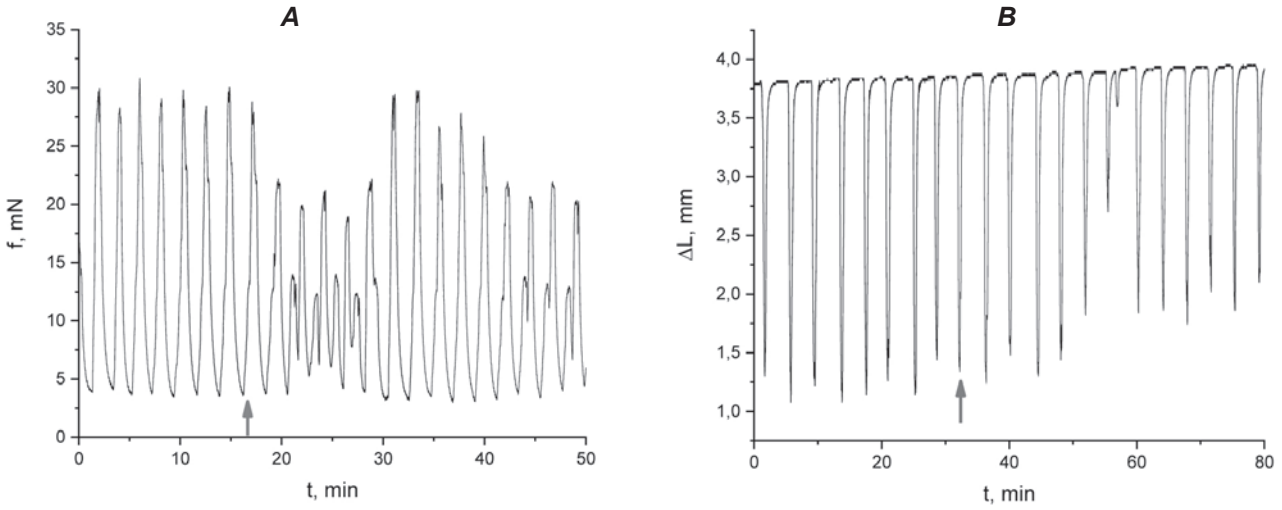



*Fig. 10. The spontaneous contractile activity of rat myometrium (**A** – in the isometric mode and **B** – in the isotonic mode of registration) in control and against the background of the impact of C-1087 (10 μM). The initial moment of C-1087 application is indicated with the asterisk. Typical mechanograms are presented*

To exercise a more convenient comparison with our previous results on the impact of calixarene C-90 (10 μM) [39], we estimated the normalized (amplitude-independent) maximal velocities of the phases of contraction ($V_{nc}$) and relaxation ($V_{nr}$) using the mechanokinetic analysis [32]. Under the impact of thiacalixarene C-1087, the parameter $V_{nr}$ was 51.3% on average regarding the control ($P < 0.05$, $n = 6$), whereas under the impact of calixarene C-90, it was 78.0% on average ($P < 0.05$, $n = 7$). Thus, if the same concentrations are used, thiacalixarene C-1087 causes a reliable, more considerable slowing down of the process of myometrium SM relaxation as compared to calixarene C-90.

In the presence of C-1087, there were no changes in the kinetics of the force accumulation (isometric contraction and isotonic shortening): in all cases, the maximal velocities of the contraction phase ($V_c$ and $V_{nc}$) did not demonstrate statistically significant changes as compared with the control. It should be noted that similar kinetic effects were also observed against the background of calixarenes C-90 and C-716 [39, 40].

In the following series of experiments, we investigated kinetic regularities in the contractile activity of rat uterine SM, caused by the application of the hyperkalemic solution (80 mM), under the impact of C-1087 in the concentration of 10 μM (Fig. 11). It is known that under this technique, the activation of SM contractions is achieved via the release of $Ca^{2+}$ ions via potential-governed $Ca^{2+}$-channels, and the processes of decreasing the intracellular concentration of this cation (at the level of the relaxation phase) occur due to the functioning of the energy-dependent transportation systems [6].

