EXPERIMENTAL WORKS

UDC 577.332.4 doi: https://doi.org/10.15407/ubj90.01.025

CALIX[4]ARENE C-956 IS EFFECTIVE INHIBITOR OF H^+-Ca^{2+}-EXCHANGER IN SMOOTH MUSCLE MITOCHONDRIA

G. V. DANYLOVYCH[1], O. V. KOLOMIETS[1], Yu. V. DANYLOVYCH[1], R. V. RODIK[2], V. I. KALCHENKO[2], S. O. KOSTERIN[1]

[1]Palladin Institute of Biochemistry, National Academy of Science of Ukraine, Kyiv; e-mail: danylovych@biochem.kiev.ua;
[2]Institute of Organic Chemistry, National Academy of Science of Ukraine, Kyiv

It was shown that calix[4]arene C-956 exhibited a pronounced concentration-dependent (10-100 μM) inhibitory effect on the H^+-Ca^{2+}-exchanger of the inner mitochondrial membrane of rat uterine myocytes (K_i 35.1 ± 7.9 μM). The inhibitory effect of calix[4]arene C-956 was accompanied by a decrease in the initial rate (V_0) and an increase in the magnitude of the characteristic time ($\tau_{1/2}$) of the ΔpH-induced Ca^{2+} release. At the same time, it did not affect the potential-dependent accumulation of Ca^{2+} in mitochondria. Thus, the action of calix[4]arene C-956 might be directed on increasing the concentration of Ca ions in the mitochondrial matrix. The calculation of basic kinetic parameters of the Ca^{2+} transport from isolated organelles (in the case of its non-zero stationary level), based on changes in fluorescence of Ca^{2+}-sensitive dye Fluo-4 AM in mitochondria was performed. The proposed approach can be used for the kinetic analysis of the exponential decrease of the fluorescence response of any probes under the same experimental conditions.

K e y w o r d s: calix[4]arenes, H^+-Ca^{2+}-exchanger, mitochondria, smooth muscle, uterus.

Mitochondria play a central role in many fundamental biological processes, such as energy supply of cellular functions, oxidative metabolism, steroidogenesis, programmed cell death, etc. [1-4]. Changes in the Ca^{2+} concentration in mitochondria are major factors in the regulation of biochemical processes in the matrix and the functioning of the electron transport chain. An increase in Ca^{2+} concentration within mitochondrial matrix activates the ATP synthesis and enzymes of the tricarboxylic acid cycle while overloading the organelles with a cation induces cell death. It has been established that mitochondria play an essential role in the intracellular Ca^{2+} signaling in smooth muscle cells owing to their ability to accumulate and release significant amounts of Ca ions [1, 5-7]. The vital task of modern membranology is the search for exogenous non-toxic compounds that could efficiently and selectively regulate Ca^{2+} transport in the inner mitochondrial membrane and, thus, modulate Ca^{2+} homeostasis.

In recent years, much attention has been paid to the supramolecular macrocyclic polyphenol compounds calix[4]arenes, particularly their ability to effectively alter the metabolic, energy and transport processes in subcellular organelles [8]. Modern research is also focused on studying of calix[4]arenes derivatives that specifically affect the cation-transport systems in subcellular structures of smooth muscle. In particular, it has been established that the calix[4]arenes C-97, C-99 and C-107 alter the polarization of the inner membrane, the redox state of purine and flavin nucleotides of the electron transport chain, as well as mitochondrial Ca^{2+}-homeostasis [9, 10].

The mitochondria of uterine smooth muscle cells are high capacious Ca^{2+}-storage units, and able, along with the Ca^{2+}-pumps of the plasma membrane

and sarcoplasmic reticulum, to terminate the Ca^{2+} signal and maintain a low physiological cation concentration in the cytosol [11]. The regulation of the mitochondrial Ca^{2+} concentration is based on the coordinated functioning of systems that support potential-dependent calcium accumulation and cation release from the matrix into the cytosol. Two systems that regulate the Ca^{2+} concentration in the matrix have been identified in the myometrial mitochondria: a Ca^{2+}-uniporter and an H^+-Ca^{2+}-exchanger [11]. The main mechanism for the Ca^{2+} uptake into the mitochondrial matrix is the functioning of the Ca^{2+}-uniporter, whose activity is optimal at micromolar Ca^{2+} concentrations out of mitochondria and which support a potential-dependent (electrophoretic), highly capacious, ruthenium red-sensitive cation accumulation pathway [12]. In smooth muscles, unlike other electroexcitable tissues, Na^+-dependent Ca^{2+} transport from mitochondria is less significant compared to the H^+-Ca^{2+}-exchanger [11], since the myocytes excitation and, hence, the development of the action potential occurs due to not only sodium but also calcium inward currents [13, 14]. It was found that in the sarcolemma of the uterine myocytes Ca^{2+}-independent Mg^{2+}-ATPase, whose function may be a production of H^+ in the myoplasm, exhibited a high activity [15]. These data indicate the primary role of the H^+-Ca^{2+}-exchanger in the mitochondria of the uterus smooth muscle cells. A similar transport system operates in liver and kidney cells [16]. The H^+-Ca^{2+}-exchanger ensure a ΔpH-dependent decrease in Ca^{2+} concentration in the mitochondria. The molecular basis of this process is the protein Mdm38/Letm1 (leucine-zipper-EF hand-containing transmembrane region) of the mitochondrial inner membrane [17]. Overloading with Ca^{2+} and de-energization of mitochondria are accompanied by the opening of a non-specific permeability transition pore through which cation may also be transported [12, 17, 18].

