REVIEW

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DEPARTMENT OF MUSCLE BIOCHEMISTRY: CALIXARENES AS MODULATORS OF ENERGY-DEPENDENT Ca^{2+}-TRANSPORTING PUMPS IN SMOOTH MUSCLES

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Dedicated to dear colleagues
and friends of all generations,
who have worked and are working
at the Department of Muscle Biochemistry

In this scientific-historical review devoted to the recent achievements of the Muscle Biochemistry Department of the Palladin Institute of Biochemistry, the NAS of Ukraine, we synthesize findings from interdisciplinary investigations of intracellular calcium homeostasis in smooth muscle (exemplified by the myometrium) conducted at the interface of biochemistry, physical and organic chemistry, biophysics, and mathematical/computational modeling. We emphasize that the selected calix[4]arenes considered here act selectively as inhibitors of the Mg^{2+},ATP-dependent calcium and sodium pumps – ion-transporting ATPases (electroenzymes Ca^{2+},Mg^{2+}-ATPase and Na^{+},K^{+}-ATPase) – of the plasma membrane of smooth-muscle cells, enabling controlled modulation of intracellular Ca^{2+} homeostasis and the contractile activity of the myometrium. The data obtained also indicate that the selected calix[4]arenes can be regarded as compounds suitable for efficient investigation of mitochondrial function in smooth-muscle cells, in particular the mechanisms of transmembrane Ca^{2+} exchange, the principles governing membrane-potential formation, and the contribution of these subcellular structures to the control of the mechanokinetics of the contraction–relaxation cycle. It is shown that some calix[4]arenes act as effectors of the ATPase activity of contractile proteins and protect this activity from the inhibitory influence of heavy-metal ions. Taken together, these results outline biochemical approaches to the fine regulation of calcium fluxes and smooth-muscle contractility and underscore the potential of calix[4]arenes as selective "molecular platforms" useful for addressing fundamental and applied (biomedical) problems in contemporary physico-chemical muscle biology.

K e y w o r d s: smooth muscle; myometrium; Ca^{2+},Mg^{2+}-ATPase; Na^{+},K^{+}-ATPase; calix[4]arenes; plasma membrane; mitochondria; Ca^{2+} signaling; spontaneous contractions.

Palladin Institute of Biochemistry celebrates its significant anniversary – 100 years since the day of its foundation. The studies of muscle biochemistry have been a classic direction in the scientific research of the Institute practically since the very first years of its activity.

All muscles are known to be divided into three large groups: 1) skeletal or striated muscles, which perform a locomotor function and constitute a part of the musculoskeletal system; 2) myocardium (the cardiac muscle) which actually is a muscle pump, ensuring the pumping of blood; 3) smooth (non-striated) muscles of internal organs, innervated by the vegetative nervous system, i.e. their contractions occur spontaneously and cannot be regulated consciously. These muscles ensure the functioning of internal organs and their systems. First and foremost, these are vascular, respiratory, lymphatic, urinary,

genital systems, gastrointestinal tract, gland ducts of external and internal secretion, sphincter muscle of the pupil, etc.

From the thermodynamic standpoint, the performance coefficient (PC) of muscles is rather high, reaching 20–30% on average, but there are unique cases in nature, e.g., the performance coefficient of the skeletal muscle of a turtle can be as high as 65–75%.

The fundamental contribution to solving the problem of muscle biochemistry was made by a prominent scientist and founder of our Institute, the full member of the NASU, O.V. Palladin, and his disciples and colleagues – the corresponding member of the NASU D.L. Ferdman, the full members of the NASU M. F. Hulyi and R. S. Chagovets. The studies in the field of muscle biochemistry were initiated by O.V. Palladin in the Department of Physiological Chemistry of the Kharkiv Medical Institute in 1921–1922. Mostly, these were related to the investigation of the chemical dynamics of muscle contractions and the biochemical aspects of muscles being tired or trained. The Department of Muscle Biochemistry was founded in our Institute (as a laboratory, at first) in May 1944. Till January 1970, the organizer and irreplaceable head of the Department was a leading biochemist, the corresponding member of the AS of USSR, Doctor of Biosciences, Professor D.L. Ferdman. From January 1970 till May 1973, the duties of the head of the Department were fulfilled by Ph.D. in Biosciences, V.A. Hryhorieva, and from May 1973 till July 1996, the Department was headed by Doctor of Biosciences, Professor M.D. Kurskyi. In September 1988, after the staff reorganization of the Department of Muscle Biochemistry, pursuant to the decision of the Scientific Council of the Institute, a new department was established – the Department of Biochemical Kinetics (the head – Doctor of Biosciences S.O. Kosterin, now the full member of NAS of Ukraine, Professor), the specialists of which studied the dynamic regularities of the transmembrane exchange of Ca ions in smooth muscle cells, and in July 1996, after the structural reorganization of the Institute, pursuant to the decision of the Scientific Council, the Department of Muscle Biochemistry was disbanded, while the Department of Biochemical Kinetics was renamed as the Department of Muscle Biochemistry. So, one may assume that the Department of Muscle Biochemistry has been working in the Institute for almost 80 years.

At the stages of its genesis, the scientific achievements of the Department in the sphere of classic muscle biochemistry were first and foremost associated with the creative activity of the corresponding member of the AS of USSR, Professor D.L. Ferdman, Professor M.D. Kurskyi, Professor H.I. Sylakova, Doctor of Biosciences S.F. Epstein, Doctor of Biosciences Z.Yu. Nechyporenko, Ph.D. in Biosciences V.A. Hryhorieva, Ph.D. in Biosciences O.N. Medovar. Later, O.M. Fedoriv, N.H. Himmelreikh, H.P. Diadiusha, V.Y. Kocherha, M.P. Dmytrenko, V.A. Tuhai, H.O. Pkhakadze, N.M. Slinchenko, O.L. Konoplytska, K.L. Sanina, O.M. Bukhanevych, O.O. Lytvynenko, V.B. Piskariov, L.I. Meshkova came to the Department. And then the Department was joined by the researchers of the new generation – S.O. Kosterin, T.P. Kondratiuk, H.A. Osypenko, H.M. Popova, Z.D. Vorobets, I.I. Romas, L.H. Babich, S.H. Shlykov, V.P. Fomin, N.M. Shevchuk, T.I. Polishchuk, H.O. Popenkova, N.F. Bratkova, I.B. Chervonenko, L.O. Pryshchepa, S.M. Marchenko, V.P. Zimina, O.Ya. Shaturskyi,

D.L. Ferdman

V.A. Hryhorieva

M.D. Kurskyi

S.O. Kosterin

O.V. Titus, F.V. Burdyga, L.A. Borysova, Yu.V. Danylovych, O.B. Vadziuk, T.O. Veklich, H.V. Danylovych, R.D. Labyntseva, T.T. Taran, T.V. Ulianenko, I.H. Chernysh, O.P. Shynlova, A.P. Chornyi, O.A. Shkrabak, A.A. Bevza, N.V. Naumova, N.A. Rovenets, Yu.Yu. Mazur, V.F. Horchev, S.O. Karakhim, O.Yu.Chunikhin, O.V. Tsymbaliuk and others.

Throughout the history of the Department, its specialists paid the main attention to the following issues:

– investigating carbohydrate-phosphorus and nitrogen metabolism in muscles, studying their bioenergy, studying the processes of adenine nucleotide conversion and ammonia formation in muscle tissue (D.L. Ferdman, Z.Yu. Nechyporenko, N.H. Himmelreikh, H.I. Silakova, S.F. Epstein, M.P. Dmytrenko, M.D. Kurskyi and others);

– detecting the specificities of metabolism under muscle pathology (D.L. Ferdman, V.A. Hryhorieva, H.I. Silakova, S.F. Epstein, M.D. Kurskyi, O.N. Medovar, H.O. Pkhakadze and others);

– studying the biochemical and physical-chemical properties of energy-dependent membrane-bound Ca^{2+}-transporting and contractile proteins of muscle cells, investigating the impact of various effectors on the activity of enzymes, including ATP-hydrolases in muscles (M.F. Kurskyi, S.O. Kosterin, H.I. Sylakova, V.A. Hryhorieva, O.M. Fedorov, N.H. Himmelreikh, H.P. Diadiusha, V.Y. Kocherha, M.P. Dmytrenko, V.A. Tuhai and others).

However, it is noteworthy that since the 1970s-1980s, scientific research in the field of biochemical membranology of muscles has become the dominant direction in the Department.

At present, the Department is proud to have one full member of the NAS of Ukraine, 7 Doctors of Science, 3 Ph.D., 4 engineers, and 2 postgraduates.

A detailed scientific and historical description of the achievements of the Department in Muscle Biochemistry from the day of its foundation till 1975 was presented in the article of M.D. Kurskyi in the monograph "The O.V. Palladin Institute of Biochemistry" (Kyiv: "Naukova Dumka", 1975. 210 p.), published on the 50th anniversary of the Institute. The results, obtained in the field of muscle biochemistry from 1975 till 1995, were highlighted in the articles of M.D. Kurskyi and S.O. Kosterin ("The Department of Muscle Biochemistry" and "The Department of Biochemical Kinetics", respectively), published in the jubilee issue of the Ukrainian Biochemical Journal, dedicated to the 70th anniversary of the Institute (1995, V. 67, No. 3, pp. 59-69 and 70-75, respectively). And, finally, the results in the area of biochemistry and biophysical chemistry of muscle cells, obtained by the scientists of the Department in 1995–2005, were highlighted by S.O. Kosterin in his essay "The Department of Muscle Biochemistry" in the monograph "The O.V. Palladin Institute of Biochemistry, 1925–2005" (Kyiv: "Alfa-Prime", 2005. 496 p.).

This article presents the laconic analysis and summary of selected experimental results, obtained in the Department of Muscle Biochemistry in 2005–2025, mostly in the field of studying the urgent issues of biochemistry and biophysical chemistry of smooth muscle cells with the focus on the investigation of the systems of energy-dependent transmembrane transfer of Ca ions therein.

I. Some general provisions

There are undoubted reasons to believe that the twenty-first century will be the century of transdisciplinary research in the field of natural sciences. Indeed, our *Nature* is *the only one* we have! It "does not know" that we differentiate our approaches to its cognition into *Biology*, *Chemistry*, and *Physics* according to our education, knowledge, interests, focus on solving specific scientific problems, and preferences. It is quite obvious that the most interesting problems of modern *Nature study* are localized at

Borys Paton, the President of the NAS of Ukraine in 1962–2020, the full member of the NAS of Ukraine, is awarding Serhii Kosterin, the full member of the NAS of Ukraine, with the award of the Honoured Worker of Science and Technology of Ukraine, 2015

the "crossroads" of various sciences and scientific fields. How else can one explain the fact that in recent years there has been a progressive development of such "crossover" sciences and scientific trends as biophysical chemistry, physical biochemistry, chemical biophysics, biochemical physics, physical and chemical biology, physics of the living, mathematical biophysics, molecular physiology, theoretical biology, bioinformatics, artificial intelligence in biology and medicine, etc. A transdisciplinary approach envisages a synergistic combination of theoretical and experimental methodologies of different sciences, going beyond the scope of individual disciplines. This approach is usually based on the use of mathematical models.

One of the classic objects of transdisciplinary biochemical and biophysical studies is muscles. A smooth muscle cell can indeed be interpreted as an open, receptor, uneven, tensoelectromechanochemical, practically isothermic, thermodynamic system, capable of Ca^{2+}-dependent contraction.