Similar to the isometric spontaneous contractions, the myometrium contractions, activated by the application of the hyperkalemic solution (80 mM) (Fig. 11, *A*), were characterized by a considerable decrease in the parameter of the normalized maximal velocity of the relaxation phase $V_{nr}$ (on average, to 46.7% regarding the control, accepted as 100%, $P < 0.01$, $n = 5$).

Similar changes were also observed for the kinetics of myometrium contractions, activated by the application of hyperkalemic solution (80 mM), which were registered in the isometric mode. In this case, the parameter $V_R$ was 32.9% on average regarding the control, accepted as 100% ($P < 0.001$, $n = 5$).

It should also be noted that in the isometric mode of registering the contractile activity, the amplitude of SM shortening under the impact of thiacalix[4]arene C-1087 decreased considerably (Fig. 11, *B*).

Considering the mechanisms by which C-1087 changes the mechanokinetic parameters of contractile responses of myometrium, it is relevant to keep in mind that at present, there are no selective blockers of the calcium pump of PM, and we can rely only on the data, obtained using the models with a disrupted expression of specific isoforms of this enzyme [9, 12, 41]. For instance, as shown by the research of Liu L. et al. [9, 12], in the case of using mice with PMCA4$^{-/-}$, PMCA1$^{+/-}$ and PMCA1$^{+/-}$/4$^{-/-}$,

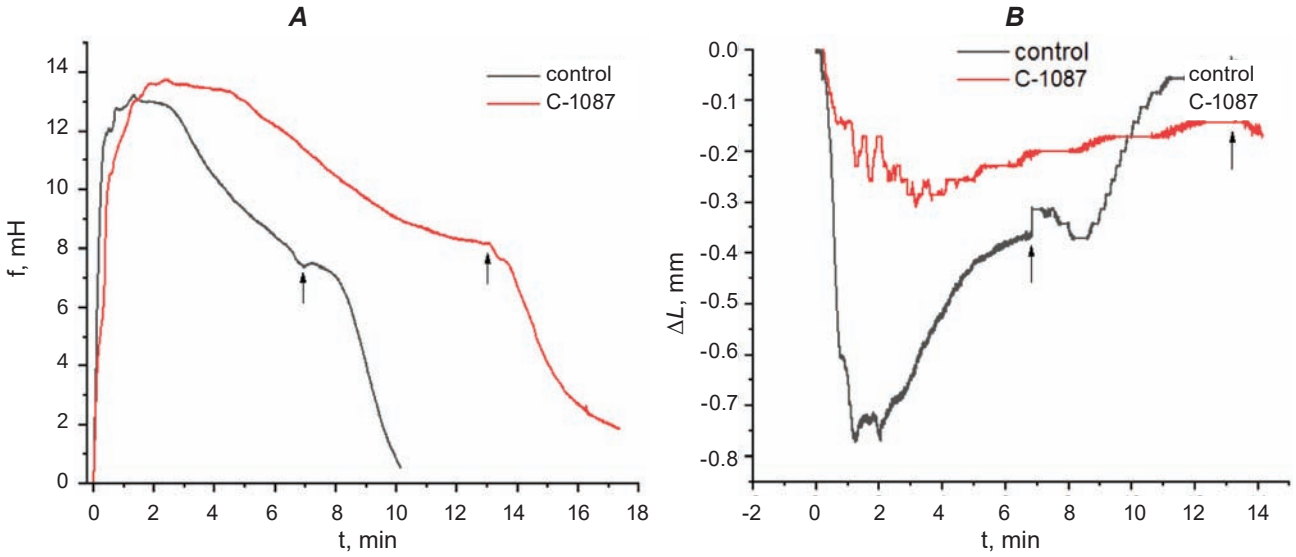



*Fig. 11. The contractile activity of rat myometrium (**A** – under the isometric mode and **B** – under the isotonic mode of registration), induced by the application of hyperkalemic solution (80 mM) in control and against the background of the impact of C-1087 (10 μM). The moment of washing the hyperkalemic solution is indicated with asterisks. Typical mechanograms are presented*

as for SM of the urinal bladder with PMCA1$^{+/-}$ and PMCA1$^{+/-}$/4$^{-/-}$ there was a considerable slowing down of the relaxation phase for the contractile responses to the application of the hyperkalemic solution and the agonist of muscarinic cholinoreceptors, carbachol. So, a considerable decrease in the maximal velocities of relaxation ($V_r$ and $V_{nr}$), induced by C-1087, may be related to the targeted inhibition of the calcium pump in PM.