Due to the importance of biochemical processes within mitochondria for the functioning of the whole cell, as well as the direct relationship between mitochondrial dysfunction and the development of smooth muscle pathologies, the search for non-toxic and selective modifiers of the mitochondrial Ca-transport systems is a focus of research interest. The ability of some supramolecular macrocyclic calix[4]-arenes, in particular, C-90 and C-956, to inhibit the activity of the plasma membrane and sarcoplasmic reticulum Ca^{2+}-pumps in smooth muscle draws interest to these compounds as possible regulators of Ca^{2+} homeostasis in myocytes.

Given the above-mentioned, the aim of this work was to study the effect of calix[4]arenes C-90 and C-956 on Ca^{2+}-transport systems in isolated mitochondria of rat uterine smooth muscle.

Materials and Methods

Synthesis of calix[4]arenes. Compounds C-90 (5,11,17,23-tetra(trifluoro)methyl(phenylsulfonylimino)-methylamino-25,26,27,28-tetrapropoxy-calix[4]arene) and C-956 (5.11, 17.23-tetra(trifluoro)methyl (phenylsulfonylimino)-methylamino-25,27-dioctyloxy-26,28-dipropoxy calix[4]arene) were synthesized and characterized using NMR and IR spectroscopy in the Phosphoranes Chemistry Department of the Institute of Organic Chemistry NASU.

Isolation of myometrium mitochondria. The rats were anesthetized by inhalation of diethyl ether and then decapitated. All animal experiments were performed in accordance with the rules and regulations of The European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

Mitochondria were isolated from rat myometrium using differential centrifugation [19]. According to electron microscopy, the isolated mitochondria had round shape of 500 nm in size with cristae, i.e., they had morphological features of intact organelles. Their hydrodynamic diameter measured by photon correlation spectroscopy was 549 ± 20 nm. This value increased significantly to 804 ± 35 nm at mitochondrial inner membrane rupture by antibiotic alamethicin indicating that mitochondria remained intact during isolation. Isolated mitochondria were functionally active and supported energy-dependent Ca^{2+} accumulation in the presence of succinate and Mg-ATP^{2-} [19].

The protein concentration in the mitochondrial fraction was determined by the Bradford assay [20]. Its average value was 2 mg/ml, and in the sample – 25 μg/ml.

Procedure for loading fluorescent probe Fluo-4 AM into mitochondria. The loading fluorescent probe Fluo-4 AM at a final concentration of 2 μM into mitochondria was carried out in a medium containing 10 mM Hepes (pH 7.4; 37 °C), 250 mM sucrose, 0.1% bovine serum albumin for 30 min at 37 °C. To facilitate the loading, the dye was mixed with Pluronic F-127 (0.02%) [19].

Studying ionized Ca^{2+} content in mitochondria by spectrofluorimetry. The assessment of the relative

values of the Ca^{2+} level in the myometrium mitochondrial matrix loaded with Fluo-4 AM (λ_{ex} = 490 nm, λ_{fl} = 520 nm) was performed using Quanta Master 40 PRTI (Canada) and FelixGX 4.1.0.3096 software.

The potential-dependent Ca^{2+} accumulation in mitochondria was carried out in a medium composed of: 20 mM Hepes (pH 7.4, 37 °C), 250 mM sucrose, 2 mM potassium phosphate buffer (pH 7.4, 37 °C), 3 mM $MgCl_2$, 3 mM ATP, 5 mM sodium succinate; the Ca^{2+} concentration was 80 μM.

The ΔpH-induced release of Ca^{2+} after 5th min of their potential-dependent accumulation was triggered by diluting an aliquot of the suspension (100 μl) in a Ca^{2+} release medium (2 ml) composed of: 20 mM Hepes (pH 6.5, 37 °C), 250 mM sucrose, 2 mM potassium phosphate buffer (pH 6.5, 37 °C), 5 mM sodium succinate, 5 μM cyclosporin A.

The Fluo-4 fluorescent signal was presented in relative units as $(F\text{-}F_0)/F_0$, where F_0 is the fluorescence intensity at the start of the experiment. F is the fluorescence intensity recorded as the experiment runs.

The apparent inhibition constant K_i and Hill coefficient were calculated by the Hill method in coordinates $\{(1\text{-}F/F_0)_{control}\text{-}(1\text{-}F/F_0)_{calix})/(1\text{-}F/F_0)_{calix}$; lg [calix[4]arene]}. K – control in the absence of calix[4]arene, F_0 – initial fluorescence, F – fluorescence at appropriate intervals.

The studied calix[4]arenes (10 μl aliquots in DMSO) were introduced directly into the Ca^{2+} accumulation/release medium.

Statistical data analysis was performed with common statistical methods [21] and Student's *t*-test using the IBM standard software.

The following reagents were used in the experiments: Hepes, DMSO, cyclosporin A, sodium succinate, sucrose, ATP, bovine serum albumin (Sigma, USA); Fluo-4 AM, Pluronic F-127 (Invitrogen, USA); mineral salts of domestic production. The solutions were prepared with double distilled water (conductivity under 1.5 μS/cm. The water conductivity was measured by a conductivity meter type OK-102/1 (Hungary).

