# EXPERIMENTAL WORKS

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## THIACALIX[4]ARENE C-1087 EFFECTS ON THE $Ca^{2+}$ EXCHANGE IN MITOCHONDRIA

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***Background.*** *Calixarenes are supramolecular compounds with a unique three-dimensional structure, the biological activity of which is determined by the chemical groups on the upper or lower rim. It was found that thiacalix[4]arene C-1087 selectively (compared to other ATPases) inhibits plasma membrane $Ca^{2+}$,$Mg^{2+}$-ATPase activity that playis a crucial role in maintaining $Ca^{2+}$ homeostasis in myometrial cell. To expand our understanding of the thiacalix[4]arene C-1087 effects on $Ca^{2+}$ exchange in myometrial cells, it is necessary to analyze its possible effects on $Ca^{2+}$ exchange in intracellular pools.* ***Objective.*** *Therefore, the aim of this study was to investigate whether thiacalix[4]arene C-1087 affects Ca ion exchange in myometrial mitochondria.* ***Methods.*** *Mitochondria from myometrium of non-pregnant rats were isolated. Ionized calcium concentration in the mitochondria matrix was determined using the fluorescent probe Fluo-4 AM. Baseline $Ca^{2+}$ concentration in the absence of exogenous (added) $Ca^{2+}$ and $Ca^{2+}$ accumulation in its presence were determined. $Ca^{2+}$ concentration in the incubation medium was determined using fluorescent probe 1 μM Fluo5F, Pentapotassium Salt. Relative value of mitochondria membrane potential (Δψ) was assayed using a fluorescent probe 100 nM TMRM. The Microsoft Office Excel software package was used for data processing.* ***Results.*** *It was proven that mitochondria incubation in $Mg^{2+}$- and $Mg^{2+}$,ATP-medium with 10 μM thiacalix[4]arene C-1087 was accompanied by an increase of both the baseline $Ca^{2+}$ concentration and accumulation of this cation in the matrix and did not affect $Ca^{2+}$ efflux from mitochondria. Mitochondria incubation with 10 μM thiacalix[4]arene C-1087 resulted in reducing of TMRM-loaded mitochondria fluorescence intensity, indicatings the depolarizing effect of this compound.* ***Conclusions.*** *The results obtained indicate the possibility of thiacalix[4]arene C-1087 modulating effect on the $Ca^{2+}$ exchange in mitochondria and polarization of its membranes.*

*K e y w o r d s: mitochondria, $Ca^{2+}$ concentration, $Ca^{2+}$ accumulation and efflux, membrane polarization, thiacalix[4]arene C-1087.*

Intracellular $Ca^{2+}$ distribution is a tightly regulated process. Numerous $Ca^{2+}$ chelating, storage, and transport mechanisms are required to maintain normal cellular physiology [1]. $Ca^{2+}$ channels, ATP-driven pumps, and exchangers assist the binding proteins in transferring the ions to and from appropriate cellular compartments [1]. Two P-type Ca transporters, the plasma membrane Ca-ATPase (PMCA) and the sarcoplasmic reticulum (SR) Ca-ATPase (SERCA), play a crucial role in maintaining Ca homeostasis, controlling contractility and contributing to excitably and cell signaling in smooth muscle cells [2]. Located in plasma membranes, ATP hydrolases are involved in several dynamic transport processes, helping to control the movement of ions across cell membranes. ATP hydrolase acts as a

transport protein, converting energy from ATP hydrolysis into transport molecules against their concentration gradients [3]. Therefore, there is an urgent need to study compounds that regulate the activity of this pump. The enzymatic and kinetic analyses were used to demonstrate that thiacalix[4]arene C-1087 effectively inhibited the $Ca^{2+}$,$Mg^{2+}$-ATPase activity of the rat myometrium cells plasma membrane with no effect on the relative activity of other membrane ATPases [4, 5]. However, it cannot be ruled out that thiacalix[4]arene C-1087 also affects other Ca-transporting systems of myometrial cells, in particular, $Ca^{2+}$ exchange in mitochondria. Therefore, the aim of this study was to investigate whether thiacalix[4]arene C-1087 affects $Ca^{2+}$ ion exchange in myometrial mitochondria.

## Materials and Methods

*Animals.* Female non-pregnant rats were used. Experiments were performed using white female rats (weight 170-240 g). Rats were kept under the stationary vivarium conditions at constant temperature and basic allowance. Animals were narcotized with chloroform and then sacrificed using cervical dislocation.

*Compliance with ethical standards statement.* The treatment of the lab animals was carried out according to "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and the Law of Ukraine "On protection of animals from cruelty".

*Ethics approval.* The Bioethics Commission of the Palladin Institute of Biochemistry (Order No. 15 of September 5, 2025) approved all manipulations with laboratory animals.