Therefore, the data of this study may serve as a foundation for the use of thiacalix[4]arene C-1087 as a selective and effective inhibitor of $Ca^{2+}$,$Mg^{2+}$-ATPase of PM, which, in its turn, will have great importance for further investigation of the membrane mechanisms of control over $Ca^{2+}$-exchange in SM, for instance, while studying the role of PM in ensuring electromechanical conjugation in them. The obtained results may also be useful for further studies directed at finding the correlation between the structure of calixarenes and their impact on different cation-transporting enzymatic systems, which are the basis for improving the selectivity and efficiency of their potential new inhibitors based on calixarenes. In addition, these data may serve as a foundation for the elaboration of potential pharmacological means capable of modulating the contractile function of the uterus in the pathologies of SM contractile activity.

*Conflict of interest.* The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

*Funding.* This work was financially supported by the grants with the state registration numbers: 0119U002508, 0123U100894.

# ТІАКАЛІКС[4]АРЕН С-1087 – СЕЛЕКТИВНИЙ ІНГІБІТОР КАЛЬЦІЄВОЇ ПОМПИ ПЛАЗМАТИЧНОЇ МЕМБРАНИ КЛІТИН ГЛАДЕНЬКИХ М'ЯЗІВ

*Т. О. Векліч[1]✉, Р. В. Родік[2], О. В. Цимбалюк[3], О. А. Шкрабак[1], О. В. Малюк[1], С. О. Карахім[1], С. Г. Вишневський[2], В. І. Кальченко[2], С. О. Костерін[1]*

[1]Інститут біохімії ім. О. В. Палладіна НАН України, Київ;
✉e-mail: veklich@biochem.kiev.ua
[2]Інститут органічної хімії НАН України, Київ;
[3]Навчально-науковий інститут високих технологій, Київський національний університет імені Тараса Шевченка, Київ, Україна

За допомогою методів ензимного та кінетичного аналізів показано, що 5,11,17,23-тетра(трифтор)метил(фенілсульфоніліміно)метиламіно-25,27-дигексилокси-26,28-дигідрокситіакалікс[4]арен С-1087 ефективно інгібує $Ca^{2+}$,$Mg^{2+}$-АТРазну активність плазматичної мембрани клітин міометрія щурів ($I_{0,5}$ = 9,4 ± 0,6 мкМ). При цьому ефектор не впливає на відносну активність інших мембранних АТРаз. За допомогою конфокальної мікроскопії та $Ca^{2+}$-чутливого флуоресцентного зонда fluo-4 було показано, що дія тіакалікс[4]арену С-1087 на іммобілізовані міоцити матки збільшує цитозольну концентрацію $Ca^{2+}$. Тензометричні дослідження гладеньких м'язів матки щурів із подальшим механокінетичним аналізом показали, що тіакалікс[4]арен С-1087 значно знижує максимальну швидкість розслаблення як спонтанної скоротливої відповіді, так і скорочення, спричиненого гіперкалієвим розчином.

Ключові слова: $Ca^{2+}$,$Mg^{2+}$-АТРаза, плазматична мембрана, гладеньком'язові клітини, міометрій, ензиматичний гідроліз АТР, кінетичні параметри, швидкість фази розслаблення, тіакалікс[4]арени.