Results and Discussion

Calix[4]arenes C-90 and C-956 are structural analogs (Fig. 1) that differ in existence of two elongated aliphatic chains in the C-956 structure that facilitates interaction of C-956 with hydrophobic parts of subcellular membranes and penetration into a lipid bilayer.

It was found that calix[4]arene C-956 at a concentration of 100 μM almost completely blocked the ΔpH-induced Ca^{2+} release from isolated rat myometrium mitochondria and did not affect the potential-dependent accumulation of this cation. Its structural precursor, C-90, at the same concentration exhibited slight inhibitory effect ($P < 0.1$) on both Ca^{2+} exchange systems in the inner mitochondrial membrane (Fig. 2).

It was shown that the studied calix[4]arenes markedly affect the Ca^{2+} transport and, respectively, Ca^{2+}-homeostasis in the smooth muscle subcellular organelles [22]. Thus, C-90 inhibited Ca^{2+}, Mg^{2+}-ATPase of the plasma membrane ($I_{0.5}$ = 20 μM) and sarcoplasmic reticulum ($I_{0.5}$ = 57 μM), but did not inhibit other plasma membrane-localized ATP-hydrolases. Treatment uterine myocytes with C-90

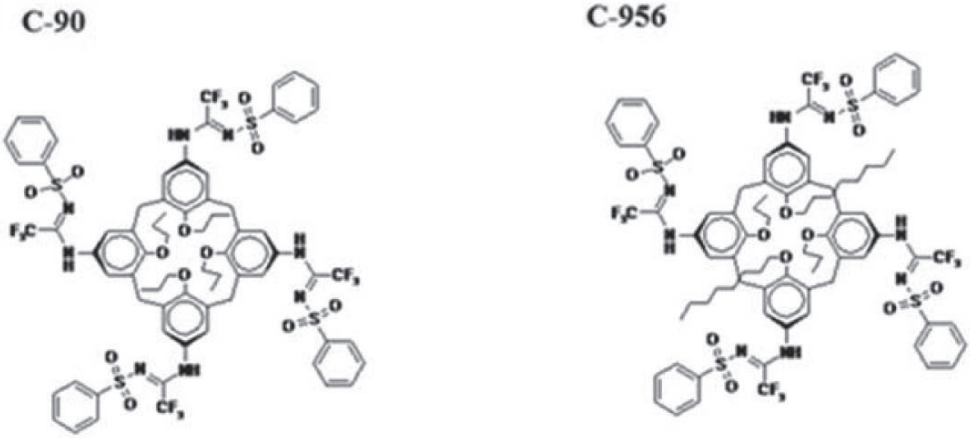

Fig. 1. Structural formulas of the studied calix[4]arenes

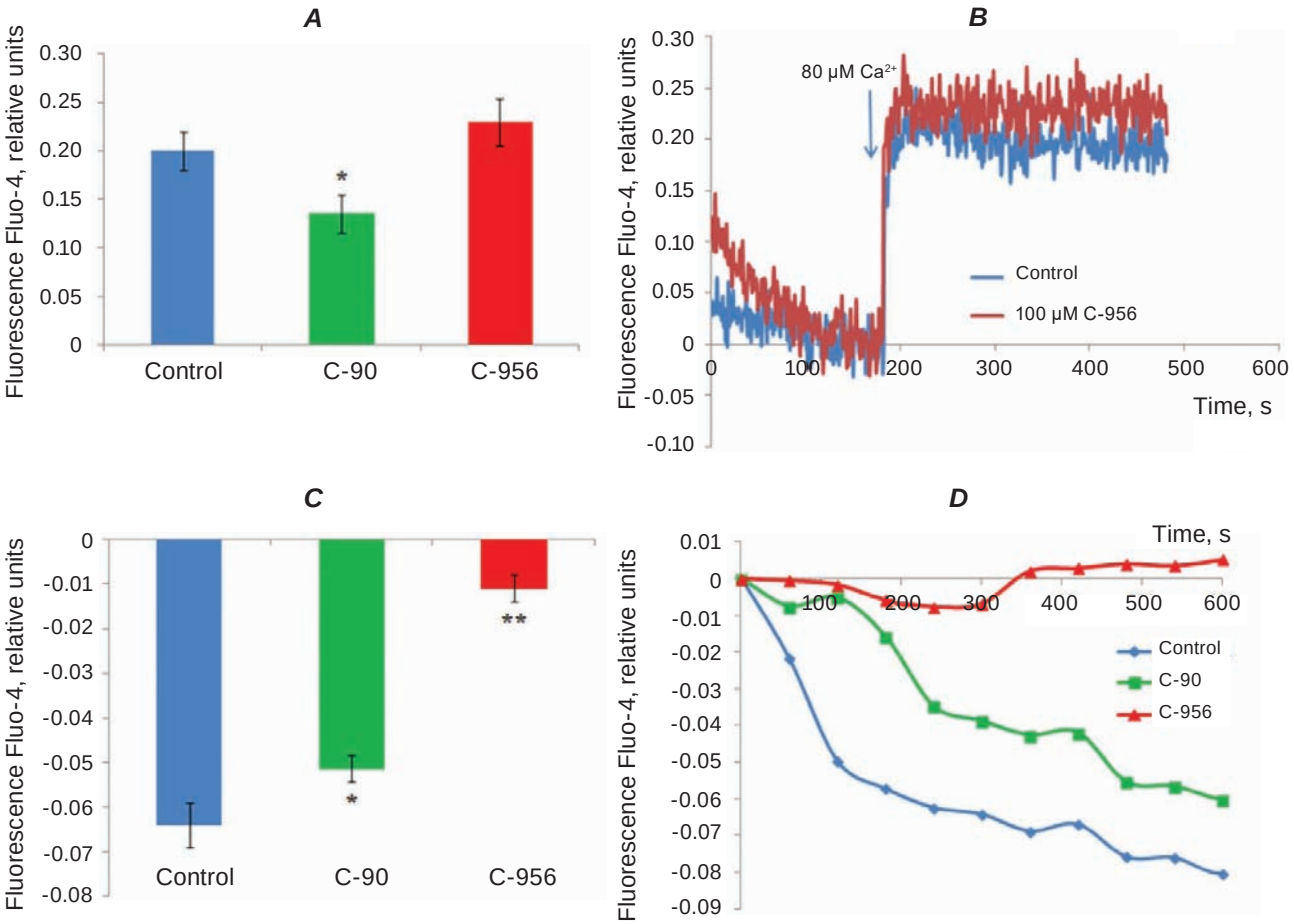

*Fig. 2. Energy-dependent accumulation of Ca^{2+} in isolated rat myometrium mitochondria (**A**, **B**) and ΔpH-induced Ca^{2+} release from them (**C**, **D**) in the presence of calix[4]arenes C-90 and C-956 (100 μM) in the incubation medium. **A**, **C** – statistical processing of the results, * $P < 0.1$, ** $P < 0.05$, relative to control ($M \pm m$, $n = 4$-6); **B**, **D** – the data of a typical experiment*

(20 μM) led to an increase in Ca^{2+} concentration in myoplasm (according to the changes in the fluorescence intensity of the Ca^{2+}-sensitive Fluo-4 AM dye and confocal microscopy), however, the Ca^{2+} concentration decreased over time to basal level indicating a trigger for cellular mechanisms for reducing the level of ionized Ca^{2+} in myoplasm. This phenomenon might be account for the function of mitochondria as a potent Ca^{2+} store and/or Na^+-Ca^{2+}-exchanger of the plasma membrane [23]. These authors showed that calix[4]arene C-956 inhibited the plasma membrane Ca^{2+}, Mg^{2+}-ATPase even more effectively, and caused a transient increase in Ca^{2+} concentration in myoplasm. In view of the results presented in this study, the inhibition of H^+-Ca^{2+}-exchanger by C-956 compound may lead to a shift in the equilibrium in the inner mitochondrial membrane Ca^{2+} transport toward the calcium accumulation and a corresponding decrease in the Ca^{2+} concentration in the myoplasm after the transient increase.