It is well known that the concentration of Ca ions in the extracellular space $[Ca^{2+}]_e$ is ~ 1-3 mM, and in an unexcited cell, the concentration of these ions $[Ca^{2+}]_i$ ~ 100 nM, which, considering the value of electric potential $\Delta\Psi$ on the plasma membrane, corresponds to the value of Gibbs energy ΔG_{PM} for the transmembrane calcium gradient, aimed into the cell, $\Delta G_{PM} = RT\ln\{[Ca^{2+}]_e/[Ca^{2+}]_i\}+2F\Delta\Psi$ = 40 kilojoule/mol (R – universal gas constant, T – absolute temperature, °K, F – Faraday constant). In case of excitation, Ca ions energy-independently penetrate the cytoplasm via various calcium channels of the plasma membrane according to the abovementioned transmembrane calcium gradient, due to which the concentration of Ca^{2+} in the myocyte increases, approximating 1 μM. This increase in the concentration of Ca ions in a muscle cell from 100 nM to 1 μM induces a series of complex biochemical processes which result in launching the process of a smooth muscle contraction. On the other hand, to ensure the relaxation of smooth muscles, nature envisaged the presence of very important energy-dependent Ca^{2+}-transporting systems, localized in the plasma membrane – Mg^{2+},ATP-dependent calcium pump (electroenzyme Ca^{2+},Mg^{2+}-ATPase) and Na^+-Ca^{2+}-exchanger, which remove Ca ions from the cell against the electrochemical calcium gradient and control the process of muscle relaxation. In addition, the smooth muscle cell has peculiar intracellular calcium depots, where Ca^{2+} gets accumulated and reversely released. The role of such storages of Ca ions is played by intracellular organelles – sarcoplasmic reticulum (which has its own localized Mg^{2+},ATP-dependent calcium pump that differs in its properties from Mg^{2+},ATP-dependent calcium pump of the plasma membrane), and mitochondria (with the localized Ca^{2+}-uniporter, ensuring the electrophoretic accumulation of Ca ions in the mitochondrial matrix, as well as $H^+(Na^+)$-Ca^{2+} exchanger). So, the complex nature of the control mechanisms for Ca^{2+} concentration in the smooth muscle cell in the dynamics of the contraction-relaxation process is evident. In principle, intracellular calcium homeostasis can be viewed as a general biological phenomenon with the properties of a cooperative, non-linear, non-additive, synergistic phenomenon, which is characterized by a network of various positive and negative feedback.

It will be demonstrated later how, using the transdisciplinary experimental and theoretical approaches, one can study the biochemical mechanisms of Ca^{2+}-dependent regulation of contractibility of smooth muscles (using the example of a smooth muscle of the uterus – myometrium) and the regularities of the targeted modulation of this regulation by macrocyclic compounds – calixarenes as effectors of the systems of energy-dependent transportation of Ca ions.

Generally, the search for molecules, capable of targeted "attacking" specific Ca^{2+}-transporting proteins, increasing or decreasing their functional activity, is an extremely important task in fighting pathologies under which the functioning of these proteins is impaired. From this standpoint, the calixarenes we have been working with for quite a long time have proven to be rather interesting and promising objects.

It should be highlighted that we have conducted our studies in systemic, close, and mutually beneficial creative cooperation with the scientific laboratory of V.I. Kalchenko, the full member of the NAS of Ukraine (the Institute of Organic Chemistry, the NAS of Ukraine), under whose guidance calixarenes were synthesized and their physical-chemical and chemical properties were studied. In addition, while studying the dynamics of Ca^{2+}-dependent contraction of smooth muscles, the Department of Muscle Biochemistry has been actively cooperating with Professor O.V. Tsymbaliuk from the Institute of High Technologies of the Taras Shevchenko National University of Kyiv. The elaboration of mathematical models has been done in active cooperation between

our Department and Professor P.F. Zhuk, Doctor of Sciences in Physics and Mathematics from the National Aviation University.

The transdisciplinary approach provided for our organic and efficient combination of biochemical, physical-chemical, physical, and mathematical methods of investigation in our work on the study of the functioning of energy-dependent Ca^{2+}-transporting proteins of smooth muscle cells. These are the methods of preparative and analytical biochemistry, biochemical membranology, enzymology, isotope technique ($^{45}Ca^{2+}$), photon correlation spectroscopy, spectrophotometry, spectrofluorimetry, flow cytofluorometry, light and electron microscopy, confocal microscopy, mass-spectrometry, computerized docking-modelling and molecular dynamics, chemical kinetics and thermodynamics, biochemical kinetics, mathematical modelling. Our colleagues from the Institute of Organic Chemistry of the NAS of Ukraine, where calixarenes were synthesized, used the methods of organic synthesis, nuclear magnetic resonance, infrared spectroscopy, reversed-phase high-performance liquid chromatography, and toxicology. The method of tensometry and mechanokinetics were applied at the Institute of High Technologies of the Taras Shevchenko National University of Kyiv.

We managed to achieve relevant results while studying the transmembrane exchange of Ca ions in the smooth muscles (on the example of myometrium) in the experiments using various (biochemical, first and foremost) experimental models, such as solubilized Ca^{2+}-transporting proteins; reconstructed Ca^{2+}-transporting proteins (proteoliposomes); isolated contractile proteins and their fragments; isolated subcellular membrane structures (fragments of plasma membrane, sarcoplasmic reticulum, mitochondria); suspensions of intact smooth cells and those, permeabilized with digitonin, – myocytes. Some studies were conducted using isolated smooth muscle stripes and intact animals (rats).

As stated above, we also actively used the method of scanning laser confocal microscopy in our experimental studies. For instance, we investigated the changes in Ca^{2+} concentration, induced by uterotonic peptide hormone oxytocin (so-called "calcium transient") in myometrium cells. In these experiments, myocytes were previously burdened with Ca^{2+}-sensitive fluorescent probe Fluo-4AM. As seen in Fig. 1, at the effect of oxytocin (concentration of 100 nM), there is an increase in the concentration of Ca ions in the cell in time (due to the uptake of Ca^{2+} into the cell via calcium channels of the plasma membrane), followed by relaxation ("termination")

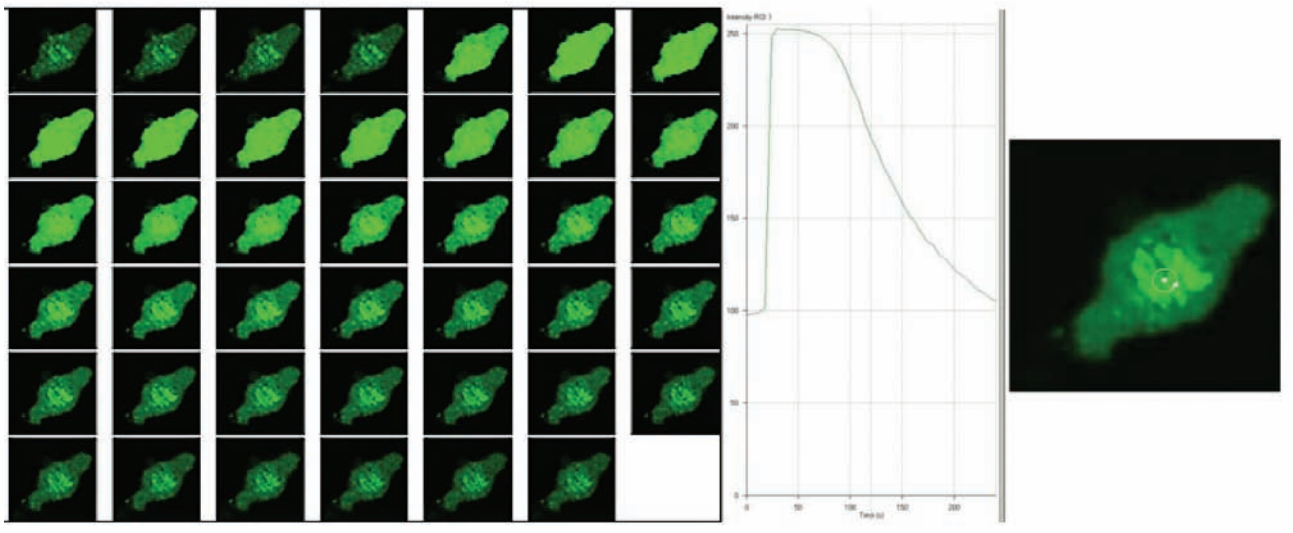


Fig. 1. The dynamic change in Ca^{2+} concentration in the myometrium cell of rats, induced by oxytocin. The method of scanning laser confocal microscopy, the Ca^{2+}-sensitive fluorescent probe Fluo-4AM was used. On the left panel each shot (from the left to the right) corresponds to the increase in the temporal coordinate. The aliquot of the oxytocin solution (100 nM) was added at the level of shot 4 (upper series of shots). The right panel demonstrates the kinetics of changes in the relative fluorescence response of the Ca^{2+}-sensitive fluorescent probe Fluo-4 in a myocyte in a selected area of the myoplasm [1]

of the calcium signal (for instance, due to the functioning of the systems of active transportation of Ca ions, first and foremost, Mg^{2+},ATP-dependent calcium pump of the plasma membranes; these systems ensure the reverse decrease in the concentration of Ca^{2+} in the cytoplasm) [1].

Another useful applied approach is based on the study of the simultaneous colocalization of various fluorescent probes in smooth muscle cells, such as dyes, to test the membrane potential and the proton gradient at the level of subcellular membrane structures. It was found that in the uterine myocyte, the distribution of probes specific to the mitochondrial membrane (NAO probe), membrane electric potential (DIOC-6(3) probe), and transmembrane gradient of protons (probe 9-aminoacridine) is practically the same. This methodological approach allows for a deeper understanding of the mechanisms of the transmembrane (in particular, pH-dependent) exchange of Ca ions in mitochondria in smooth muscle cells.

Immanuel Kant said, "In every department of physical science, there is only so much science, properly so-called, as there is mathematics." Indeed, quantitative interpretation of experimental results and theoretical ideas deepens our understanding of natural phenomena and allows for their characterization in terms of objective indices. The Department of Muscle Biochemistry of the O.V. Palladin Institute of Biochemistry, the NAS of Ukraine, developed the mathematical model for regulating Ca ions concentration in the smooth muscle cells of the uterus. While elaborating this model, we used our experimentally obtained views on Ca^{2+}-transporting systems of myometrium cells and their kinetic parameters. Of course, as we obtain new experimental data, this model is constantly improved; for instance, it has already helped us explain the phenomena of so-called intracellular calcium oscillations and spontaneous Ca^{2+}-dependent mechanic activity of the uterus. In addition, it is worth noting that experimental and theoretical research in the Department aimed at studying the biochemical foundations of regulating the ionized Ca concentration in myocytes was accompanied by the development of new methods for kinetic analysis of enzymatic and transport processes.

The fundamental problems we are studying, as discussed above, also have an important practical aspect. After all, some very dangerous pathologies of the contractile function of smooth muscles are often associated with impaired calcium ion metabolism in myocytes. In particular, this applies to hypotension and hypertension, intestinal atony and other intestinal motility disorders, asthma, diseases of the genitourinary system, including uterine hypotension and hypertension, miscarriages, spontaneous abortions, etc. Thus, the search for novel non-toxic/low-toxicity reverse effectors (inhibitors, activators) – selective and high-affinity regulators of membrane-bound Mg^{2+}-dependent Ca^{2+}-transporting ATP-hydrolases and ATP-hydrolases of contractile proteins is extremely urgent. Potentially, these effectors may serve as "molecular platforms" for the elaboration of new-generation medicine which would normalize the contractile function of smooth muscles in case of its impairment under pathologies.

Thus, as mentioned above, in our fundamental research in the field of biochemistry and biophysical chemistry of intracellular calcium homeostasis in smooth muscle, we tried to use a comprehensive interdisciplinary approach "at the intersection" of Biochemistry, Physical and Organic Chemistry, as well as Mathematical and Computer Modeling.

Later, we will touch upon the results obtained by us while studying the energy-dependent cation (Ca^{2+}, Na^{+})-transporting systems of smooth muscle cells in more detail.