## References

1. Wray S. Insights from physiology into myometrial function and dysfunction. *Exp Physiol.* 2015; 100(12): 1468-1476.
2. Kosterin SO, Babich LG, Shlykov SG, Danylovych IuV, Veklich TO, Mazur YuYu. Biochemical properties and regulation of smooth muscle cell $Ca^{2+}$-transporting systems. K.: Science opinion, 2016. 210 p.
3. Noble D, Herchuelz A. Role of Na/Ca exchange and the plasma membrane $Ca^{2+}$-ATPase in cell function. *EMBO Rep.* 2007; 8(3): 228-232.
4. Futai M, Wada Y, Kaplan J. Catalytic and transport mechanism of the sarco-(endo)plasmic reticulum $Ca^{2+}$-ATPase (SERCA). Handbook of ATPases: biochemistry, cell biology, pathophysiology. Ed. John Wiley & Sons. – Weinheim: Wiley-VCH, 2004; 63-84.
5. Karaki H, Ozaki H, Hori M, Mitsui-Saito M, Amano K, Harada K, Miyamoto S, Nakazawa H, Won KJ, Sato K. Calcium movements, distribution, and functions in smooth muscle. *Pharmacol Rev.* 1997; 49(2): 157-230.
6. Brini M, Carafoli E. The plasma membrane $Ca^{2+}$ ATPase and the plasma membrane sodium calcium exchanger cooperate in the regulation of cell calcium. *Cold Spring Harb Perspect Biol.* 2011; 3(2): a004168.
7. Sanborn BM. Hormonal signaling and signal pathway crosstalk in the control of myometrial calcium dynamics. *Semin Cell Dev Biol.* 2007; 18(3): 305-314.
8. Penniston JT, Enyedi A. Modulation of the plasma membrane $Ca^{2+}$ pump. *J Membr Biol.* 1998; 165(2): 101-109.
9. Liu L, Ishida Y, Okunade G, Shull GE, Paul RJ. Role of plasma membrane $Ca^{2+}$-ATPase in contraction-relaxation processes of the bladder: evidence from PMCA gene-ablated mice. *Am J Physiol Cell Physiol.* 2006; 290(4): C1239-C1247.
10. Cartwright EJ, Oceandy D, Austin C, Neyses L. $Ca^{2+}$ signalling in cardiovascular disease: the role of the plasma membrane calcium pumps. *Sci China Life Sci.* 2011; 54(8): 691-698.
11. Brini M. Plasma membrane $Ca^{2+}$-ATPase: from a housekeeping function to a versatile signaling role. *Pflugers Arch.* 2009; 457(3): 657-664.
12. Liu L, Ishida Y, Okunade G, Pyne-Geithman GJ, Shull GE, Paul RJ. Distinct roles of PMCA isoforms in $Ca^{2+}$ homeostasis of bladder smooth muscle: evidence from PMCA gene-ablated mice. *Am J Physiol Cell Physiol.* 2007; 292(1): C423-C431.
13. Uterine contractility. Ed. by R.E. Garfield - Serano Symposia, VSA, Norwell, Massachusetts, 1990. 388 p.