Thus, the results obtained indicate that calix[4]arene C-956 affects the H^+-Ca^{2+}-exchanger of myometrium mitochondria, but not the system of potential-dependent Ca^{2+} accumulation in these subcellular structures.

The unique property of the calix[4]arenes is their specific structure-dependent effect on the cation-transport systems of subcellular organelles. In particular, we showed that the high-affinity inhibitors of Na^+, K^+-ATPase namely, C-97, C-99 and C-107 depolarized the plasma membrane of rat uterine myocytes and transiently hyperpolarized the internal mitochondrial membrane [9]. The latter effect testifies to the penetration of calix[4]arenes into the cell and their direct interaction with mitochondria. The indicated calix[4]arenes were shown to effec-

tively block the potential-dependent Ca^{2+} accumulation and reduce the cation concentration in the myometrium mitochondria, probably, stimulating the H^+-Ca^{2+}-exchanger [10]. In this respect, the effects of mentioned calix[4]arenes, as well as C-956, on the Ca^{2+} transport in mitochondria differ significantly, that may be due to different chemical nature, size, hydrophobicity, charge and relative position of the substituents on the calix[4]arene cup.

We studied the concentration and time dependences of the calix[4]arene C-956 inhibitory effect on the ΔpH-induced Ca^{2+} release from isolated mitochondria in concentration range of 10-100 μM (Fig. 3, in 3 *A* – data are given only for three concentrations)

The calculated apparent inhibition constant K_i for calix[4]arene C-956 effect on the mitochondrial H^+-Ca^{2+}-exchanger was 35.1 ± 7.9 μM, and the Hill coefficient (n_H) was close to 1 (Fig. 3, *B*).

The time dependence of the cation release for both the control and in the presence of calix[4]arene is an exponential curve, and the level of the cation in the matrix, according to the changes in Fluo-4 fluorescence, reaches a constant level and, hence, does not reach zero values (Fig. 4). In the case, when the cation release from the matrix becomes constant over time and does not tend to zero, the calculation of the kinetic parameters of the transport process is not trivial. Therefore, we proposed a methodology for calculating the initial rate (V_0) and the characteristic time ($\tau_{1/2}$) of ΔpH-induced Ca^{2+} release from isolated mitochondria. It is based on the assumption that a decrease in the fluorescence of the Ca^{2+}-sensitive Fluo-4 probe loaded into mitochondria adequately reflects a decrease in the Ca^{2+} concentration in the matrix.