*Isolation of myometrium mitochondria.* Mitochondria from myometrium of non-pregnant rats were isolated using differential centrifugation method [6]. The mitochondria were suspended in a medium with the following composition: 250 mM sucrose, 1 mM EGTA, 20 mM Hepes, and buffered pH 7.4 at 4°C. Protein concentration of the mitochondrial fraction was determined by Bradford assay [7]. The concentration of mitochondrial protein in the sample was 25 μg/ml.

*Determination of ionized calcium concentration in the mitochondria matrix.* Ionized calcium concentration in the mitochondria matrix was determined using the QuantaMaster™ 40 spectrofluorometer (Photon Technology International) and the fluorescent probe Fluo-4, AM ($\lambda_{exc}$ = 490 nm, $\lambda_{em}$ = 520 nm) ($K_d$ for $Ca^{2+}$ = 345 nM). Baseline $Ca^{2+}$ concentration ($[Ca^{2+}]_b$) was determined in the absence of exogenous (added) $Ca^{2+}$ and $Ca^{2+}$ accumulation ($[Ca^{2+}]_m$) in its presence. The dissociation constant of the Ca probe (Fluo-4, AM) is 345 nM, which allows measuring the cation concentration in the range of 34-3400 nM. Myometrium mitochondria were loaded with 2 μM Fluo-4, AM for 30 min at 37°C in a medium the composition of which is indicated above. Thereafter, the suspension of mitochondria was diluted (1:10) in the same medium containing no fluorescence probe followed by centrifugation. The pellet was resuspended in the same medium containing no fluorescence probe. The $[Ca^{2+}]_m$ was measured in a medium containing: 250 mM sucrose, 2 mM $K^+$-phosphate buffer, 5 mM sodium succinate, 3 mM $MgCl_2$, ±3 mM ATP, ±0.1 mM $CaCl_2$, 20 mM Hepes, pH 7.4. To exclude the possibility of the medium acidification by adenosine triphosphate addition, we normalized the pH of ATP stock solution with 1 M tris. The calibration of the Fluo-4 fluorescence was performed at the end of each testing probe by adding 0.1% Triton X-100 (at the presence of 100 μM $CaCl_2$) and, in 1 min, 5 mM EGTA (fluorescence intensities $F_{max}$ and $F_{min}$, respectively). $[Ca^{2+}]_b$ and $[Ca^{2+}]_m$ were calculated using the Grynkiewicz equation [8].

*Determination of ionized calcium concentration in the incubation medium.* Ionized Ca concentration in the incubation medium ($[Ca^{2+}]_o$) was determined using the QuantaMasterTM 40 spectrofluorometer (Photon Technology International) and the fluorescent probe 1 μM Fluo5F, Pentapotassium Salt, cell impermeant ($\lambda_{exc}$ = 490 nm, $\lambda_{em}$ = 520 nm) ($K_d$ for $Ca^{2+}$ = 2.3 μM). The dissociation constant of the Ca probe (Fluo5F, Pentapotassium Salt) is 2.3 μM, which allows measuring the cation concentration in the range of 0.23-23 μM. The $[Ca^{2+}]_o$ was measured in a medium containing: 250 mM sucrose, 2 mM $K^+$-phosphate buffer, 5 mM sodium succinate, 3 mM $MgCl_2$, ±3 mM ATP, 20 mM Hepes, pH 7.4. To exclude the possibility of the medium acidification by adenosine triphosphate addition, we normalized the pH of ATP stock solution with 1 M tris. The calibration of the Fluo5F, Pentapotassium Salt fluorescence was performed at the end of each testing probe by adding 5 mM EGTA and, in 1 min, 5 mM $CaCl_2$ (fluorescence intensities $F_{min}$ and $F_{max}$, respectively). $[Ca^{2+}]_o$ was calculated using the Grynkiewicz equation [8].

*Measurements of mitochondria membrane polarization*. To record mitochondria membrane polarization a DxFLEX (Beckman Coulter Biotechnology) flow cytometer with an argon laser ($\lambda_{ex}$ 488 nm) was used. Experimental data was analyzed using the CytExpert. Samples autofluorescence was measured after mitochondria incubation for 2 min with thiacalix[4]arene C-1087 at concentrations of 1 or 10 μM in a medium containing: 250 mM sucrose, 2 mM $K^+$-phosphate buffer, 5 mM sodium succinate, 3 mM $MgCl_2$, 20 mM Hepes, pH 7.4. An aliquot of solvent (DMSO) was added to control samples. Relative values of mitochondria membrane potential ($\Delta\psi$) were assayed using a fluorescent probe 100 nM TMRM ($\lambda_{ex}$ = 488 nm, $\lambda_{em}$ = 590 nm). Mitochondria suspension (25 μg/ml) was loaded with 100 nM TMRM for 2 min and then immediately analyzed on the flow cytometer at a wavelength of 590 nm. Each measurement was represented as the average mean of 3000 events fluorescent intensity. The results were calculated in relative units (the average fluorescence intensity value of mitochondria loaded with TMRM minus the average autofluorescence value of mitochondria) and presented in % relative to the control sample containing an aliquot of the solvent (DMSO) used to prepare the thiacalix[4]arene C-1087 solutions.