14. Hertelendy F, Zakar T. Regulation of myometrial smooth muscle functions. *Curr Pharm Des.* 2004; 10(20): 2499-2517.
15. Calixarenes in the Nanoworld. Ed. by Vicens J, Harrowfield J, Baklouti L. Springer, Dordrecht, The Netherlands, 2007. 395 p.
16. Calixarenes and Beyond. Ed. by Neri P, Sessler JL, Wang MX. Springer International Publishing, Switzerland, 2016. 1062 p.
17. Español ES, Villamil MM. Calixarenes: Generalities and Their Role in Improving the Solubility, Biocompatibility, Stability, Bioavailability, Detection, and Transport of Biomolecules. *Biomolecules.* 2019; 9(3): 90.
18. Rodik RV, Boyko VI, Kalchenko VI. Calixarenes in biotechnology and bio-medical researches. Front Med Chem. Eds. Atta-ur-Rahman, Choudhary MI, Reitz AB. Bentham Science Publishers. 2016; 8: 206-301.
19. Pan YC, Hu XY, Guo DS. Biomedical Applications of Calixarenes: State of the Art and Perspectives. *Angew Chem Int Ed Engl.* 2021; 60(6): 2768-2794.
20. Fan X, Guo X. Development of calixarene-based drug nanocarriers. *J Mol Liquids.* 2021; 325: 115246.
21. Coleman AW, Jebors S, Cecillon S, Perret P, Garin D, Marti-Battle D, Moulin M. Toxicity and biodistribution of para-sulfonato-calix[4]-arene in mice. *New J Chem.* 2008; 32: 780-782.
22. Kosterin SO, Kalchenko VI, Veklich TO, Babich LG, Shlykov SG. Calixarenes as modulators of ATP-hydrilizing systems of smooth muscles. K.: Science opinion, 2019. 256 p.
23. Rassukana YV, Onys'ko PP, Grechukha AG, Sinitsa AD. N-(Arylsulfonyl)trihaloacetimidoyl Chlorides and Their Reactions with Phosphites. *Eur J Org Chem.* 2003; 2003(21): 4181-4186.
24. 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).
25. Kondratiuk TP, Bychenok SF, Prishchepa AA, 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).
26. 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.
27. 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): C47-C54.
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. Veklich TO, Shkrabak OA, Nikonishyna YuV, Rodik RV, Kalchenko VI, Kosterin SO. Calix[4]arene C-956 selectively inhibits plasma membrane $Ca^{2+}$,$Mg^{2+}$-ATPase in myometrial cells. *Ukr Biochem J.* 2018; 90(5): 34-42.
30. Magocsi M, Penniston JT. $Ca^{2+}$ or $Mg^{2+}$ nucleotide phosphohydrolases in myometrium: two ecto-enzymes. *Biochim Biophys Acta.* 1991; 1070(1): 163-172.
31. Amédée T, Mironneau C, Mironneau J. Isolation and contractile responses of single pregnant rat myometrial cells in short-term primary culture and the effects of pharmacological and electrical stimuli. *Br J Pharmacol*. 1986; 88(4): 873-880.
32. Kosterin S, Tsymbalyuk O, Holden O. Multiparameter analysis of mechanokinetics of the contractile response of visceral smooth muscles. *Ser Biomechan*. 2021; 35(1): 14-30.
33. Burdyga TV, Kosterin SA. Kinetic analysis of smooth muscle relaxation. *Gen Physiol Biophys.* 1991; 10(6): 589-598.
34. Iki N, Narumi F, Fujimoto T, Morohashi N, Miyano S. Selective synthesis of three conformational isomers of tetrakis[(ethoxycarbonyl)methoxy]thiacalix[4] arene and their complexation properties towards alkali metal ions. *J Chem Soc Perkin Trans 2.* 1998; (12): 2745-2750.
35. Lang J, Dvořáková H, Bartošová I, Lhotak P, Stibor I, Hrabal R. Conformational flexibility of a novel tetraethylether of thiacalix[4]arene. A comparison with the "classical" methylene-bridged compounds. *Tetrahedron Lett.* 1999; 40(2): 373-376.
36. Kasyan O, Healey ER, Drapailo A, Zaworotko M, Cecillon S, Coleman AW, Kalchenko V. Synthesis, Structure and Selective Upper Rim Functionalization of Long Chained Alkoxythiacalix[4]arenes. *J Incl Phenom Macrocycl Chem.* 2007; 58: 127-132.

37. Veklich TO, Shkrabak OA, Nikonishyna YuV, Rodik RV, Kalchenko VI, Kosterin SO. Calix[4]arene C-90 as a promising supramolecular compound to regulate the activity of plasma membrane $Ca^{2+}$,$Mg^{2+}$-ATPase of smooth muscle cells. *Nanosist Nanomater Nanotehnol*. 2017; 15(2): 373-380. (In Ukrainian).
38. Danylovych IuV, Chunikhin OIu, Danylovich 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).
39. Tsymbalyuk OV, Kosterin SO. Influence of calixarene C-90 jn contractile activity of rat myometrium smooth muscles. *Studia Biologica*. 2013; 7(3): 87-98.
40. Tsymbalyuk OV. Kinetics of relaxation of rat myometrium in conditions of inhibition of plasma membrane calcium pump and systems of active $Ca^{2+}$ transport of intracellular $Ca^{2+}$-depot. *Studia Biologica*. 2018; 12(2): 3-12.
41. Ishida Y, Paul RJ. $Ca^{2+}$ clearance in smooth muscle: lessons from gene-altered mice. *J Smooth Muscle Res.* 2005; 41(5): 235-245.