Assuming that F_0 is the initial fluorescence, F is the fluorescence at the appropriate time interval, and F' is the constant level of fluorescence reached over time as a result of the release of a certain amount of Ca^{2+} from the matrix, we obtain the equation:

$$\frac{F}{F_0} = \frac{F'}{F_0} + \left(1 - \frac{F'}{F_0}\right) e^{-kt}, \quad (1)$$

where t – time, k – rate constant.

Particular cases:

1) $t \to \infty$, thus $\frac{F}{F_0} = \frac{F'}{F_0}$, $t \to 0$, thus $\frac{F}{F_0} = 1$

$$\frac{F}{F_0} - \frac{F'}{F_0} = \left(1 - \frac{F'}{F_0}\right) e^{-kt} \quad (2)$$

$$\frac{F - F'}{F_0} = \frac{F_0 - F'}{F_0} e^{-kt} \quad (3)$$

$$\frac{F - F'}{F_0 - F'} = e^{-kt} \quad (4)$$

$$\ln\left(\frac{F_0 - F'}{F - F'}\right) = kt \quad (5)$$

According to the equation (5), we obtain a typical linear dependence in the coordinates {ln ((F_0-F')/(F-F')); t} (Fig. 5).

From the resulting equation, we calculated the characteristic time ($\tau_{1/2}$) and the initial rate V_0 of the ΔpH-induced Ca^{2+} release (equations (6) and (7)):

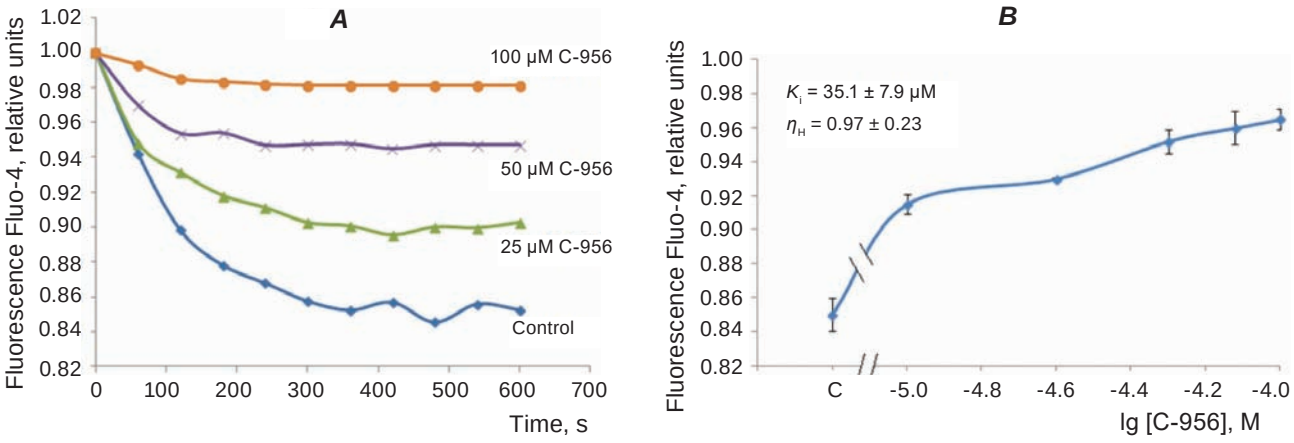

Fig. 3. Concentration and time dependences of calix[4]arene C-956 effect on ΔpH-induced Ca^{2+} release from rat myometrium isolated mitochondria. ***A*** *– the time dependences of Ca^{2+} release at different concentrations of calix[4]arene C-956, the data of a typical experiment (n = 5).* ***B*** *– concentration dependence of the inhibitory effect of calix[4]arene C-956, (M ± m, n = 5).* ***C*** *– control in the absence of calix[4]arene*