*Statistical analysis*. Results are reported as means ± SEM of 3-4 independent experiments (biological replicates). Statistical analysis was performed using paired Student's *t*-test; $P < 0.05$ was taken as the level of significance. The Microsoft Office Excel software package was used for data processing.

*Chemicals and reagents*. The following reagents were used: EGTA, Hepes, D-(+)-sucrose, sodium succinate – by "Sigma-Aldrich"; Fluo-4 AM, Fluo5F, Pentapotassium salt, TMRM – by "Invitrogen"; ATP – by "Fluka".

## Results

*Thiacalix[4]arene C-1087*. Thiacalix[4]arene C-1087 was obtained via three step procedure (Fig. 1) [4].

Thiacalix[4]arene C-1087 and mitochondria matrix ionized Ca concentration increase. Before studying the thiacalix[4]arene C-1087 effects on $Ca^{2+}$ exchange in mitochondria, we need to determine whether it directly affects the fluorescence of the $Ca^{2+}$-sensitive probe. Thiacalix[4]arene C-1087 effects on the Fluo5F pentapotassium salt fluorescence (a water-soluble analog of Fluo-4 AM) was

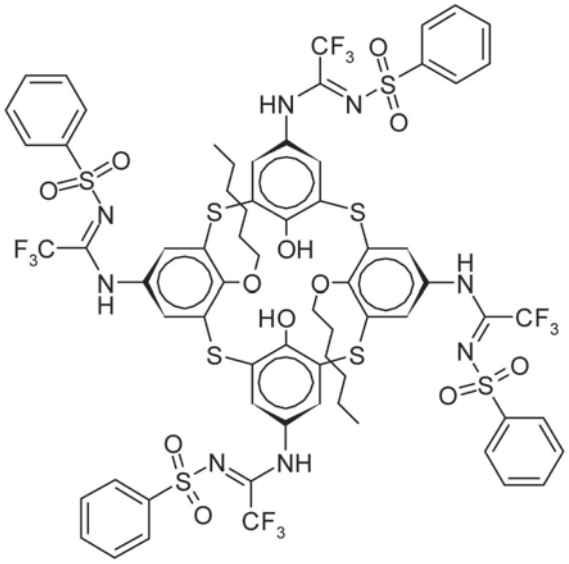


*Fig. 1. Thiacalix[4]arene C-1087 structural formula*

studied. As can be seen from the results presented in Fig. 2. under 100 μM $Ca^{2+}$ addition the fluorescence spectrum of the Fluo5F pentapotassium salt did not change significantly when aliquots of 1 or 10 μM thiacalix[4]arene C-1087 were added. EGTA addition effectively blocked the fluorescence of the $Ca^{2+}$-sensitive probe.

Myometrium mitochondria, loaded with fluorescent probe Fluo-4 AM, were incubated in $Mg^{2+}$- medium at the absence (baseline $Ca^{2+}$ concentration, $[Ca^{2+}]_b$) and in the presence of exogenous $Ca^{2+}$ ($Ca^{2+}$ accumulation, $[Ca^{2+}]_m$) (Fig. 3, *a*). It was shown that mitochondria incubation with 1 μM thiacalix[4]arene C-1087 did not change as $[Ca^{2+}]_b$ so $[Ca^{2+}]_m$. At the same time mitochondria incubation with 10 μM thiacalix[4]arene C-1087 resulted in increase of $[Ca^{2+}]_b$ and approximately 3 times increase of $[Ca^{2+}]_m$ compared to the control (Fig. 3, *a*). The addition of ATP to the incubation medium was accompanied by a more than 2-fold increase of $[Ca^{2+}]_b$ (Fig. 3, *b*). It should be noted that incubation of mitochondria with thiacalix[4]arene C-1087 did not affect the ATP-induced increase of $[Ca^{2+}]_b$ (Fig. 3, *b*). At the same time mitochondria incubation with 10 μM thiacalix[4]arene C-1087 resulted in approximately 2 times increase of $[Ca^{2+}]_m$ compared to the control (Fig. 3, *b*).