II. Mg^{2+},ATP-dependent calcium and sodium pumps of the plasma membrane and calix[4]arenes

First of all, we became interested in Mg^{2+},ATP-dependent calcium pump of the plasma membrane. It releases Ca ions against the considerable electrochemical gradient (as stated above – Gibbs free energy ΔGPM for this gradient is about 40 kilojoule/mol). When smooth muscles are in a dormant state, Mg^{2+},ATP-dependent calcium pump functions at the background of the stationary basal flow of calcium ions (10^{-15}–10^{-14} mol Ca^{2+}/sq.cm. per one second), which comes into the unexcited myocytes by the gradient of electrochemical potential and is capable of ensuring long-term stationary maintenance of the concentration of Ca^{2+} in the myoplasm at the level of 10^{-7}–10^{-6} M. Thus, this pump is the most relevant element of the total mechanism of membrane control for the basal tone of the smooth muscle. However, considering the fact that the saturation of intracellular depots with calcium ions in excited myocytes occurs after about 100 contractions, activated by a series of depolarizing impulses due to the uptake of

extracellular Ca^{2+} into the cells via calcium channels of the plasma membrane, one may conclude that if this pump were not there, the myocytes would pass into the state of a stable contracture relatively fast. Thus, Mg^{2+},ATP-dependent calcium pump of the plasma membrane, is of fundamental relevance for ensuring Ca^{2+}-dependent control for the muscle tension of excited myocytes.

In the case of myometrium, it was found that, for instance, Mg^{2+},ATP-dependent calcium pump (Ca^{2+},Mg^{2+}-ATPase) of the plasma membrane had an interesting specificity. The uterotonic peptide hormone, oxytocin, is known to stimulate the uptake of Ca ions into the uterus cells, mediated by the interaction with the relevant receptor of the plasma membrane, via calcium channels. However, we found that there is also an inhibiting impact of oxytocin on the kinetics of Mg^{2+},ATP-dependent accumulation of Ca^{2+} in the inverted (*inside-out*) vesicles of the plasma membrane of myometrium cells. It was found that this hormone, getting into the inside-out vesicles of the plasma membrane on the stage of their formation during the tissue homogenization partially inhibits Mg^{2+},ATP-dependent pumping of Ca ions into such vesicles. If these results are extrapolated on the events, occurring in an intact smooth muscle cell, one should expect that the mentioned hormone, via the interaction with the corresponding receptor, located on the outer side of the plasma membrane, ensures partial inhibition of Mg^{2+},ATP-dependent release of Ca ions from the cells; the presence of this unique positive reverse association will promote the maintenance of the increased concentration of Ca ions in the excited myocytes, and thus, promote the myometrium contraction.

There are also other useful experimental models, which provide a possibility of investigating the properties and mechanisms of the functioning of Mg^{2+},ATP-dependent calcium pump of the plasma membrane. In particular, these are proteoliposomes – lipid vesicles (i.e. made of azolectin), containing the built-in Ca^{2+}-transporting Ca^{2+},Mg^{2+}-ATPase, which was previously solubilized from the plasma membrane of myometrium cells by the method of affinity chromatography. In the presence of Mg ions and ATP, these proteoliposomes are capable of ensuring the active accumulation of Ca ions. Using the relevant experimental models (inverted vesicles of the plasma membrane; proteoliposomes, containing the built-in transporting Ca^{2+},Mg^{2+}-ATPase; solubilized Ca^{2+},Mg^{2+}-ATPase), we have studied the properties (kinetic, catalytic, energetic) of Mg^{2+},ATP-dependent calcium pump of the plasma membrane of myometrium cells. For instance, it was proven that this pump has a high affinity to Ca ions (the value of the activation constant for Ca ions, Ca K_{Ca}, is 0.3–0.4 μM) and is potential-dependent.

As already stated, the Department of Muscle Biochemistry studied the regularities of the targeted regulation of the functional activity of energy-dependent Ca^{2+}-transporting systems by calixarenes. Calixarenes are non-toxic macrocyclic compounds, the molecules of which have a “cup”-like form, intramolecular highly orderly lipophilic cavities, and demonstrate a unique trait of recognizing and binding cations, anions, and neutral molecules into stable complexes of “guest-host” type. These properties of calixarenes are widely used in the elaboration of catalysts and sensors, the mentioned compounds have a wide spectrum of biological activity, namely, they can be either inhibitors or activators of enzymes, have membranotropic, antithrombotic, antitumor, antituberculosis effects, impact the functioning of receptor and transport proteins, and inhibit the adhesion of cells [2].

It should be noted that prior to the publication of our results, there were no data in the world about low-molecular selective inhibitors of Mg^{2+},ATP-dependent calcium pump of the plasma membrane. It was found that among the great diversity of calixarenes, investigated by us, thiacalix[4]arene C-1087 (Fig. 2) is a selective inhibitor of Mg^{2+},ATP-dependent calcium pump of the plasma membrane [3]. It was synthesized and characterized using the methods of NMR- and IR-spectroscopy under the guidance of V.I. Kalchenko, the full member of the NAS of Ukraine, in the Institute of Organic Chemistry, the NAS of Ukraine.

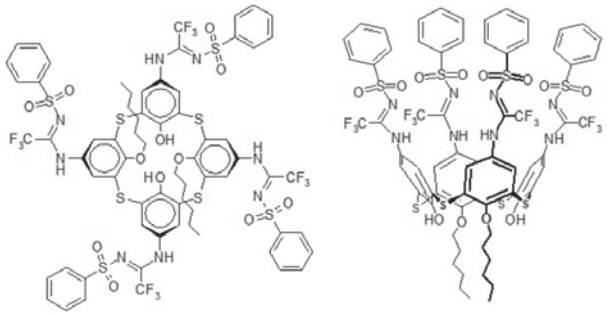


Fig. 2. The structural formula of thiacalix[4]arene C-1087 [3]

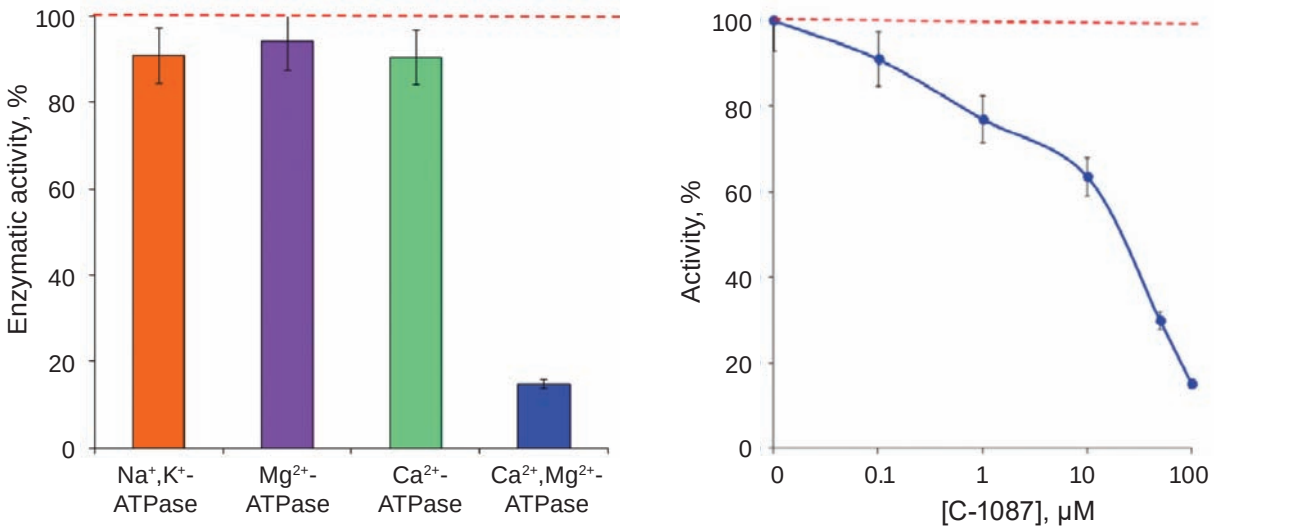


Fig. 3. The selectivity (left panel) and affinity (right panel) of the inhibiting effect of thiacalix[4]arene C-1087 on the enzymatic activity of the transporting Ca^{2+},Mg^{2+}-ATPase of the plasma membrane of myometrium cells of rats. In the case of the left panel, the concentration of thiacalix[4]arene is 100 nM. The value of specific enzymatic activity in the absence of thiacalix[4]arene in the incubation medium is accepted as 100% [3]

In Fig. 3, it is evident in the left panel how effectively thiacalix[4]arene C-1087 (100 nM) inhibits the activity of the transporting Ca^{2+},Mg^{2+}-ATPase (fourth column). However, the effect of the mentioned thiacalix[4]arene on other ATP-hydrolases of the plasma membrane (Na^+,K^+-ATPase, Ca^{2+}-independent Mg^{2+}-ATPase, Mg^{2+}-independent Ca^{2+}-ATPase) was practically not observed. This selectivity in the effect of thiacalix[4]arene C-1087 on Mg^{2+}-dependent Ca^{2+}-transporting ATP-hydrolase is very important since modern medicine requires target medical means, e.g., the ones attacking one specific Ca^{2+}-transporting protein and not affecting other proteins. The inhibiting effect of thiacalix[4]arene C-1087 on the activity of the transporting Ca^{2+},Mg^{2+}-ATPase occurs with rather a high affinity to this enzyme – according to the chart, presented in the right panel of Fig. 3, the value of the inhibition coefficient $I_{0.5}$ for the effect of thiacalix[4]arene C-1087 on Mg^{2+}-dependent Ca^{2+}-transporting ATP-hydrolase is 10 μM. Thus, it is possible to assume that thiacalix[4]arene C-1087 is selective, at least at the level of the plasma membrane, and a rather high-affinity inhibitor of Mg^{2+},ATP-dependent calcium pump, localized in the mentioned subcellular structure.

To come closer to understanding the mechanism of high selectivity of the inhibiting effect of thiacalix[4]arene C-1087 on the enzymatic activity of the transporting Ca^{2+},Mg^{2+}-ATPase of the plasma membrane, we conducted the comparative study on the effect of a calixarene "cup" specifically and the functional groups at its upper brim on the above-mentioned activity. It was found that separately, neither the "cup" itself nor functional groups at its upper brim practically had any impact on the enzymatic activity; thus, the inhibitory effect of thiacalix[4]arene C-1087 on Mg^{2+},ATP-dependent calcium pump is ensured by the complex effect of the calixarene "cup" and the functional groups, modifying its upper brim.

Using the technologies of docking and molecular dynamics, we found the two most probable sites of thiacalix[4]arene C-1087 binding to functionally active sites of Mg^{2+},ATP-dependent calcium pump (Fig. 4 and 5). It was found that steric and electrostatic interactions as well as hydrogen bonds play a relevant role in stabilizing the complex of "Ca^{2+},Mg^{2+}-ATPase-thiacalix[4]arene C-1087".

It was found that the selective inhibitor of Mg^{2+},ATP-dependent calcium pump – Ca^{2+},Mg^{2+}-ATPase of the plasma membrane, thiacalix[4]arene C-1087, caused an increase in the concentration of Ca ions in smooth muscle cells of the uterus; these data were obtained using the method of confocal microscopy (involving Ca^{2+}-sensitive fluorescent probe Fluo-4AM) (Fig. 6).