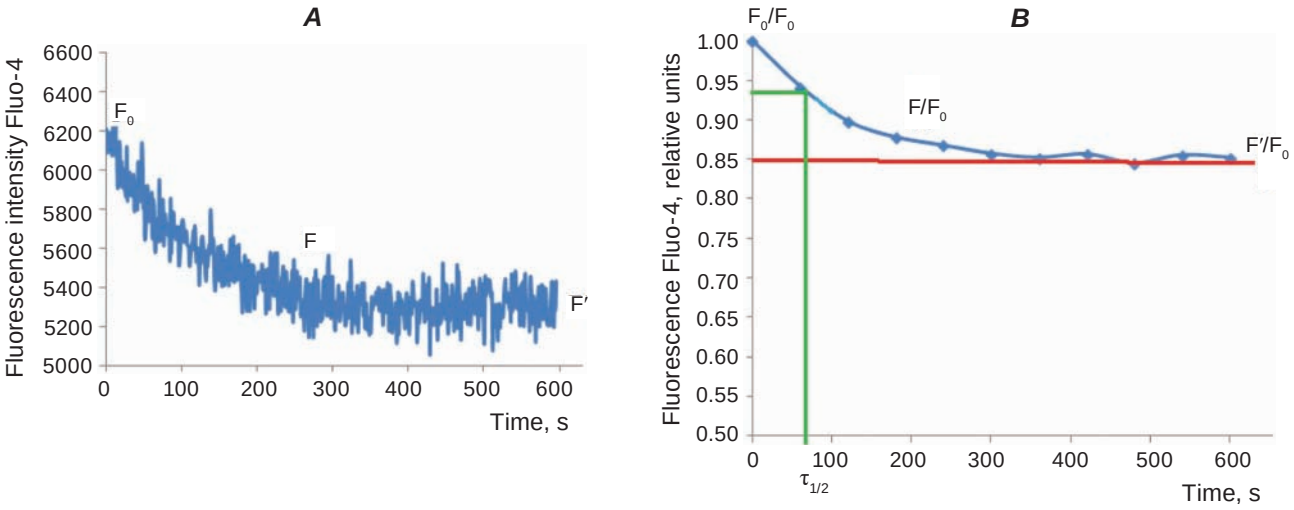

*Fig. 4. The ΔpH-induced Ca^{2+} release from rat myometrium isolated mitochondria. **A** – initial experimental data, spectrofluorometry method, Ca^{2+}-sensitive fluorescence probe Fluo-4; **B** – experimental data (selected points for a certain period of time) are rebuilt in relative units of fluorescence F/F_0, ∂e F_0 – initial fluorescence, F – fluorescence at appropriate intervals, F′ – stationary fluorescence level*

$$\tau_{1/2} = \frac{ln2}{k} \quad (6)$$

$$V_0 = k\left(\frac{F_0 - F'}{F_0}\right) \quad (7)$$

We performed calculations with the use of equations (6) and (7), and obtained the corresponding dependences (Fig. 6).

The calix[4]arene C-956 was found to reduce the initial rate V_0 (i.e., the number of the H^+-Ca^{2+}-exchanger cycles) and increase the characteristic time $\tau_{1/2}$ of ΔpH-induced Ca^{2+} release in a concentration-dependent manner (10-100 μM) (Fig. 6).

Our previous results [19] demonstrated that the H^+-Ca^{2+}-exchanger of the inner mitochondrial membrane of myometrium cells is an electrogenic system involved in calcium ions transport in the stoichiometric ratio $1H^+ : 1Ca^{2+}$. Plasma membrane Ca^{2+}, Mg^{2+}-ATPase – another subcellular transport system of uterine smooth muscles – also functions in the electrogenic mode and can transport one Ca ion from the cell in exchange for H^+ [24]. In each case, H^+ can be the counter-ion upon Ca^{2+} transport across the membrane. Taking into account that both systems are highly sensitive to the inhibitory effect of C-956 and have similar inhibitory constants (tens of μM) [23], it can be suggested that the calix[4]arene C-956 targets specifically at the H^+-transport component of these systems. More detailed studies of the transporter structure along with further research of the mechanisms for the C-956 inhibitory effect can support or disprove our assumption.

Thus, the results obtained indicate the following: calix[4]arene C-956 (derivative of calix[4]arene C-90) in the range of micromolar concentrations exhibited a pronounced inhibitory effect on the H^+-Ca^{2+}-exchanger, which was a decrease in the initial rate V_0 and, as a result, an increase in the characteristic time $\tau_{1/2}$ of ΔpH-induced Ca^{2+} release from mitochondria. Though, C-956 did not affect

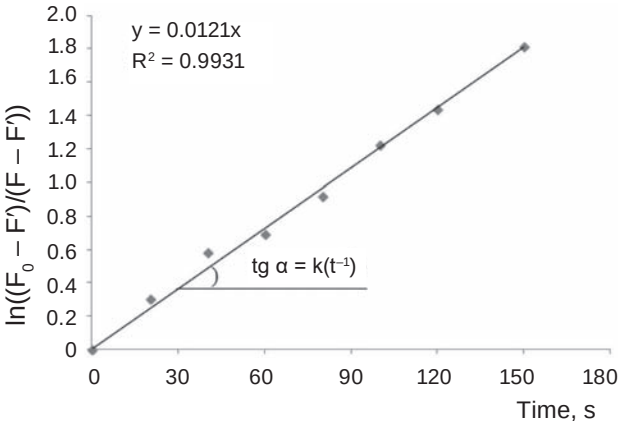

Fig. 5. The linear dependence of changes in the logarithmic value of the relative fluorescence signal Fluo-4 in mitochondria on time was subsequently used to calculate the constant of the rate of ΔpH-induced Ca^{2+} release from mitochondria. The data of a typical experiment

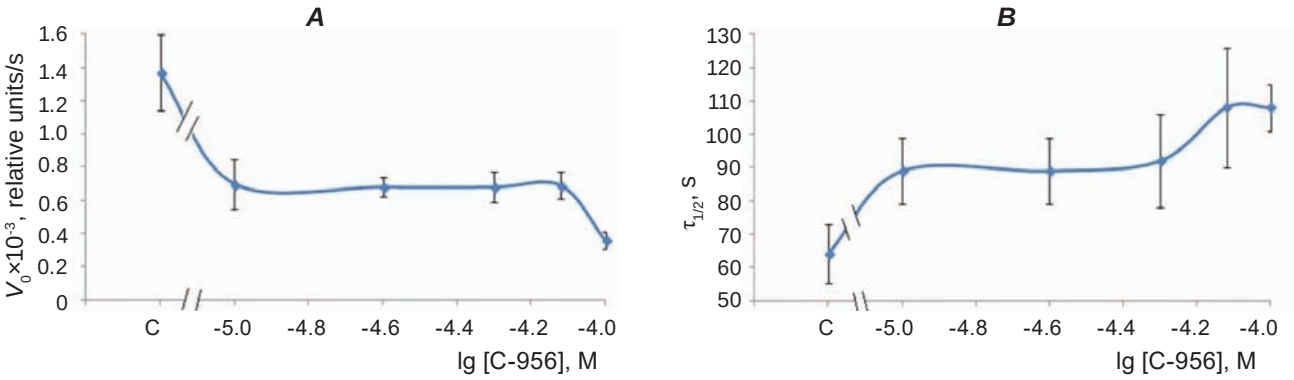

*Fig. 6. The dependence of the initial rate V_0 (**A**) and the values of the characteristic time $\tau_{1/2}$ (**B**) of the ΔpH-induced Ca^{2+} release from isolated rat myometrium mitochondria on the concentration of calix[4]arene C-956. C – control in the absence of calix[4]arene (M ± m, n = 5)*