*Thiacalix[4]arene C-1087 and $Ca^{2+}$ efflux from mitochondria*. Cell impermeant fluorescent probe Fluo5F, Pentapotassium Salt, was used to evaluate $Ca^{2+}$ efflux from mitochondria ($[Ca^{2+}]_o$) (Fig. 4). It

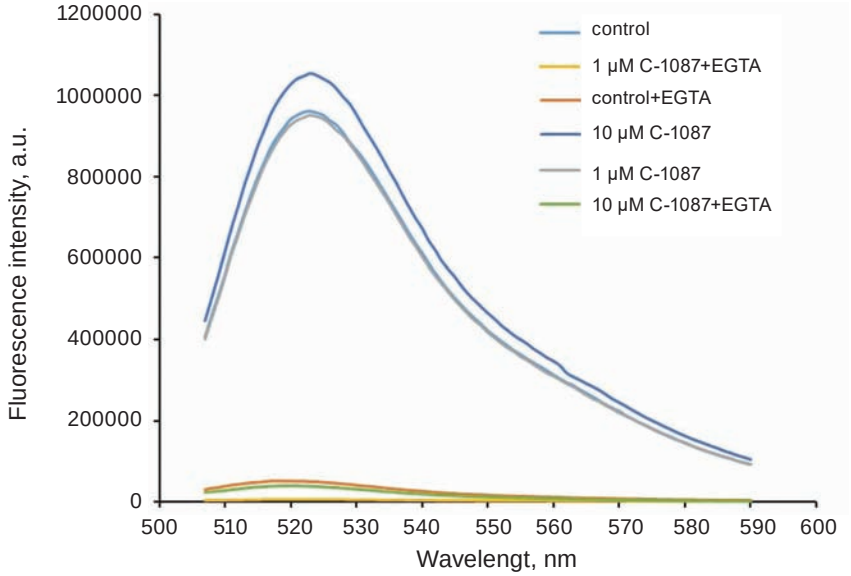


*Fig. 2. Thiacalix[4]arene C-1087 effects on fluorescence spectra of the Fluo5F pentapotassium salt: testing was carried out in Mg-medium in the presence of 100 μM $Ca^{2+}$ followed by the addition of 1 mM EGTA*

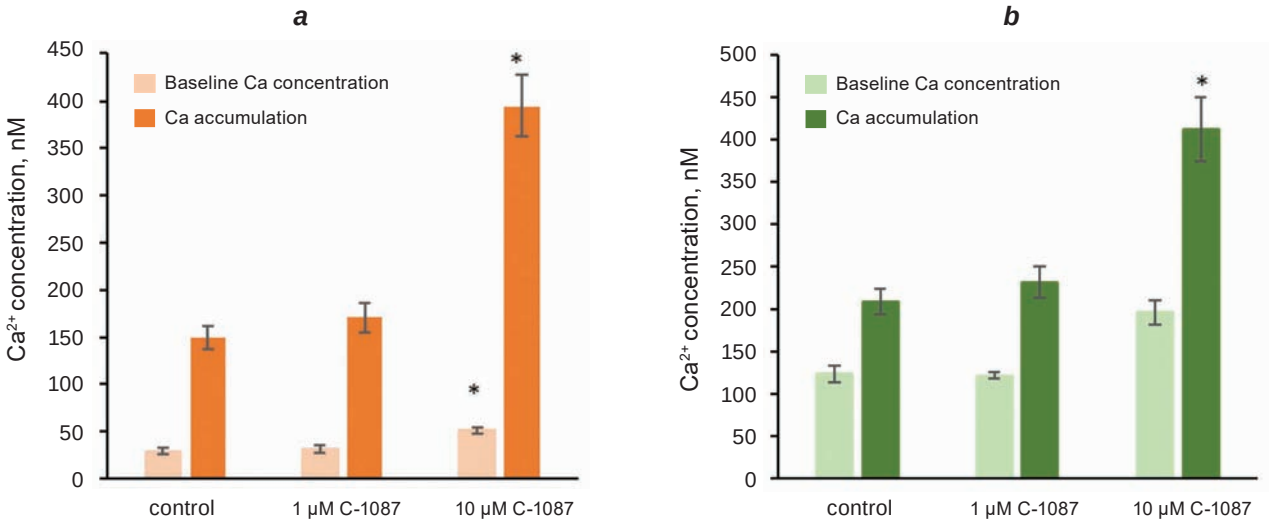


*Fig. 3. Mitochondrial matrix $[Ca^{2+}]_b$ and $[Ca^{2+}]_m$: **a** – $Mg^{2+}$-medium; **b** – $Mg^{2+}$,ATP-medium. Means ± SEM, n = 4, * P < 0.05 (compared to control)*

was shown, that $[Ca^{2+}]o$ at mitochondria incubation in $Mg^{2+}$- and $Mg^{2+}$,ATP-medium was not affected by 1 or 10 μM thiacalix[4]arene C-1087. It should be noted that incubation of mitochondria with thiacalix[4]arene C-1087 did not affect the ATP-induced increase of $Ca^{2+}$ efflux from organelle (Fig. 4).