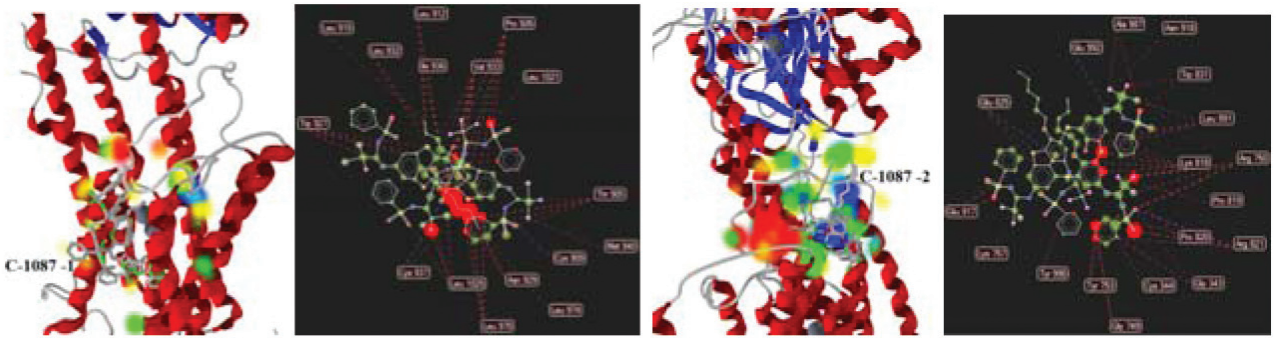


Fig. 4. Different types of interactions, ensuring the contact between thiacalix[4]arene C-1087 and Mg^{2+}-ATP-dependent calcium pump in the regions 1 (on the left) and 2 (on the right). Amino acid residues, forming H-bonds with the inhibitor, are marked in brown. Green color – steric interactions; turquoise color – hydrogen acceptors; yellow – hydrogen donors; red-blue – electrostatic interactions [3]

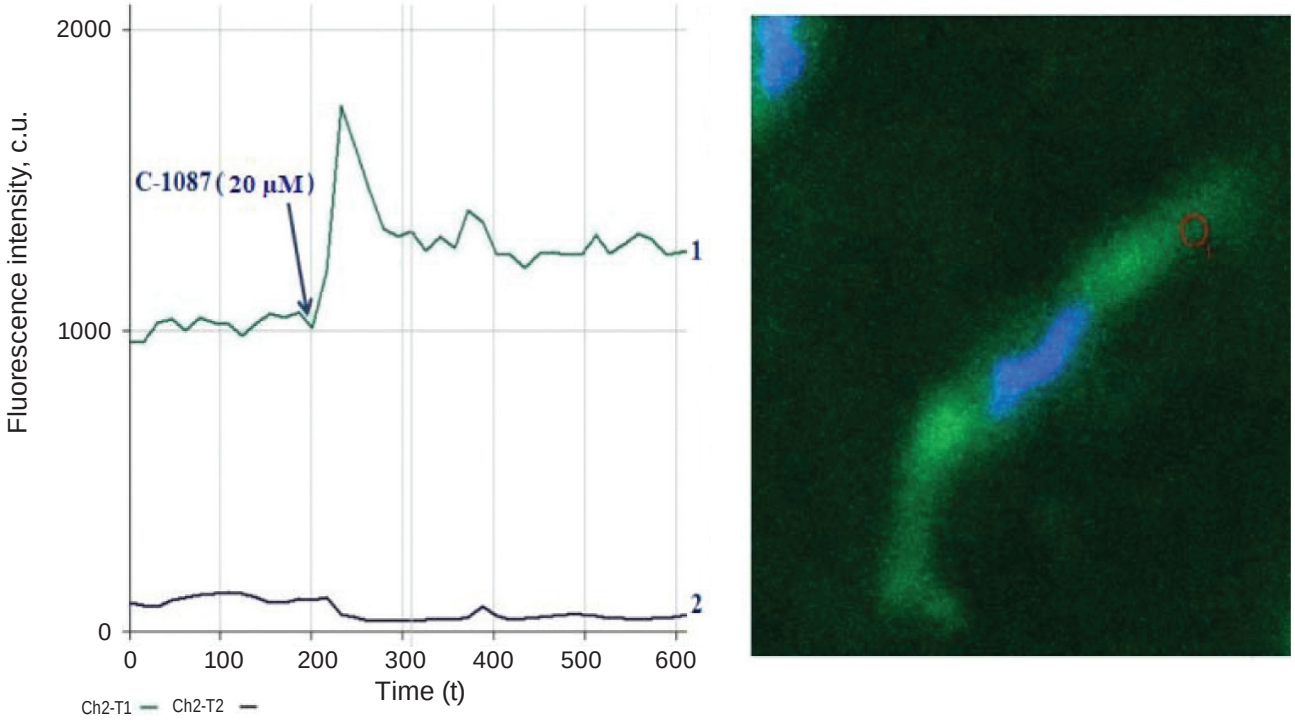


Fig. 5. The effect of the selective inhibitor of Mg^{2+},ATP-dependent calcium pump of the plasma membrane, thiacalix[4]arene C-1087 (20 μM) on the concentration of Ca^{2+} in myometrium cells of rats. The concentration of thiacalix[4]arene is 20 nM. The method of scanning laser confocal microscopy with Ca^{2+}-sensitive fluorescent probe Fluo-4AM (chart 1). DNA-sensitive fluorescent probe Hoechst was used to visualize the cell nucleus (chart 2) [3]

The method of laser photon-correlation spectroscopy was used to demonstrate that thiacalix[4]arene C-1087 and its analogs cause a decrease in the effective hydrodynamic diameter of smooth muscle cells of the uterus. It is an independent confirmation of the inhibitory effect of the mentioned calix[4]arenes on Mg^{2+}-dependent Ca^{2+}-transporting ATP-hydrolase, which removes Ca ions from the cells since this inhibition is accompanied by the increase in the concentration of Ca^{2+} in the myoplasm (Fig. 5) and thus, the contraction of muscle cells is stimulated.

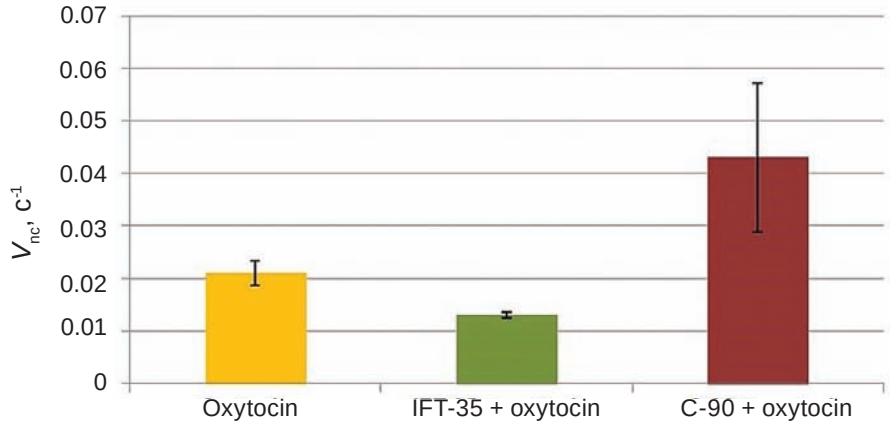


Fig. 6. The multidirectional change in the normalized velocity V_{nc} of the isometric contractions of the myometrium of rats in vitro under the effect of oxytocin (0.1 IU/ml) in conditions, impacting the muscle preparation of one of the selective inhibitors of Mg^{2+},ATP-dependent calcium pump of the plasma membrane, calix[4]arene C-90 (100 μM), and the activator of the calcium pump of the plasma membrane of the compound IFT-35 (100 μM) [2]

Indeed, the purely physiological aspect of the effect of the inhibitor of Mg^{2+}-dependent Ca^{2+}-transporting ATP-hydrolase of the plasma membrane, thiacalix[4]arene C-1087, and its analogs on the intact smooth muscle is very important (Fig. 6). For instance, it was proven that one of selective inhibitors of Mg^{2+},ATP-dependent calcium pump of the plasma membrane, calix[4]arene C-90, activates oxytocin-induced isometric contraction of myometrium (in these experiments, we determined the velocity of smooth muscle contraction, normalized according to the amplitude of the contractile response – parameter V_{nc}).

Our colleagues from the Institute of Organic Chemistry of the NAS of Ukraine determined in their experiments, conducted using the perioral way of administrating this substance to rats, that the inhibitor of Mg^{2+},ATP-dependent calcium pump of the plasma membrane, thiacalix[4]arene C-1087, is a low-toxic substance (according to the classification of L.I. Medved, it belongs to class IV of toxicity). Usually, in medical practice, when the labor is slow, and oxytocin is used to stimulate uterine contractions but does not achieve the desired effect, the doctors perform a cesarean section. Still, according to the results obtained by us, there are grounds to hope that the application of relevant calixarenes – inhibitors of Mg^{2+},ATP-dependent calcium pump of the plasma membrane – potentially may allow for avoiding this surgical intervention.

It was found in our studies that calix[4]arenes C-97, C-99, and C-107 effectively inhibited the activity of Mg^{2+},ATP-dependent sodium pump of the plasma membrane of myometrium. It was determined that the mentioned calixarenes inhibit the enzymatic activity of Na^+,K^+-ATPase effectively in the fraction of the plasma membranes and do not impact the activity of Mg^{2+}-ATPase. Compared to ouabain, these compounds inhibit the activity of Na^+,K^+-ATPase in a dose-dependent and a much more efficient way: the values of apparent inhibition coefficients $I_{0.5}$ for these calix[4]arenes < 0.1 μM (the value of $I_{0.5}$ for ouabain – a specific inhibitor, acting from the extracellular side of the membrane is 20–30 μM) [4]. However, it was found that the structural fragments of calix[4]arene C-107 (calixarene "cup" C-150 and compound M-3 – aminophosphonic residue) in a wide range of concentrations had practically no impact on the enzymatic activity of Na^+,K^+-ATPase of the plasma membrane. Thus, the inhibiting effect of calix[4]arene C-107 on the sodium pump is related to the joint effect of two aminophosphonic groups, spatially oriented on the calixarene platform, but not to the effect of the tetraphenol macrocycle as such or the action of a specific aminophosphonic residue.

It was found that calix[4]arene C-97 decreases the affinity of the enzyme to Na ion (a cation, activating Na^+,K^+-ATPase from the cytoplasmic side of the membrane), but enhances the affinity of the enzyme to ouabain. At the same time, this

calix[4]arene does not impact the affinity of the enzyme to another univalent activating cation – K ion, stimulating the activity of the enzyme from the external side of the cell.

In recent years, it has been determined in the Department that thiacalix[4]arene C-1193 (thiacalix[4]arene-bis-hydroxymethylphosphonic acid) is another effective affinity inhibitor of the sodium pump, which increases the intracellular concentration of Ca ions and modifies the contractile activity of myometrium [5]. For instance, using the methods of enzymatic and kinetic analysis, it was demonstrated that C-1193 has a higher efficient inhibitory effect on Na^+,K^+-ATPase activity in the plasma membrane of myometrium cells ($I_{0.5}$ = 42.1 ± 0.6 nM) as compared to other calix[4]arenes – inhibitors of this enzyme, and has practically no impact on relative activities of other ATPases, localized in this subcellular structure. The method of confocal microscopy with Ca^{2+}-sensitive fluorescent probe Fluo-4 was used to demonstrate that thiacalix[4]arene C-1193 conditioned the increase in the intracellular concentration of Ca^{2+} in the immobilized myocytes of the uterus. The tensometric studies proved that thiacalix[4]arene C-1193 (10 and 100 μM) modified the mechanokinetics of spontaneous isometric contractions in the myometrium [6]. Against the background of C-1193 (10 μM), we observed an increase in the isometric phasic contractions, induced via the paths of electromechanical conjugation (depolarization with hyperkalemic solution) and pharmacomechanic conjugation (on condition of the effect of the uterotonic hormone, oxytocin, a neuromediator acetylcholine and selective agonist of muscarinic acetylcholine receptors of M3-type, cevimeline). It is expected that the experimental data, obtained using thiacalix[4]arene C-1193 – a selective and effective inhibitor of Na^+,K^+-ATPase, may be relevant for the research on determining the membrane mechanisms of cation (in particular, sodium-calcium) exchange in smooth muscles while investigating the role of the plasma membrane in ensuring the electro- and pharmaco-mechanic conjugation in them, and in the regulation of ion homeostasis in myocytes.