the potential-dependent accumulation of the calcium ions by these subcellular organelles. The methodology for calculating the basic kinetic parameters of the Ca^{2+} transport from isolated mitochondria, in the case when the transport process reaches a nonzero constant level, was proposed. This approach can be used for the kinetic analysis of the exponential decrease in the fluorescent response of any probe.

This work was financed by the target complex multidisciplinary research program of the NAS of Ukraine "Molecular and cellular biotechnologies for the needs of medicine, industry and agriculture" (State Registration No. 0115U003639).

КАЛІКС[4]АРЕН С-956 – ЕФЕКТИВНИЙ ІНГІБІТОР H^+-Ca^{2+}-ОБМІННИКА В МІТОХОНДРІЯХ ГЛАДЕНЬКОГО М'ЯЗА

Г. В. Данилович[1], О. В. Коломієць[1], Ю. В. Данилович[1], Р. В. Родік[2], В. І. Кальченко[2], С. О. Костерін[1]

[1]Інститут біохімії ім. О. В. Палладіна НАН України, Київ;
e-mail: danylovych@biochem.kiev.ua;
[2]Інститут органічної хімії НАН України, Київ

Показано, що калікс[4]арен С-956 виявляв виражений концентраційнозалежний (10–100 мкМ) гальмівний вплив на H^+-Ca^{2+}-обмінник внутрішньої мітохондріальної мембрани міоцитів матки щурів (K_i 35,1 ± 7,9 мкМ). Інгібувальний ефект калікс[4]арену С-956 супроводжувався зниженням початкової швидкості V_0 та збільшенням величини характеристичного часу $\tau_{1/2}$ ΔpH-індукованого виходу Ca^{2+}. Водночас зазначений калікс[4]арен не впливав на енергозалежну акумуляцію Ca^{2+} мітохондріями. Отже, дія калікс[4]арену може бути спрямована на зростання концентрації Ca^{2+} в матриксі мітохондрій. За змінами флуоресценції Ca^{2+}-чутливого барвника Fluo-4 в мітохондріях, що відображають транспорт Ca^{2+} з ізольованих органел, запропоновано розрахунок основних кінетичних параметрів транспортного процесу для випадків ненульового стаціонарного рівня. Такий підхід можна застосовувати для кінетичного аналізу експоненціального зниження флуоресцентної відповіді будь-якого зонда за схожих експериментальних умов.

Ключові слова: калікс[4]арени, H^+-Ca^{2+}-обмінник, мітохондрії, гладенькі м'язи, матка.

КАЛИКС[4]АРЕН С-956 – ЭФФЕКТИВНЫЙ ИНГИБИТОР H^+-Ca^{2+}-ОБМЕННИКА В МИТОХОНДРИЯХ ГЛАДКОЙ МЫШЦЫ

А. В. Данилович[1], О. В. Коломиец[1], Ю. В. Данилович[1], Р. В. Родик[2], В. И. Кальченко[2], С. А. Костерин[1]

[1]Институт биохимии им. А. В. Палладина НАН Украины, Киев;
e-mail: danylovych@biochem.kiev.ua;
[2]Институт органической химии НАН Украины, Киев

Показано, что каликс[4]арен С-956 имел выраженный концентрационнозависимый (10–100 мкМ) ингибиторный эффект на H^+-Ca^{2+}-обменник внутренней митохондриальной мембраны миоцитов матки крыс (K_i 35,1 ± 7,9 мкМ). Ингибиторный эффект каликс[4]арена С-956 сопровождался снижением начальной скорости (V_0) и увеличением величины характеристического времени ($\tau_{1/2}$) ΔpH-индуцированного выхода Ca^{2+}. В то же время исследуемый каликс[4]арен не влиял на энергозависимую аккумуляцию Ca^{2+} митохондриями. Таким образом, действие каликс[4]арена может быть направлено на увеличение концентрации Ca^{2+} в матриксе митохондрий. По изменениям флуоресценции Ca^{2+}-чувствительного зонда Fluo-4 в митохондриях, отображающих транспорт Ca^{2+} из изолированных органел, предложен расчет основных кинетических параметров транспортного процесса для случаев ненулевого стационарного уровня. Данный подход можно использовать для кинетического анализа экспоненциального снижения флуоресцентного ответа любого зонда при подобных экспериментальных условиях.

Ключевые слова: каликс[4]арены, H^+-Ca^{2+}-обменник, митохондрии, гладкие мышцы, матка.