*Thiacalix[4]arene C-1087 and polarization of myometrium mitochondria membranes.* Flow cytometry and a voltage sensitive probe (100 nM TMRM) were used to test the polarization of mitochondrial membranes. It was shown, that TMRM probe is loaded into myometrial mitochondria quite quickly (during 2 min incubation), that indirectly indicates their functional activity (Fig. 5, *a*). Mitochondria incubation with 1 μM thiacalix[4]arene C-1087 did not significantly affect either the autofluorescence of mitochondria or the fluorescence of probe-loaded mitochondria (Fig. 5, *b, c*). Mitochondria incubation with 10 μM thiacalix[4]arene C-1087 resulted in reducing of TMRM-loaded mitochondria fluorescence intensity, that indicates the depolarizing effect of this compound. This effect may be associated at least with an

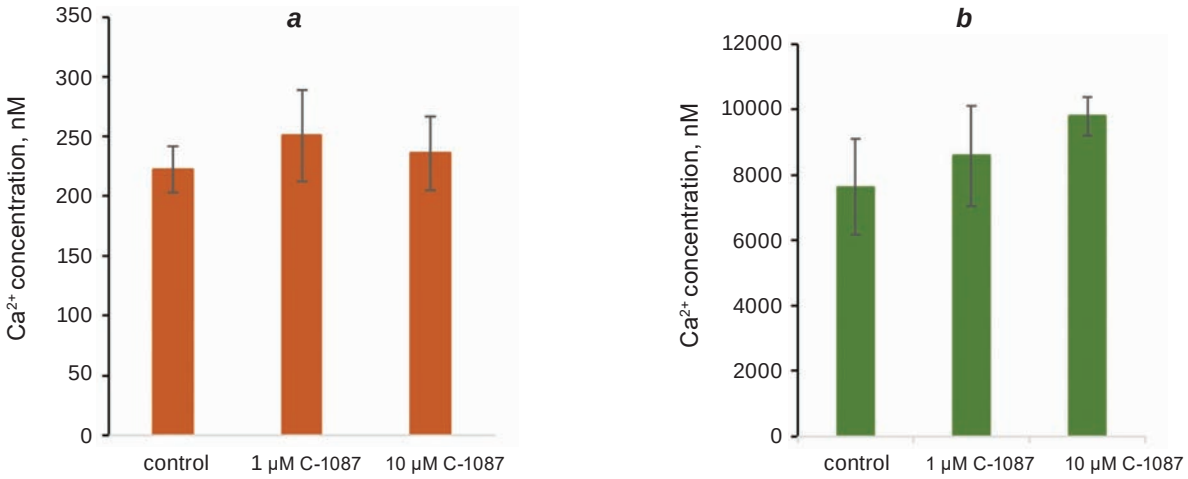


*Fig. 4. $Ca^{2+}$ efflux from mitochondria:* ***a*** *– $Mg^{2+}$-medium;* ***b*** *– $Mg^{2+}$,ATP-medium. Means ± SEM, n = 3*

increase of baseline $Ca^{2+}$ concentration that occurred during incubation of mitochondria with 10 μM thiacalix[4]arene C-1087 in $Mg^{2+}$-medium.

## Discussion

Nanotechnology has been widely studied in biomedical applications in the last decade. The revolution in nanotechnology triggers the fabrication of nanomaterials with novel properties and functionalities, making the research in nanosensors and biomedical rapidly expanding. Nanosensor application has improved the sensitivity by enhancing their catalytic activity, conductivity, and biocompatibility. Calixarene is excellent as a sensing element used as a sensor due to its unique host-guest properties. Three major types of calixarene which are extensively studied are calix[4]arene, calix[6]arene, and calix[8]arene [9].

Macrocyclic arenes including calixarenes, resorcinarenes, cyclotriveratrylene, pillararenes and so on have emerged as highly attractive synthetic macrocyclic hosts due to their unique structures, facile functionalization, and broad range of applications [10].

It was shown that the application of thiacalix[4]arene C-1087 to the uterus myocytes increased the cytosolic $Ca^{2+}$ concentration [4]. 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 [4].

In smooth muscle, $Ca^{2+}$ signals arise from two major sources. The first is the extracellular space from which $Ca^{2+}$ enters the cell via channels such as voltage-dependent $Ca^{2+}$ channels, store-operated $Ca^{2+}$ (SOC) channels and various members of the transient receptor potential channel family. The second major $Ca^{2+}$ source is the internal $Ca^{2+}$ store (sarcoplasmic reticulum; SR). Activation of either $Ca^{2+}$influx or $Ca^{2+}$ release results in an increase of the cytoplasmic $Ca^{2+}$ concentration [11]. In native smooth muscle cells, mitochondria contribute to the localization of $Ca^{2+}$ signals and to the modulation of the amplitude of $Ca^{2+}$ signals. Mitochondria regulate these local signals by the organelles' ability to take up and release the ion. Mitochondrial $Ca^{2+}$ uptake and efflux may regulate cytoplasmic $Ca^{2+}$ concentrations both directly and indirectly. Direct regulation occurs by alteration of bulk $Ca^{2+}$ levels. Indirect regulation occurs as a result of mitochondrial influence on the activity of SR or plasma membrane $Ca^{2+}$ channels [11].