Therefore, the selected calix[4]arenes are promising regulators of smooth muscle contractility. For comparison, Fig. 6 also presents the results, obtained by us in cooperation with the employees of the Institute of Pharmacology and Toxicology of the NAMS of Ukraine (Professor M.A. Mokhort and his colleagues) in the experiments on using the low molecular selective activator of Mg^{2+},ATP-dependent calcium pump of the plasma membrane – compound IFT-35. Contrary to calix[4]arenes, selective inhibitors of the calcium pump, this compound inhibited oxytocin-induced isometric contractions of the myometrium.

Therefore, the use of two selective effectors of Mg^{2+},ATP-dependent calcium pump of the plasma membrane – the pump inhibitor, calix[4]arene C-90 and the pump activator, compound IFT-35, opens the perspectives for multidirectional regulation of the contractile activity of the uterus.

However, the control experiments, conducted to implement the abovedescribed investigations, aimed at the study of the impact of calix[4]arenes on the enzymatic hydrolysis of ATP, demonstrated that some of these macrocyclic compounds, in particular, calix[4]arene C-107, are capable of hydrolyzing nucleoside triphosphate in the non-enzymatic process [7]. This initiated the idea of a possible mechanism for the reaction of calix[4] and the arene-induced hydrolysis of ATP. We managed to elaborate the kinetic model, which explains the specificities of this reaction, in particular, the phenomenon of its spontaneous termination on the 45th minute of the incubation, when the incubation medium still contains ATP in the amount of ~ 90% of its initial content. It is assumed that during the calixarene-induced hydrolysis of ATP, the molecules of calix[4]arene C-107, which formed a complex with nucleoside-triphosphate and ensured the release of inorganic phosphate P_i, switch into the non-reactive states; being in these states, these inactive molecules of calix[4]arene are not capable of forming a complex with ATP anymore, and thus to ensure the hydrolysis of nucleoside triphosphate. The kinetic analysis of the postulated mechanism (in the permanent mode) was used to obtain quantitative equations for the dependence between the accumulation of the reaction product *p* (inorganic phosphate P_i) and the time and period of ATP half-conversion $\tau_{0.5}$ on the concentration of nucleoside triphosphate a_0, the dependence of the initial velocity v_0 of the ATP hydrolysis reaction on the concentration of calixarene q_0 and the concentration of ATP a_0; the magnitude of individual velocity constants of specific stages of the suggested mechanism was evaluated, including the stages, ensuring the change in the properties of calix[4]arene C-107 during the ATP hydrolysis reaction. The results of the quantitative analysis of the suggested mechanism of this reaction were used to explain its kinetic properties,

determined in the experimental original studies, as follows: – an insignificant yield of the reaction product – inorganic phosphate P_i after the process completion (up to 10% from the initial amount of ATP); – satisfactory description of the complete kinetic curve in the linearized format; – quantitative regularities of the plateau (time-wise) accumulation of the product reaction pmax under the change in the concentration of calix[4]arene C-107 q_0 (under the stable concentration of ATP a_0) and under the change in the concentration of ATP a0 (under the stable concentration of calix[4]arene C-107 q_0); – the reciprocal dependence of the ATP half-conversion period $\tau_{0.5}$ under the effect of calix[4]arene C-107 from the concentration of nucleoside triphosphate a_0; – the conformity of the reaction course (in the mode of measuring its initial velocity) to a Michaelis-Menten-type equation for the dependence of the initial reaction velocity v_0 on the concentration of calix[4]arene q_0 and ATP a_0. Some (also rather remote) similarity between the suggested mechanism of calixarene-induced cleaving of ATP and a typical enzymatic reaction was determined from the standpoint of kinetic and energetic interpretation. The final solution to the issue regarding the mechanism of calix[4]arene-induced hydrolysis of ATP requires further experimental and theoretical studies.

Thus, our interdisciplinary studies have demonstrated that the selected calix[4]arenes can serve as selective and sufficiently affinity inhibitors of Mg^{2+},ATP-dependent calcium and sodium pumps of the plasma membrane of smooth muscle cells and, accordingly, modulators of intracellular calcium homeostasis and contractile activity of the myometrium.

III. Transmembrane exchange of Ca ions in mitochondria and calix[4]arenes

Our studies confirmed the key role of the electrochemical potential on the internal membrane of mitochondria in regulating Ca^{2+}-homeostasis in myometrium cells. Using Ca^{2+}-sensitive fluorescent probes-dyes, interacting with mitochondria, and the method of laser scanning confocal microscopy, we demonstrated that the dissipation of electrochemical potential on the internal membrane of uterine myocytes in the presence of protonophore CCCP – carbonyl cyanide m-chlorophenyl hydrazone (10 μM) and the inhibitor IV of the respiratory chain complex, sodium azide (1 mM), is accompanied by a considerable increase in Ca^{2+} concentration in the myoplasm only in case of the action of protonophore, not sodium azide. Using two independent fluorimetric methodological approaches, namely, laser confocal microscopy and laser flow cytometry, and Ca^{2+}-sensitive fluorescent probe Fluo-4 AM on the models of freshly isolated myocytes and the mitochondria of the uterine smooth muscle, isolated by differential centrifugation, we proved a relevant role of the electrochemical potential on the internal membrane of these organelles in the mechanisms supporting Ca^{2+}-homeostasis in the myometrium cells.

The method of flow cytometry was used to investigate the impact of calmodulin antagonists, calmidazolium and trifluoperazine, on the membrane potential of myometrium mitochondria. It was determined that calmidazolium in the concentration of 1–10 μM induced a dose-dependent decrease in the membrane potential of mitochondria. At the same time, trifluoperazine in the concentrations of 10–100 μM had different effects: 10 μM increased the polarization, whereas 100 μM induced almost complete depolarization of mitochondrial membranes. Further experiments were conducted using the method of confocal microscopy, potential-sensitive fluorescent probes TMRM and MTG and myometrium cells in the culture. TMRM and MTG are lipophilic cations, accumulated in the membrane structures depending on the level of membrane potential of these structures. Since the internal membrane of mitochondria has a high negative potential (about -180 mV), the probes diffuse to the cytosol of myometrium cells and are mostly accumulated in mitochondria. The behavior of these probes is different under changes in the membrane potential of mitochondria. The MTG probe is accumulated in the polarized mitochondria and covalently bound to mitochondrial proteins. Therefore, under the dissipation of the membrane potential, the probe remains in mitochondria. On the contrary, the TMRM probe does not interact with the membrane structures of proteins of mitochondria, so the dissipation of the membrane potential is accompanied by the exit of the probe from the mitochondria and, thus, the decrease in the level of its fluorescence. It was demonstrated that loading the myometrium cells with a potential-sensitive MTG probe led to fluorescence in the "green" range of radiation. The introduction of another probe, TMRM, resulted in the fluorescence in the "red" range of the radiation. The depolarization of the mitochondria with protonophore CCCP or 10 mM NaN_3 was accompanied by a decrease

in the intensity of the "red" fluorescence, while the "green" fluorescence was preserved. The introduction of 10 μM calmidazolium or 100 μM trifluoperazine into the incubation medium was accompanied by practically the complete extinction of the fluorescent signal of the TMRM probe. Thus, it was demonstrated that calmodulin antagonists modulate the membrane potential of mitochondria in myometrium cells [8].

Further experiments were conducted using the spectrofluorimetry method. The polarization of the mitochondria membranes in the myometrium was studied using the mode of fluorescence extinction and potential-sensitive probe, 1 μM TMRM. The concentration-dependent effect of trifluoperazine on the polarization level of mitochondrial membranes was demonstrated. $K_{0.5}$ is 24.4 ± 5 μM (n = 10). Hill's coefficient is 2.0 ± 0.2, which demonstrates the presence of two binding centers of trifluoperazine on the membrane of mitochondria.

It turned out that the calmodulin antagonist, trifluoperazine (100 μM), increases the content of ionized Ca in the matrix of mitochondria. The preliminary incubation of mitochondria suspensions with 100 μM Ca^{2+}, prior to the introduction of trifluoperazine to the incubation medium, partially inhibits the impact of the latter on the cation concentration in the matrix. The incubation of myometrium cells (the primary culture) with another calmodulin antagonist, calmidazolium (10 μM), is accompanied by the depolarization of the mitochondrial membrane and the increase in the concentration of ionized Ca in the cytosol. Therefore, it was demonstrated using two models, the suspensions of mitochondria and intact myometrium cells, that calmodulin antagonists induce the depolarization of mitochondria membranes and the increase in the concentration of ionized Ca both in the mitochondria matrix and in the cytosol of cells.

The changes in the concentration of the ionized Ca in myometrium mitochondria were also studied when these subcellular structures were incubated in the media of different compositions, namely, the medium not containing ATP and Mg ions (0-medium), the medium containing 3 mM Mg (Mg-medium) and the medium containing 3 mM Mg + 3 mM ATP (Mg, ATP-medium). It was determined that the composition of the incubation medium affected the values of the ionized Ca concentration in mitochondria in the absence of exogenous Ca^{2+} and did not affect them in the presence of this cation. The preliminary incubation of mitochondria in the media of different compositions with 25 μM trifluoperazine did not affect the values of the concentration of ionized Ca in mitochondria both prior to and after the addition of 100 μM Ca^{2+} to the incubation medium. Therefore, trifluoperazine causes the depolarization of mitochondrial membranes, and this effect depends on its concentration. However, the preliminary incubation of mitochondria with 25 μM trifluoperazine, accompanied by a 50 % decrease in the polarization of membranes, does not affect the values of the concentration of ionized Ca in mitochondria both prior to and after the addition of 100 μM Ca^{2+} to the incubation medium.

Ca ions are known to regulate the accumulation of Ca^{2+} in mitochondria. However, the mechanism of this phenomenon is still under discussion. The experiments, conducted in the Department of Muscle Biochemistry, demonstrated that Ca ions regulate the level of the polarization of mitochondria membranes in myometrium directly or indirectly. For instance, the introduction of 100 μM Ca^{2+} to the incubation medium is accompanied by the depolarization of mitochondrial membranes. We also studied the effect of Ca^{2+} on the concentration of ionized Ca in mitochondria. The isolated mitochondria of myometrium were previously incubated in the absence or presence of 10 μM Ca^{2+}, with the subsequent addition of 100 μM Ca^{2+} into the incubation medium. The experiments were conducted in three media, namely, no ATP or Mg^{2+} (0-medium), the presence of 3 mM Mg^{2+} (Mg-medium) and 3 mM Mg^{2+} + 3 mM ATP (Mg, ATP-medium). It was demonstrated that the effects of 10 μM Ca^{2+} were different in different media, namely, in 0- and Mg-medium, the values for the concentration of ionized Ca in mitochondria increased, whereas in Mg, ATP-medium, no statistically significant changes were registered. The preliminary incubation of mitochondria with 10 μM Ca^{2+} did not affect the values for the concentration of ionized Ca in mitochondria after the introduction of 100 μM Ca^{2+}into the incubation medium. The values for the concentration of ionized Ca in mitochondria after the introduction of 100 μM Ca^{2+} were the same for the incubation of mitochondria in 0- and Mg, ATP-media, and somewhat lower in Mg-medium. It should also be mentioned that the preliminary incubation of mitochondria with 10 μM Ca^{2+} in 0- and Mg-media decreased the changes in the values of the normalized fluorescence of Ca^{2+}-sensitive Fluo probe, induced by the addition of

100 μM Ca^{2+}, but in Mg, ATP-media these changes were not registered. The conclusion was drawn that the composition of the incubation medium has a considerable effect on the concentration of ionized Ca in mitochondria in the absence of the cation in the external environment. It is assumed that an insignificant increase in the concentration of ionized Ca in mitochondria prior to the addition of 100 μM Ca^{2+} can have a positive effect on the functional activity of mitochondria.