References

1. Kostyuk PG, Kostyuk OP, Lukyanets EA. Intracellular calcium signaling: structures and functions. Kyiv: Naukova Dumka, 2010. 175 p. (In Ukrainian).
2. Bernardi P, Rasola A. Calcium and cell death: the mitochondrial connection. *Subcell Biochem.* 2007; 45: 481-506.
3. Graier WF, Frieden M, Malli R. Mitochondria and Ca^{2+} signaling: old quests, new functions. *Eur J Physiol.* 2007; 455(3): 375-396.
4. Mitochondrial signaling in health and disease. Eds. S. Orrenius, L. Packer, E. Cadenas. N.Y.: CRC Press, 2012. 493 p.
5. Szabadkai G, Duchen MR. Mitochondria: the hub of cellular Ca^{2+} signaling. *Physiology (Bethesda).* 2008; 23(2): 84-94.
6. Chalmers S, Nicholls DG. The relationship between free and total calcium concentrations in the matrix of liver and brain mitochondria. *J Biol Chem.* 2003; 278(21): 19062-19070.
7. Takeuchi A, Kim B, Matsuoka S. The destiny of Ca(2+) released by mitochondria. *J Physiol Sci.* 2015; 65(1): 11-24.
8. Giuliani M, Morbioli I, Sansone F, Casnati A. Moulding calixarenes for biomacromolecule targeting. *Chem Commun (Camb).* 2015; 51(75): 14140-14159.
9. Danylovych GV, Danylovych YuV, Kolomiets OV, Kosterin SO, Rodik RV, Cherenok SO, Kalchenko VI, Chunikhin AJu, Gorchev VF, Karakhim SA. Changes in polarization of myometrial cells plasma and internal mitochondrial membranes under calixarenes action as inhibitors of plasma membrane Na^+, K^+-ATPase. *Ukr Biokhim Zhurn.* 2012; 84(6): 37-48. (In Ukrainian).
10. Danylovych HV, Danylovych YuV, Rodik RV, Kalchenko VI, Chunikhin AJu. Calix[4]arenes as modulators of energy-dependent Ca^{2+}-accumulation and functioning of the electron transport chain in smooth muscle mitochondria. *Fisiol Zh.* 2016; 62(5): 27-36. (In Ukrainian).
11. Kolomiets OV, Danylovych YuV, Danylovych HV, Kosterin SO. Ways and mechanisms of transmembrane exchange of Ca^{2+} in mitochondria. *Fisiol Zh.* 2017; 63(4): 87-104. (In Ukrainian).
12. Csordás G, Várnai P, Golenár T, Sheu SS, Hajnóczky G. Calcium transport across the inner mitochondrial membrane: molecular mechanisms and pharmacology. *Mol Cell Endocrinol.* 2012; 353(1-2): 109-113.
13. Yoshino M, Wang SY, Kao CY. Sodium and calcium inward currents in freshly dissociated smooth myocytes of rat uterus. *J Gen Physiol.* 1997; 110(5): 565-577.
14. Seda M, Pinto FM, Wray S, Cintado CG, Noheda P, Buschmann H, Candenas L.

Functional and molecular characterization of voltage-gated sodium channels in uteri from nonpregnant rats. *Biol Reprod.* 2007; 77(5): 855-863.

15. Kosterin SA. The possible H^+-dependent functional connection between cell membrane and mitochondria in smooth muscle cells. *Ukr Biokhim Zhurn*. 1998; 70(6): 152-160. (In Russian).
16. Vovkanich LS, Dubitsky LO. Kinetic properties of H^+-stimulated exit of Ca^{2+} from liver mitochondria. *Exp Clin Physiol Biochem.* 2001; 3(15): 34-37. (In Ukrainian).
17. Jiang D, Zhao L, Clapham DE. Genome-wide RNAi screen identifies Letm1 as a mitochondrial Ca^{2+}/H^+ antiporter. *Science*. 2009; 326(5949): 144-147.
18. Santo-Domingo J, Wiederkehr A, De Marchi U. Modulation of the matrix redox signaling by mitochondrial Ca^{2+}. *World J Biol Chem*. 2015; 6(4): 310-323.
19. Kolomiets OV, Danylovych YuV, Danylovych HV, Kosterin SO. Ca^{2+}/H^+-exchange in myometrium mitochondria. *Ukr Biochem J.* 2014; 86(3): 41-48. (In Ukrainian).
20. 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: 248-254.
21. Applied Statistics in Biological Research. Basic concepts, related problems and common methods / Dr. Karin Schmid. CSF vienna BIOCENTER. 2014/ http://www.vbcf.ac.at/fileadmin/user_upload/BioComp/training/Applied_Stat_Intro_Script.pdf
22. Veklich TO. The inhibitory influence of calix[4]-arene of C-90 on the activity of Ca^{2+},Mg^{2+}-ATPases in plasma membrane and sarcoplasmic reticulum in myometrium cells. *Ukr Biochem J.* 2016; 88(2): 5-15.
23. Veklich T, Shkrabak O, Nikonishyna Yu. Calix[4]aren C-956 selectively inhibits Ca^{2+},Mg^{2+}-ATPases of plasma membrane. The 4th international scientific conference current problems of biochemistry and cell biology. Program and abstracts. 5-6 October, 2017. Dnipro, Ukraine: 233-234. (In Ukrainian).
24. Babich LG, Fomin VP, Kosterin SA. Effect of the membrane potential on the Mg^{2+},ATP-dependent transport of Ca^{2+} across smooth muscle sarcolemma. *Biokhimiia*. 1990; 55(10): 1890-1901. (In Russian).

Received 29.11.2017