In the previous works, using mitochondria fraction and myometrium cells perforated with digitonin, we have shown that calix[4]arene chalcone amides are able to influence both the level of mitochondria membrane polarization [12, 13] and the $Ca^{2+}$ concentration in the myometrial mitochondria matrix [12]. This study showed that mitochondria incubation with 1 μM thiacalix[4]arene C-1087 did not change as baseline $Ca^{2+}$ concentration so $[Ca^{2+}]_m$. At the same time mitochondria incubation with 10 μM thiacalix[4]arene C-1087 resulted in increase of baseline $Ca^{2+}$ concentration and approximately 3 times increase of $[Ca^{2+}]_m$ compared to the control.

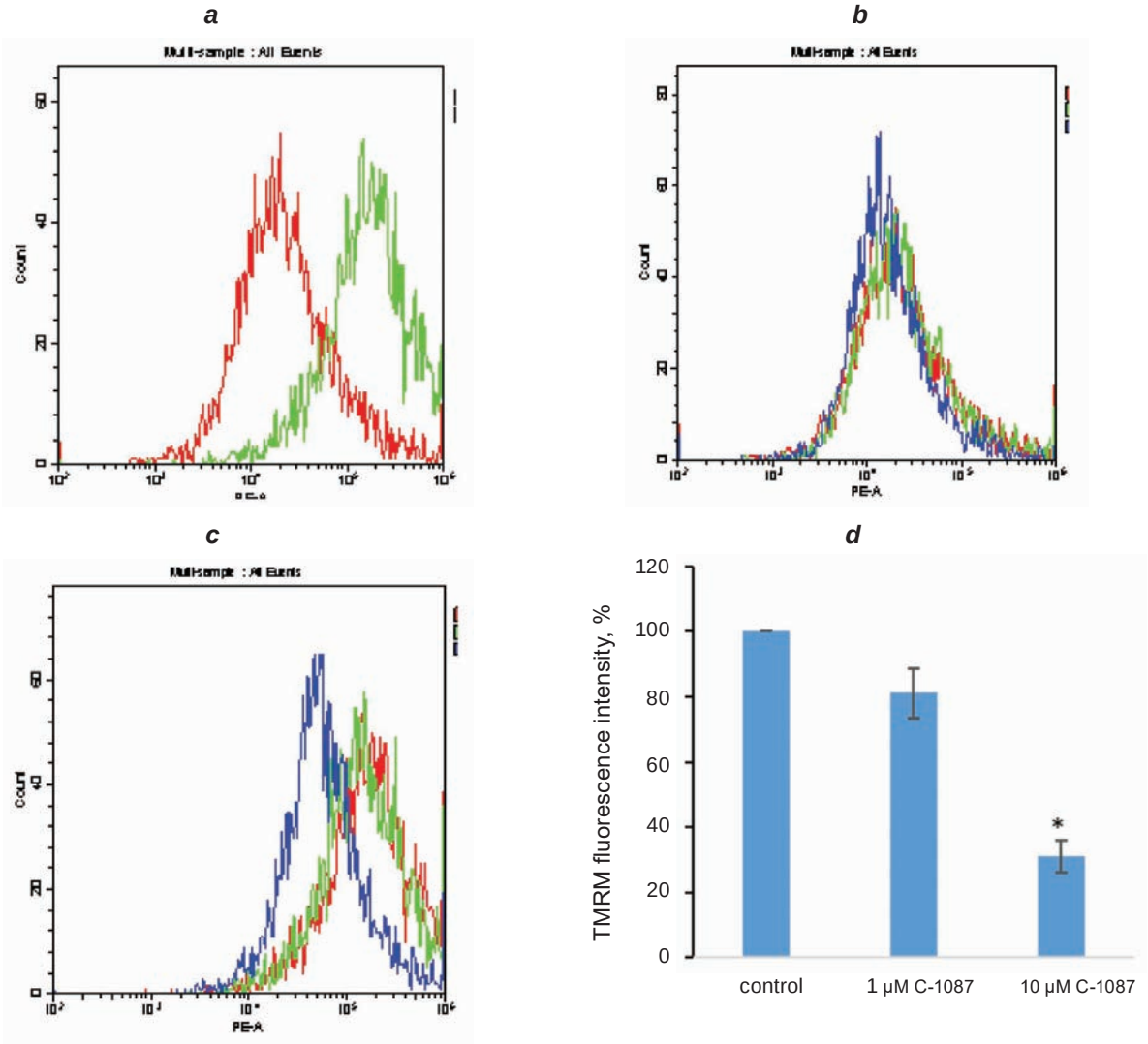


*Fig. 5. Flow cytometry analyses of mitochondria membrane polarization upon incubation with 1 and 10 μM thiacalix[4]arene C-1087. (**a**) red graph – mitochondria autofluorescence; green graph – TMRM loaded mitochondria. (**b**) red graph – mitochondria autofluorescence (control); green graph – mitochondria autofluorescence after incubation with 1 μM thiacalix[4]arene C-1087; blue graph – mitochondria autofluorescence after incubation with 10 μM thiacalix[4]arene C-1087. (**c**) red graph – TMRM loaded mitochondria (control); green graph – TMRM loaded mitochondria + 1 μM thiacalix[4]arene C-1087; blue graph – TMRM loaded mitochondria + 10 μM thiacalix[4]arene C-1087. (**d**) TMRM fluorescence intensity *P < 0.05 (as compared to control). Means±SEM, n = 4. The results of a typical experiment are presented (**a-c**), n = 4*