It was determined that the incubation of myometrium mitochondria in the medium, containing 3 mM Mg^{2+}, leads to a low concentration of ionized Ca in mitochondria. Further addition of 100 μM Ca^{2+} was accompanied by an 8-fold increase in the concentration of ionized Ca, yet, under these conditions, a low level of total accumulation of this cation is registered. The normalized fluorescence of Ca^{2+}-sensitive probe Fluo-4 in response to the addition of Ca^{2+} was over 2.5 conventional units. At the same time, the value for the concentration of ionized Ca in mitochondria was much higher than that for the incubation of mitochondria in the presence of 3 mM ATP and 3 mM Mg^{2+}. Further addition of 100 μM Ca^{2+} was accompanied by the increase in the concentration of ionized Ca only 2.4 times, but a high level of the total accumulation of this cation was registered. In the presence of 3 mM ATP and 3 mM Mg^{2+} the normalized fluorescence of Ca^{2+}-sensitive Fluo-4 probe in response to the addition of Ca^{2+} was under 1.3 conventional units.

Further experiments were conducted using spectrofluorimetry and dynamic light dissipation. The concentration of ionized Ca in myometrium mitochondria was determined in Mg^{2+}- and Mg^{2+},ATP-media. The ATP concentration-dependent increase in the ionized Ca concentration in the matrix of mitochondria was demonstrated in the absence of exogenous Ca^{2+}, which ensured a high level of total Ca accumulation after the introduction of the exogenous cation. Hill's coefficient equals 3.18 ± 0.27, and the activation constant for ATP is 0.97 ± 0.07 mM. The ATP effect on the ionized Ca concentration in mitochondria did not depend on blocking the mitochondrial pore by cyclosporine A and on the inhibition of H^+-ATPase/ATP-synthase by oligomycin. Instead, the introduction of 10 mM theophylline 30 мМ $NaHCO_3$ into the Mg^{2+}-medium was accompanied by an increase in the ionized Ca concentration in the mitochondria matrix. It was also shown that under low concentration of the ionized Ca in the matrix (Mg^{2+}-medium), mitochondria have a rather bigger size, whereas, under a higher concentration of the cation in the matrix (Mg^{2+},ATP-medium), mitochondria are smaller. The activation of soluble adenylate cyclase $NaHCO_3$ against the simultaneous inhibition of phosphodiesterase with theophylline is accompanied by a decrease in the normalized fluorescence of Ca^{2+}-sensitive probe under conditions of the introduction of exogenous Ca^{2+}. We assumed that soluble adenylate cyclase may be involved in regulating the ionized Ca concentration in the mitochondrial matrix [9].

As known, there are some considerable differences in the metabolism of smooth muscles compared to other types of cells. Oxidative phosphorylation directly maintains contractile activity, whereas the regulation of ion exchange is ensured by ATP, which is formed as a result of anaerobic metabolism. It was proven that the functioning of ion channels and transporters is mostly regulated by glycolytically synthesized ATP. It was previously demonstrated in the Department of Muscle Biochemistry that the ionized Ca concentration in the mitochondrial matrix in the absence of exogenous Ca^{2+} is regulated by ATP: in the case of Mg^{2+},ATP-containing medium, this value is several orders higher than in case of the Mg^{2+}-containing one. The concentration increase in ionized Ca in mitochondria depended on ATP concentration. It was found that ATP-induced increase in the concentration of ionized Ca in mitochondria is accompanied by enhanced release of Ca^{2+} from mitochondria. Cyclosporine A (5 μM), ruthenium red (10 μM), or oligomycin (1 μg/ml) affected neither the concentration of Ca^{2+} in the matrix, nor the release of the cation from mitochondria. The flow cytometry and fluorescent TMRM probe, sensitive to the membrane potential, were used to show that the mitochondria membranes are polarized under incubation both in Mg^{2+}-, and Mg^{2+},ATP-medium. All the abovementioned demonstrate that the transition pore of mitochondrial permeability is not involved in the investigated effects of ATP.

At present, protein regulators of Ca^{2+} exchange in mitochondria are well-described. At the same time, the role of lipid regulators has not been studied sufficiently. The mitochondrial membranes contain various phospholipids, the most specific and common among which are cardiolipins. In our experiments, the content of cardiolipin in mitochondria was determined by two methods: flow cytometry with cardiolipin-specific fluorescent probe (NAO)

and thin-layer chromatography. It was demonstrated that cardiolipin content in the mitochondrial membranes decreased under the incubation of organellas in Mg^{2+},ATP-medium compared to Mg^{2+}-medium. In addition, we observed a decrease in phosphatidylcholine content and an increase in the content of lysophosphatidylcholine under the incubation in Mg^{2+},ATP-medium. We assumed a possible involvement of the lipid pore in the effect of ATP on Ca^{2+} exchange in mitochondria. The summary of our data confirms the critical role of the extramitochondrial ATP in regulating the concentration of ionized Ca in mitochondria and cardiolipin content [10].

The biochemical properties of H^+-Ca^{2+}-exchanger in myometrium mitochondria were investigated using the methods of spectrofluorimetry and laser correlational spectroscopy. The fluorescent probes Fluo-4 AM and BCECF AM were used in the experiment respectively to register the changes in the ionized Ca^{2+} and pH in the matrix. It was confirmed that instead of Na^+-Ca^{2+}-exchanger, H^+-Ca^{2+}-exchanger functions in the myometrium. It is activated at the physiological pH value according to 1:1 stoichiometry and is electrogenic. This transporting system is modulated by magnesium ions and the diuretic amiloride, but it was not sensitive to changes in the concentration of extramitochondrial potassium ions. The functioning of H^+-Ca^{2+}-exchanger is inhibited by antibodies against protein LETM1. It was demonstrated that protein calmodulin can act as a regulator of H^+-Ca^{2+}-exchanger, inhibiting the latter [11, 12].

Considerable attention was paid to the identification and study of the biochemical properties of the reaction of nitrogen oxide synthesis, NO, in myometrium mitochondria. The conditions for conducting NO-synthase reaction in isolated mitochondria were selected, and some of its kinetic parameters were studied. It was determined that NO synthesis is inhibited by the inhibitors of Ca^{2+}-dependent constitutive NO-synthases, depends on the efficiency of the uptake of Ca ions into the matrix and the functional activity of the complexes of electron-transporting chain, and is inhibited by calmodulin antagonists. Our experimental data regarding the colocalization of MitoTracker Orange CMTMRos and DAF-FM dyes in the uterine smooth muscles demonstrate that nitrogen oxide may be synthesized in mitochondria. High activity of NO-synthase in isolated mitochondria requires the presence of respiration substrates, L-arginine, Ca^{2+} and NADPH, in the incubation medium. The apparent affinity constant for L-arginine in the case of nitrogen oxide production is 28.9 ± 9.1 μM, and the activation constant for Ca^{2+} is 44.4 ± 14.5 μM. The synthesis of nitrogen oxide is inhibited by specific inhibitors of the electron transfer chain (rotenone and antimycin A) and protonophore CCCP. NO synthesis depends on the uptake of exogenous Ca^{2+} to mitochondria (inhibited by 1–10 mM Mg^{2+} and 10 μM ruthenium red), inhibited by calmodulin antagonists (0.1–10 μM calmidosolium and 1–100 μM trifluoperazine) and antibodies (2.5 μg anti-Letm1/50 μg of protein) to H^+-Ca^{2+}-exchanger of protein (protein LETM1). It is blocked by known antagonists of constitutive NO-synthases - NG-nitro-L-arginine and 2-aminopyridine with the value of half-maximal inhibition at the concentration of about 25 μM and 100 μM, respectively. The decrease in potassium ions concentration in the reaction medium under isotonic conditions and the adjustment of inhibitors of different types of potassium channels inhibited NO-synthase reaction considerably, which allows for an assumption on the relevance of potassium permeability of the internal mitochondrial membrane in the regulation of this reaction [12].

It was found that the endogenous adenylate cyclase signalling pathway regulates the nitrogen oxde synthesis in mitochondria. NO synthesis in mitochondria was enhanced at the effect of the activators of adenylate cyclase 30 mM $NaHCO_3$ and 10 μM forskolin, and the inhibitor of phosphodiesterases, 1 mM caffeine. No stimulating effect of the investigated compounds was observed after the introduction of the inhibitor of constitutive NO-synthases, L-NAME (100 μM), to the incubation medium. In the case of the inhibited activity of adenylate cyclase with compound KH7 (25 μM), the intensity of NO formation in mitochondria decreased by about 50%. NO synthesis in mitochondria also decreased considerably in the presence of the inhibitor of protein kinase A, compound PKI (10 nM).

There are reasons to believe that the products of oxidative and non-oxidative metabolism, L-arginine, nitrogen oxide, and spermin, are possible regulators of Ca^{2+} transportation in mitochondria. It was demonstrated using the fluorescent probe Fluo-4 AM that 50 μM L-arginine stimulates the energy-dependent accumulation of Ca^{2+} by mitochondria in the presence of 10 μM NADPH and BH4, required for the optimal work of mitochondrial NO synthase. A similar effect was noted for the use of direct donors of ni-

trogen oxide 100 μM SNP, SNAP, and sodium nitrate (SN). The stimulating effect was removed in the presence of the scavenger of nitrogen oxide C-PTIO, which demonstrates the NO-dependent character of the effect of the applied nitrogen compounds and products of oxidative transformation of L-arginine into Ca^{2+} transport in mitochondria. The stimulating effect of spermin in low physiological concentrations (100 μM) on the accumulation of Ca^{2+} by mitochondria was conditioned by the increase in NO synthesis, which was demonstrated using C-PTIO, inhibitors of NO-synthase (100 μM NA and L-NAME), and in the case of direct monitoring of NO synthesis by the fluorescent probe DAF-FM.

The biochemical processes in mitochondria were modeled using the mathematical apparatus of Petri nets. Using this methodology, a mathematical simulation model was developed that allows for predicting simultaneous changes in the biophysicochemical parameters of mitochondrial functioning. The model relates the time-related changes in the hydrodynamic diameter of mitochondria, the functioning of the electron transfer chain, endogenous fluorescence of adenine nucleotides, and the production of reactive oxygen species under the action of sodium azide as an indirect NO donor. For instance, the models were developed for the processes of changes in the concentration of Ca^{2+} in mitochondria (fluorescence Fluo-4), synthesis of nitrogen oxide (fluorescence DAF-FM), generation of reactive oxygen species (DCF-fluorescence) and endogenous fluorescence of NADH. The estimated values of the investigated biophysicochemical parameters corresponded to the experimentally obtained data.

As stated above, calixarenes are macrocyclic compounds, interacting with biologically active molecules and ions and, thus, causing a change in a series of biochemical and biophysical processes. It was found that selected calix[4]arenes have an effective and rather specific impact on Ca^{2+} transport, the functioning of the electron transfer chain, and the synthesis of reactive forms of nitrogen and oxygen in myometrium mitochondria, so they can be used as instruments to investigate biochemical processes in these subcellular structures.

Calix[4]arene chalcone amides C-136 and C-137 at the lower brim of the calixarene molecule contain two amidochalcone groups, and calix[4]arene chalcone amide C-138 – only one. Calix[4]arenes C-136 and C-137 differ in the presence of ether or hydroxyl groups, respectively, on the lower brim of the calixarene platform and in the length of the alkyl spacer between amidochalcone groups and the macrocycle. The experiments were conducted using the myometrium cells in the culture and the suspension of uterine myocytes, permeabilized by digitonin. It was demonstrated that calix[4]arenes C-136 and C-137 (10 μM) hyperpolarize the mitochondrial membrane. The maximal hyperpolarization effect was 173% compared to the control. At the same time, calix[4]arene C-138 did not affect the membrane potential of mitochondria.