We have shown previously that baseline $Ca^{2+}$ concentration in mitochondria matrix is regulated by ATP: in $Mg^{2+}$,ATP- medium this number is couple of times higher than in $Mg^{2+}$-one [14-16]. To elucidate the possible mechanisms that are involved in ATP-induced $[Ca^{2+}]_m$ increasing (baseline $Ca^{2+}$ concentration) different mitochondria effectors were used: cyclosporine A – mitochondrial permeability transition pore inhibitor; ruthenium red – mitochondrial $Ca^{2+}$-uniporter inhibitor; oligomycin – inhibitor of $F_1F_0$-ATPase complex; trifluoperazine – calmodulin antagonist. It was shown that $[Ca^{2+}]_m$ was not affected by 5 μM CsA, 10 μM RuR or 1μg/ml oligomycin addition [16]. This time, we tested whether thiacalix[4]arene C-1087 affects the ATP-dependent increase of baseline $Ca^{2+}$ concentration in the matrix. It was shown that adding ATP to the incubation medium increased the baseline $Ca^{2+}$ concentration

(in the absence of added Ca), but thiacalix[4]arene C-1087 had no effect on this parameter. At the same time mitochondria incubation with 10 μM thiacalix[4]arene C-1087 resulted in approximately 2 times increase of $[Ca^{2+}]_m$ compared to the control.

Mitochondria incubation in $Mg^{2+}$- and $Mg^{2+}$,ATP -medium with 1 and 10 μM thiacalix[4]arene C-1087 did not affect $Ca^{2+}$ efflux from mitochondria. We have shown previously that $[Ca^{2+}]_o$ sharply decreased with an increase of the EGTA concentration, a well-known $Ca^{2+}$ chelator. The ATP concentration-dependent increase of $[Ca^{2+}]_o$ in the absence of exogenous $Ca^{2+}$ was also shown. The Hill coefficient equals 3.69 ± 0.46 and activation constant for ATP – 2.94 ± 0.68 mM [16].

Mitochondrial membrane potential (Δψ) and morphology are considered key readouts of mitochondrial functional state [17]. Maintenance of the mitochondrial inner membrane potential ($\Delta\Psi_m$) is critical for many aspects of mitochondrial function [18]. Mitochondrial membrane potential can be studied using fluorescent dyes like tetramethylrhodamine methyl ester (TMRM). TMRM is a cell-permeant red-orange fluorescent dye that is readily sequestered by active mitochondria. Depolarization of mitochondrial membranes is accompanied by the release of TMRM, which is tested by a decrease in probe fluorescence intensity. Using flow cytometer and TMRM it was shown that mitochondria incubation with 10 μM thiacalix[4]arene C-1087 resulted in reducing of TMRM-loaded mitochondria fluorescence intensity, that indicates the depolarizing effect of this compound. Earlier we showed that myometrium mitochondria membranes were polarized in both $Mg^{2+}$- and $Mg^{2+}$,ATP-medium. However, the level of mitochondrial membranes polarization was higher in $Mg^{2+}$-medium [16].

It should be noted that thiacalix[4]arene C-1087 is insoluble in water and soluble in polar solvents, i.e. it is a lipophilic molecule. It can be assumed that the accumulation of thiacalix[4]arene C-1087 in mitochondrial membranes is accompanied by its interaction with proteins and, as a result, a decrease in the activity of respiratory chain enzymes, which leads to a decrease in the polarization of mitochondrial membranes. Within the mitochondrial matrix, $Ca^{2+}$ is heavily buffered by the presence of inorganic phosphates [19-21]. Indeed, the bound:free ratio of $Ca^{2+}$ may be as high as 100,000:1 [19]. The matrix pH was proposed to play the major role in maintaining the low matrix free calcium concentration [19]. So, partial depolarization of mitochondrial membranes is accompanied by a decrease in the matrix pH, which can lead to the breakdown of $Ca^{2+}$-phosphate complexes and an increase in the baseline $Ca^{2+}$ concentration.

*Limitations of the study.* The results presented were obtained on isolated mitochondria and should be continued using other experimental models.