It was found that the increase in the number of chalcone amide groups on the lower brim of the calixarene cup was accompanied by the increase in the polarization of the mitochondrial membranes. For instance, while studying the effects of calix[4]arenes, containing two (C-1012 and C-1021), three (C-1023 and C-1024), and four (C-1011) chalcone amide groups in their structure, it was determined that the incubation of the permeabilized myometrium cells with calix[4]arenes, containing from two to four chalcone amide groups, is accompanied by the increase in the polarization level of the mitochondrial membranes of the myometrium. It was also found that calix[4]arene chalcone amides increased the concentration of ionized Ca in the mitochondrial matrix. The accumulation of Ca^{2+} in mitochondria is a known potential-dependent process. Therefore, further experiments were aimed at investigating the effect of calix[4]arene chalcone amides on the concentration of ionized Ca in the mitochondrial matrix. It was found that all the investigated calix[4]arenes increased the concentration of ionized Ca in the matrix both in the absence and in the presence of exogenous Ca^{2+}.

Depending on the number of chalcone substituents and their polarity, the representatives of chalcone calix[4]arenes inhibit the electron transfer chain, enhance the generation of reactive oxygen species in mitochondria, inhibit the transport of Ca^{2+} in the internal mitochondrial membrane and inhibit the nitrogen oxide synthesis by these subcellular structures. For instance, chalcone calix[4]arenes C-138, C-137, C-1023, and C-1011 in the concentration of 10 μM, depending on the number of chalcone substituents (1, 2, 3, and 4, respectively) on the lower brim of the calix[4]arene cup inhibit the oxidation of NADH and $FADH_2$ in the electron transfer chain of the isolated mitochondria. Calix[4]arenes C-1023 and C-1011 enhance the generation of reactive oxygen species in mitochondria. The investigated

compounds inhibit the transport of Ca^{2+} (energy-dependent accumulation and H^+–Ca^{2+}-exchanger) in the internal mitochondrial membrane and inhibit nitrogen oxide synthesis. The determined effects also depend on the charge and polarity of the functional groups in the substituents and the very calix[4]arene cup.

Further experiments demonstrated that calix[4]arene chalcone amides modulate the value of the hydrodynamics diameter of mitochondria. The determination of the hydrodynamic diameter of mitochondria allows for testing the changes in the volume of organelles. The obtained results demonstrate that the hydrodynamic diameter of mitochondria depends on the composition of the incubation medium: it is smaller in the presence of ATP compared to its absence; the hydrodynamic diameter of mitochondria increases time-wise in case of incubating mitochondria with calix[4]arene chalcone amides; the effect of calix[4]arene chalcone amides on the value of hydrodynamic diameter of mitochondria increases along with the rise in the number of chalcone groups in the structure of calix[4]arene.

Using the methods of the laser confocal microscopy, flow cytometry, and spectrofluorimetry, it was proven that calix[4]arene chalcone amides penetrate the myometrium cells. For instance, it was illustrated using the example of calix[4]arene chalcone amide C-1070 (the fluorescent analog of calix[4]arene C-1011). The primary culture of myometrium cells with the potential-sensitive probe JC-1 was used to demonstrate the modulating impact of calix[4]arene chalcone amide with two chalcone groups on the polarization of mitochondrial membranes. The fact of calix[4]arenes penetration into myocytes was also proven using the example of calix[4]arene C-956 (this calix[4]arene has its own fluorescence in the blue-violet part of the spectrum). It was shown that it interacts with the plasmalemma of myocytes, enters the cells, and colocalizes with mitochondria. The macrocyclic compound C-956 in the concentration of 50 μM stimulates the activity of NO-synthase in the isolated mitochondria considerably and inhibits the functioning of the electron transfer chain via the oxidation inhibition of NADH and $FADH_2$. No considerable effect of the investigated compound on the generation of reactive oxygen species in the fraction of mitochondria was found.

Further investigation involved thiacalix[4]arenes C-1191 and C-1192, which differ from the previous compounds by the presence of a sulfur atom in the molecule structure and the spatial location of chalcone amide groups. It was demonstrated that thiacalix[4]arenes C-1191 and C-1192 demonstrate the hyperpolarizing effect on mitochondria. Thiacalix[4]arene chalcone amides C-1191 and C-1192, similar to calix[4]arene chalcone amide C-1011, induced the increase in the basal tension of smooth muscle preparations of rat myometrium. Thiacalix[4]arene C-1191 did not change the contraction-relaxation cycle but, similar to compound C-1011, induced a reliable and even increase in all the force (F_{max}, F_C and F_R) and velocity (V_C and V_R) parameters, and some impulse parameters (I_0 and I_R). Contrary to C-1191, thiacalix[4]arene C-1192 modified the uterine contraction-relaxation cycle considerably and enhanced the total efficiency of the spontaneous contractile activity of myometrium. Also, compound C-1192 (but not C-1191) caused a decrease in all the force (F_{max}, F_C and F_R), and some time (τ_0 and τ_R) and impulse (I_0 and I_R) parameters. The mechanokinetic effects of thiacalix[4]arenes C-1191 and C-1192 cannot be considered specific regarding some systems, involved in the realization of the contraction-relaxation cycle; it can only be predicted that the changes in some mechanokinetic parameters of the rhythm activity of the myometrium were conditioned by the change in the involvement of mitochondria in the regulation of the contractile function of the myometrium.

Calix[4]arene C-956 demonstrated a vivid concentration-dependent (10–100 μM) inhibitory effect on H^+-Ca^{2+}-exchanger of the internal mitochondrial membrane ($K_i = 35.1 \pm 7.9$ μM). The inhibitory effect was accompanied by the decrease in the initial velocity V_0 and the increase in the value of the characteristic time $\tau_{1/2}$ for ΔpH-induced release of Ca^{2+}. At the same time, the mentioned calix[4]arene did not affect the energy-dependent accumulation of Ca^{2+} by mitochondria. Thus, the effect of calix[4]arene may be directed at the increase in the concentration of Ca^{2+} in the mitochondrial matrix.

The results of the studies demonstrated that sulfur-containing calix[4]arene C-1193 may be used for targeted effect on the functional activity of mitochondria. This thiacalix[4]arene also had a concentration-dependent effect (0.01-10 μM) inhibiting NADH and $FADH_2$ oxidation in mitochondria. Depending on the concentration (0.01–100 μM), the mentioned calix[4]arene inhibited the formation of reactive oxygen species in mitochondria. At the same time, the concentrations of 1 and 10 μM of C-1193 did not change the hydrodynamic diameter of mitochondria. The selected calix[4]arene had a very effective concentration-dependent (0.001–100 μM)

inhibitory effect on the nitrogen oxide synthesis by mitochondria. The inhibition constant, estimated in Hill's coordinates, is 5.5 ± 1.7 nM (n = 7), so this compound is a high-affinity blocker of the endogenous generation of NO in mitochondria. The abovepresented results allow for an assumption that compound C-1193 is capable of both inhibiting the accumulation of Ca^{2+} in mitochondria of the uterine smooth muscles and the synthesis of reactive oxygen species of nitrogen and oxygen in these structures. We used our own experimental data to develop the imitation model of the action of C-1193 on the investigated parameters of mitochondria functioning in terms of Petri nets.

It is noteworthy that high-affinity inhibitors of the sodium pump, calix[4]arenes C-97, C-99, and C-107, are capable of inhibiting the energy-dependent accumulation of Ca^{2+} in mitochondria, modulating the functioning of their electron transfer chain and enlarging the hydrodynamic diameter of organelles. The action of the mentioned compounds (100 nM) on the functioning of isolated mitochondria of the smooth muscle was studied. Using Ca^{2+}-sensitive fluorescent dye Fluo-4 AM, it was shown that the selected calix[4]arenes inhibit the energy-dependent accumulation of Ca^{2+} by mitochondria and lead to the release of previously accumulated Ca^{2+}. The analysis of the fluorescent response of NADH and FAD in mitochondria demonstrates that the action of the investigated compounds on the functional activity of the electron transfer chain is related to the initial stimulation of the complex activity and subsequent inhibition of Ca^{2+}-dependent NAD-containing dehydrogenases of Krebs cycle. The method of photon correlation spectroscopy was used to show that selected calix[4]arenes enlarge the volume of isolated organelles.

It was found that calix[4]arene chalcone amide C-1011 has a differentiated effect on the viability of adenocarcinoma cells of the mammary gland of mice 4T1 with different levels of expression for the adapter protein Ruk/CIN85 (this research was conducted in cooperation with Professor L.B. Drobot and her colleagues). The impact of calix[4]arenes with 4 chalcone amide groups on the polarization of the membrane in mitochondria and the viability of adenocarcinoma cells of the mammary gland of mice 4T1, the surrogate model for triple-negative breast cancer of humans and on its highly malignant subline with overexpression of the adapter protein Ruk/CIN85 was investigated. It was shown that the mitochondrial membranes of control (Mock) cells had a higher polarization level (67.80 ± 8.82 c.u., n = 5) compared to 4T1 cells with overexpression of Ruk/CIN85 (RukUp cells) (25.42 ± 2.58 c.u., n = 4). After the incubation of cells with 1 μM calix[4]arene C-1011, CCCP-sensitive component of polarization of mitochondrial membranes decreased (by almost 50%) in Mock cells but did not change in RukUp cells compared to the control conditions of the incubation. It was demonstrated that 1 μM calix[4]arene C-1011 inhibited the survival of Mock cells by 45% but did not have a considerable effect on RukUp cells. The data obtained demonstrate the need for further studies on calix[4]arene chalcone amides as potential antitumor preparations.

Thus, the results of our interdisciplinary experiments made it possible to identify calix[4]arenes that are selective in their action and may be useful in studying the mitochondrion of smooth muscle cells in research, aimed at elucidating the mechanisms of transmembrane calcium exchange in mitochondria, the patterns of formation of their membrane potential, and the participation of these subcellular structures in the control of smooth muscle contraction-relaxation mechanokinetics.

IV. ATP-hydrolase of contractile proteins and calix[4]arenes

The analytical and preparative methods of biochemical studies, including spectrophotometry, vertical electrophoresis in PAAG, ion-exchange chromatography, gel-filtration, spectrofluorimetry, laser correlation spectroscopy, confocal microscopy, flow cytofluorimetry, enzymological methods and the methods of enzymatic kinetics were used in the study of the effect of calix[4]arenes on the activity of ATPases of actomyosin complex and the subfragment-1 of myosin of uterine smooth muscles.

The testing of a wide range of calix[4]arenes, functionalized by the upper brim of the macrocycle with pharmacophore amide, amidine, urea, ammonia, sulphonyl, carboxyl, and phosphoryl groups demonstrated that 10 of them: C-95, C-97, C-99, C-54, C-100, C-150, C-101, YA-012, C-107, C-91 in the concentration of 100 μM have an expressed effector (activating or inhibiting) effect on the activity of Mg^{2+}-dependent ATPase of the actomyosin complex of the uterine smooth muscle. The most noteworthy effects were registered for calix[4]arenes C-107, C-99, and C-97, so they were selected for further studies.

The studies on the complex of contractile proteins demonstrated that at the action of calix[4]arene C-107, the activity of ATPase of actomyosin increased depending on the increase in the compound concentration, and at the concentration of 100 μM reached 230 ± 12% compared to the control value (activation constant $A_{0.5}$ = 9.6 ± 0.7 μM) [4]. It was found that calix[4]arenes C-99 and C-97 had an inhibitory effect on the ATPase activity of actomyosin of myometrium, which, at the concentration of 100 μM for both compounds, was 50 ± 9% and 70 ± 8% compared to the control (inhibition constants $I_{0.5}$ were 98.8 ± 1.3 μM and 84.0 ± 2.0 μM, respectively).

A subfragment-1 (head) of myosin (about 100 kDa) was isolated from the smooth muscles via actomyosin cleaving using α-chymotrypsin with further separation on the column with DEAE-Sepharose CL-6B in the gradient of KCl solution from 0 to 0.2 M. The method of laser correlation spectroscopy was used to measure the average hydrodynamic diameter of the myosin head, which was 25 ± 3 nm.