*Conclusions.* 1) Mitochondria incubation in $Mg^{2+}$- and $Mg^{2+}$,ATP-medium with 10 μM thiacalix[4]arene C-1087 is accompanied by an increase of both the baseline $Ca^{2+}$ concentration and accumulation of this cation in the matrix. 2) Mitochondria incubation in $Mg^{2+}$- and $Mg^{2+}$,ATP -medium with 1 and 10 μM thiacalix[4]arene C-1087 did not affect $Ca^{2+}$ efflux from mitochondria. 3) Thiacalix[4]arene C-1087 did not affect the ATP-induced increase both of baseline $Ca^{2+}$ concentration and $Ca^{2+}$ efflux from organelle. 4) Mitochondria incubation with 10 μM thiacalix[4]arene C-1087 resulted in reducing of TMRM-loaded mitochondria fluorescence intensity, that indicates the depolarizing effect of this compound. Overall, the results obtained indicate the possibility of a thiacalix[4]arene C-1087 modulating effect (at 10 μM concentration) on the $Ca^{2+}$ exchange in mitochondria and polarization of its membranes.

*Author contributions.* Sergiy G. Shlykov: Conceptualization, Investigation, Formal analysis, Writing – original draft. Lidiya G. Babich: Conceptualization, Investigation, Formal analysis, Writing – review & editing. Alona I. Panchenko: Investigation, Formal analysis. Roman V. Rodik: Conceptualization, Investigation, Formal analysis, Writing – review & editing. Serhii H. Vyshnevskyi: Investigation, Formal analysis. Vitaliy I. Kalchenko: Conceptualization, Investigation, Formal analysis, Writing – review & editing, Project administration, Funding acquisition. Sergiy O. Kosterin: Conceptualization, Investigation, Formal analysis, Writing – review & editing, Project administration, Funding acquisition.

*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.

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## ВПЛИВ ТІАКАЛІКС[4]АРЕНУ С-1087 НА ОБМІН $Ca^{2+}$ У МІТОХОНДРІЯХ

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**Вступ.** Каліксарени – це супрамолекулярні сполуки з унікальною тривимірною структурою, біологічна активність яких визначається хімічними групами на верхньому або нижньому ободі. Було виявлено, що тіакалікс[4]арен С-1087 селективно (порівняно з іншими АТРазами) пригнічує активність $Ca^{2+}$,$Mg^{2+}$-АТРази плазматичної мембрани клітин міометрія, яка відіграє вирішальну роль у підтримці гомеостазу $Ca^{2+}$. Для розширення нашого розуміння впливу тіакалікс[4]арену С-1087 на обмін $Ca^{2+}$ у клітинах міометрія необхідно проаналізувати його можливий вплив на обмін $Ca^{2+}$ у внутрішньоклітинних пулах, зокрема, в мітохондріях. **Мета.** Метою цього дослідження було з'ясувати, чи впливає тіакалікс[4]арен С-1087 на іонний обмін $Ca^{2+}$ у мітохондріях міометрія. **Методи.** Мітохондрії виділяли з міометрія невагітних щурів. Концентрацію іонізованого кальцію в матриксі мітохондрій визначали за допомогою флуоресцентного зонда Fluo-4 AM. Визначали базову концентрацію $Ca^{2+}$ за відсутності екзогенного (доданого) $Ca^{2+}$ та накопичення $Ca^{2+}$ за його присутності. Концентрацію $Ca^{2+}$ в інкубаційному середовищі визначали за допомогою флуоресцентного зонда Fluo 5F, пентакалієва сіль. Відносне значення мембранного потенціалу мітохондрій ($\Delta\psi$) визначали за допомогою флуоресцентного зонда 100 нМ TMRM. Для обробки даних використовували програмний пакет Microsoft Office Excel. **Результати.** Було доведено, що інкубація мітохондрій у середовищі $Mg^{2+}$- та $Mg^{2+}$,АТР- з 10 мкМ тіакалікс[4]ареном С-1087 супроводжується збільшенням як базової концентрації $Ca^{2+}$, так і накопиченням цього катіона в матриксі. Інкубація мітохондрій у середовищі $Mg^{2+}$- та $Mg^{2+}$,АТР- з 1 та 10 мкМ тіакалікс[4]ареном С-1087 не впливала на вивільнення $Ca^{2+}$ з мітохондрій. Тіакалікс[4]арен С-1087 не впливав на АТР-індуковане збільшення як базової концентрації $Ca^{2+}$, так і на вихід $Ca^{2+}$, з органел. Інкубація мітохондрій з 10 мкМ тіакалікс[4]ареном С-1087 призвела до зниження інтенсивності флуоресценції мітохондрій, навантажених TMRM, що вказує на деполяризуючий ефект цієї сполуки. **Висновки.** Отримані результати вказують на можливість модулюючого впливу тіакалікс[4]арену С-1087 (концентрація 10 мкМ) на обмін $Ca^{2+}$ у мітохондріях та поляризацію їхніх мембран.

Ключові слова: мітохондрії, концентрація $Ca^{2+}$, поляризація мембран мітохондрій, тіакалікс[4]арен С-1087.

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