The same method helped determine the dependence of the hydrodynamic diameter of the subfragment-1 of myosin on the concentration of calix[4]arenes. When the concentration of calix[4]arene C-99 increased, there was an insignificant increase in the hydrodynamic diameter of the subfragment-1 of myosin, and in the presence of calixarene C-107, there was a more expressed concentration dependence, which demonstrates a different character of the interaction between these compounds and subfragment-1.

The experiments on investigating the impact of calix[4]arenes on the activity of ATPase of the subfragment-1 of myosin demonstrated that calix[4]arene C-107, used in the concentration range from 10 to 100 μM increases ATP hydrolysis by ATPase depending on the increase in the concentration of calix[4]arene, at the concentration of 100 μM, it activates the enzyme more than twice (activation coefficient $A_{0.5}$ = 25 ± 4 μM). According to the increase in the concentration, calix[4]arene C-99 has a dose-dependent inhibiting effect on ATP-hydrolase activity, which in the case of 100 μM was 77 ± 4% compared to the control (inhibition coefficient $I_{0.5} \approx 43 \pm 8$ μM).

To find out the mechanism of the inhibitory effect of calix[4]arene C-99 on ATPase of myosin, we performed computer modelling for the interaction between calixarene and subfragment-1, which is a functionally active part of a myosin molecule. The results of the modelling demonstrate that calix[4]arene C-99 may interact with the active center of the myosin molecule near the binding site of ATP. In this case, it involves the residues of lysine and threonine, which interact with the phosphonyl group of calixarene and Mg^{2+} ions.

We also learned selected biochemical and physical-chemical mechanisms of action of thiacalix[4]arenes as factors of activity protection for Mg^{2+},ATP-hydrolase system of subfragment-1 of myosin of the smooth muscle from the negative impact of heavy metal ions on them. The toxicity of thiacalix[4]arenes was determined for myometrium cells. In the practical aspect, thiacalix[4]arene C-800 may be viewed as a promising medical means to eliminate the toxic effects of heavy metals on the cells. Since the fluorescence of thiacalix[4]arene C-800 increased in the myocytes (in the example of myometrium cells), incubated with Zn cations, and depended on the concentration of this cation and the incubation time, the following may be stated: it is reasonable to view the mentioned thiacalix[4]arene as a fluorescent probe for qualitative and quantitative determination of intracellular Zn cations. It is important that the selectivity phenomenon regarding the cations of bivalent metals is notable for this thiacalix[4]arene, since its intracellular fluorescence was not induced by other bivalent cations (Ca^{2+},Mg^{2+}, Pb^{2+}, Cd^{2+}).

Thus, as a result of our interdisciplinary studies, it has been demonstrated that selected calix[4]arenes can serve as effectors of ATP hydrolase activity of smooth muscle contractile proteins, and some of these compounds are factors protecting this activity from the negative effects of heavy metal ions.

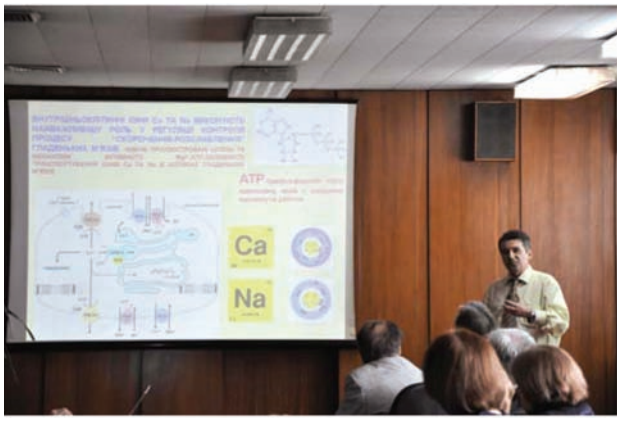
In general, the results of our investigations of the action of calix[4]arenes on energy-dependent Ca^{2+}-transporting systems, contractile proteins, and directly on contractions-relaxations of smooth muscles were reported in numerous domestic and international symposia, published in multiple articles in the leading scientific journals of Ukraine and the world, summarized in five monographs, and used to obtain two patents for an invention.

V. Mechanokinetics and thermodynamics of the contractions-relaxations of visceral smooth muscles

The Department proposed the empirical multiparameter method of complex mechanokinetic analysis of the spontaneous contractile response of

visceral smooth muscles under isometric and isotonic modes. The graphical linearization criterion of the possibility of applying the elaborated method to describe the total mechanokinetic curve of the contraction-relaxation cycle was elaborated. The quantitative regularities, subject to three universal mechanokinetic laws, were determined. The proposed method allows for determining a number of quantitative indices for a thorough description of the mechanokinetic curve. It was described how the method may be applied while studying the effect of physical-chemical (hypermagnesium), metabolic (uterotonic peptide hormone oxytocin), and pharmacological (using the example of calix[4]arene C-90) factors on electro- and pharmaco-mechanical coupling. The formulas for the change in the length of the muscle preparation in time, instant rate of contraction–relaxation, mechanic work, and muscle strength under the isotonic mode were obtained. The elaborated method was involved in the energy interpretation of the impact of acetylcholine neurotransmitter on isotonic contraction of the smooth muscle. It was demonstrated that the following mechanokinetic and energy parameters are most sensitive to the stimulating effect of acetylcholine on the isotonic contraction of the muscle preparation: the maximal contraction velocity V_C (at a time point of inflexion τ_C at the level of the contraction phase); the maximal mechanic work ΔA_{max} (done per time τ_0 of achieving the contraction amplitude); the mechanic work $\Delta A^{\tau C}$ (done per the characteristic time τ_C); the maximal contraction intensity N_{max} (at a time point); the average contraction intensity N_m. It is assumed that the proposed methodology may be promising for the universal quantitative interpretation of the effect of physical-chemical, physiologically active, and pharmacological factors on the mechanokinetics and the energetics of the smooth muscle functioning.

The staff of the Department believes that the results, presented in this review, are promising for further study of the biochemical aspects of the process of Ca^{2+}-dependent contraction-relaxation of smooth muscles, deeper understanding of the regularities of the impairment of ion, molecular, membrane, and cellular mechanisms of controlling the contractile function of the smooth muscles under pathology and for the elaboration of new pharmacological preparations based on macrocyclic compounds, calixarenes, to normalize the contractile function of smooth muscle cells in case of its impairment.



S.O. Kosterin, the full member of the NAS of Ukraine, is giving a lecture on the issues of biophysical chemistry of ion transport in the smooth muscles

Following the results of the scientific studies, in 2023, the employees of the Department of Muscle Biochemistry received the prizes named after the following outstanding scientists of the National Academy of Sciences of Ukraine: named after P.H. Kostiuk, the full member of the NASU (the full member of the NASU S.O. Kosterin, Doctor of Biosciences L.H. Babich, Doctor of Biosciences S.H. Shlykov) and named after O.V. Palladin, the full member of the NASU (Doctor of Biosciences Yu.V. Danylovych, Doctor of Biosciences H.V. Danylovych)

It should be noted that traditionally, the Department of Muscle Biochemistry of the O.V. Palladin Institute of Biochemistry of the NAS of Ukraine pays great attention to training new scientific generations in the field of muscle biochemistry and biochemical membranology. In the past ten years,

under the guidance of S.O. Kosterin, six doctorates (S.H. Shlykov, L.H. Babich, Yu.V. Danylovych, O.V. Tsymbaliuk, T.O. Veklich, H.V. Danylovych) and six Ph.D. dissertations have been defended. The Institute's specialists have been giving relevant courses of lectures in the Chairs of Biochemistry and Biophysics of the Taras Shevchenko National University of Kyiv for many years, and the Institute serves as a foundation for students to prepare their course, Bachelor, and Master research papers, dedicated to biochemistry and biophysical chemistry of muscles.

The employees of the Department of Muscle Biochemistry are involved in active systematic teaching activity, give lectures at the Taras Shevchenko National University of Kyiv (the NSC "Institute of Biology and Medicine", the Institute of High Technologies) and the National University of "Kyiv-Mohyla Academy" and to the post-graduates of the O.V. Palladin Institute of Biochemistry of the NAS of Ukraine (the full member of the NAS of Ukraine S.O. Kosterin, Doctor of Biosciences L.H. Babich, Doctor of Biosciences Yu.V. Danylovych, Doctor of Biosciences T.O. Veklich, Doctor of Biosciences H.V. Danylovych).

In this jubilee year of our Institute, the employees of the Department of Muscle Biochemistry of the O.V. Palladin Institute of Biochemistry are filled with hopes for further active work aimed at conducting interdisciplinary studies in the field of biochemistry, biophysical chemistry, and biochemical membranology of smooth muscle cells.

Selected monographs and textbooks written by employees of the Department of Muscle Biochemistry

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2. Kurskyi MD, Kosterin SO, Vorobets ZD. Regulations of intracellular calcium concentration in muscles (a monograph). Kyiv: Naukova Dumka, 1987. 144 p. (In Ukrainian).
3. Kosterin SO. Calcium transportation in smooth muscles (a monograph). Kyiv: Naukova Dumka, 1990. 216 p. (In Ukrainian).
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The scientific achievements of the employees of the Department of Muscle Biochemistry have also been published in personal and collective monographs, some of which are presented in this picture

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Conflct of interest. Authors have completed the Unified Conflicts of Interest form at http://ukr-biochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

ВІДДІЛ БІОХІМІЇ М'ЯЗІВ: КАЛІКСАРЕНИ ЯК МОДУЛЯТОРИ ЕНЕРГОЗАЛЕЖНИХ Ca^{2+}-ТРАНСПОРТНИХ НАСОСІВ У ГЛАДЕНЬКИХ М'ЯЗАХ

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У науково-історичному огляді, присвяченому новітнім здобуткам відділу біохімії м'язів Інституту біохімії ім. О. В. Палладіна НАН України, узагальнено результати міждисциплінарних досліджень внутрішньоклітинного кальцієвого гомеостазу у гладеньких м'язах (на прикладі міометрія), виконаних на стику біохімії, фізичної та органічної хімії, біофізики, а також математичного й комп'ютерного моделювання. Підкреслено, що розглянуті в роботі вибрані калікс[4]арени проявляють селективну дію як інгібітори Mg^{2+},АТР-залежних кальцієвої та натрієвої помп (електроензими Ca^{2+},Mg^{2+}-АТРаза; Na^{+},K^{+}-АТРаза) плазматичної мембрани гладенькоm'язових клітин, що надає можливість для забезпечення керованої модуляції внутрішньоклітинного Ca^{2+}-гомеостазу та скоротливої активності міометрія. Одержані дані також вказують на те, що вибрані калікс[4]арени можна розглядати як сполуки, що є доцільними для ефективного дослідження функціонування мітохондрій у гладеньком'язових клітинах, зокрема механізмів трансмембранного обміну Ca^{2+}, закономірностей формування мембранного потенціалу та внеску цих субклітинних структур у контроль механокінетики циклу «скорочення–розслаблення». Показано, що деякі калікс[4]арени діють як ефектори АТРазної активності скоротливих протеїнів; вони протектують цю активність від інгібувального впливу іонів важких металів. Сукупність результатів, що були одержані, окреслює біохімічні підходи до тонкої регуляції кальцієвих потоків і скоротливості гладенького м'яза та підкреслює потенціал калікс[4]аренів як селективних «молекулярних платформ», корисних для дослідження фундаментальних і прикладних (у галузі біомедицини) проблем сучасної фізико-хімічної біології м'язів.

Ключові слова: гладенькі м'язи; міометрій; Ca^{2+},Mg^{2+}-АТРаза; Na^{+},K^{+}-АТРаза; калікс[4]арени; плазматична мембрана; мітохондрії, Ca^{2+}-сигнал, спонтанні скорочення.

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