ISSN 2409-4943. Ukr. Biochem. J., 2020, Vol. 92, N 1

UDC 577.352.4+544.147+544.176+544.168 doi: https://doi.org/10.15407/ubj92.01.021

INHIBITION OF Na^+,K^+-ATPase AND ACTIVATION OF MYOSIN ATPase BY CALIX[4]ARENE C-107 CAUSE STIMULATION OF ISOLATED SMOOTH MUSCLE CONTRACTILE ACTIVITY

T. O. VEKLICH[1], R. D. LABYNTSEVA[1], O. A. SHKRABAK[1], O. V. TSYMBALYUK[2], R. V. RODIK[3], V. I. KALCHENKO[3], S. O. KOSTERIN[1]

[1]Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv;
[2]Institute of High Technologies, Taras Shevchenko National University of Kyiv, Ukraine;
[3]Institute of Organic Chemistry, National Academy of Sciences of Ukraine, Kyiv;
e-mail: veklich@biochem.kiev.ua; otsymbal@bigmir.net; manli@ioch.kiev.ua

Received: 04 Jule 2019; **Accepted:** 29 November 2019

The discovery of compounds that might modify myometrial contractility is an important area of researches. In our previous experiments, we found that some representatives of macrocyclic compounds family – calix[4]arenes – can modify the enzymatic and transport activity of membrane-bound cation-transport ATP hydrolases. The aim of this work was to study and compare the effect of calix[4]arene C-107 on the enzymatic activities of Mg^{2+}-dependent ATPases of the uterine smooth muscle, namely: ouabain-sensitive Na^+,K^+-ATPase, plasma membrane Ca^{2+}-independent "basal" Mg^{2+}-ATPase, ATPase of the actomyosin complex and myosin subfragment-1, with effect on the contractile activity of the myometrium. It was shown that calix[4]arene C-107 efficiently inhibited myometrium Na^+,K^+-ATPase (I_{50} = 54 ± 6 nM) selectively to other ATP-hydrolases of the plasma membrane and simultaneously activated the enzymatic activity of the myosin ATPase of smooth muscles (A_{50} = 9.6 ± 0.7 μM). Such reciprocal biochemical effects led to the stimulation of the smooth muscle contractile activity that was demonstrated by the tensometric method using different isolated smooth muscles. Calix[4]arene C-107 was shown to stimulate the increase of the tonic component of myometrium contractions induced by oxytocin, as well as contractions of the caecum muscles induced by high-potassium solution or acetylcholine, and to maintain increased tension for a long time. Thus, calix[4]arene C-107 is a prospective compound for enhancing the smooth muscle basal tone and/or contraction in case of hypotonic dysfunctions.

Keywords: Na^+,K^+-ATPase, Mg^{2+}-ATPase, myosin ATPase, plasma membrane, smooth muscle cell, myometrium, calix[4]arene.

Smooth muscles (SM) play an important role in supporting the vital functions of the human body. SM contraction and relaxation is the basis for the functioning of the blood and lymph vessels, the gastrointestinal tract, respiratory tract, eye pupil and uterus [1, 2]. For the SM contraction/relaxation, the Ca^{2+} level in the cytoplasm has to be changed dynamically from 100 nM at rest to 1 μM and even more during excitation [3, 4].

It is well known that Mg^{2+}-dependent ATP-hydrolase enzymatic systems are very important for the regulation of muscle (including SM) contraction [5, 6]. The plasma membrane (PM) of SM cells contains the following types of ATPases: Na^+,K^+-ATPase, Ca^{2+}-transporting Ca^{2+},Mg^{2+}-ATPase (with high affinity to Ca ions), Ca^{2+}-independent ("basal") Mg^{2+}-ATPase, Mg^{2+}-independent Ca^{2+}-ATPase (with low affinity to Ca ions).

The PM Na^+,K^+-ATPase is essential for cell life since it maintains a high concentration of K^+ and a low concentration of Na^+ in myoplasm, providing excitability and other cellular functions and processes [7, 8]. The Na^+,K^+-pump is involved in the regulation of the intracellular concentration of Ca ions ($[Ca^{2+}]_i$). The Na^+,K^+-pump inhibition by cardiac steroids during the treatment of heart failu-

© 2020 Veklich T. O. et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

re provides an increase of $[Ca^{2+}]_i$ level due to a reverse work of the Na^+/Ca^{2+}-exchanger induced by the elevated Na^+ level in cardiomyocytes. It is also known that there is a direct mechanical link between Na^+,K^+-ATPase (probably via its caveolar pool), Na^+/Ca^{2+}-exchanger and the protein structures of the endoplasmic reticulum membrane [9, 10]. Interactions between these Ca^{2+}-transporting structures form a structural-functional complex, known as "calcium signaling microdomain", and were found, in particular, in the cardiac and SMs [9]. It is important to emphasize that neurotransmitter receptors, such as purine receptors, muscarinic acetylcholine M3 receptors (coupled with $G_{q/11}$), and bradykinin receptors B2, are also to involve in caveolar complexes [11].

The contraction of the myometrium is associated with the function of the main structural and contractile protein of the uterine SM – actomyosin, where myosin has enzymatic activity, namely, the hydrolyzation of ATP. Myosin is called a molecular motor protein that provides energy for muscle contraction. Due to the ATP hydrolysis, myosin uses the chemical energy stored in the high-energy bonds of ATP and converts it in the directed movement of myosin relative to actin filaments [12].

The myosin molecule contains six subunits: two heavy chains and two pairs of light chains. The heavy chain consists of a globular subfragment-1 (head) and an α-helical (rod) domain, which is involved in the formation of myosin filaments. The myosin subfragment-1 (S-1) consists of a motor domain containing ATP- and actin-binding sites and a regulatory domain (lever arm) (myosin light chain binding site). Conformational changes in the myosin active site caused by ATP hydrolysis are amplified by subdomains of the motor domain: switch 1, switch 2, (relay and converter respectively) and transferred to the lever arm, which plays an important role in the generation of the force and movement [13]. The complex conformational transformations result in the myosin movement along the actin filament that leads to muscle contraction. Therefore, myosin-catalyzed ATP hydrolysis is considered to be the most important part of the molecular mechanism of muscle contraction.

Abnormal contractile function of the myometrium of women often leads to various pathologies: weak labor, spontaneous abortion, premature birth, miscarriage, atony, hypo- or hypertonic uterine [14]. The rate of preterm birth on average in the world is 9.6% [15, 16]. These pathologies are mostly caused by a disorder in the function of the membrane-bound cation transport systems and/or contractile proteins. Therefore, the searching of compounds capable of modifying the contractility of the myometrium during mentioned pathologies would be promising for medicine.

In this aspect, calixarenes are considered to be promising agents. These cup-shaped macrocyclic compounds can be obtained by facile, high-yield synthesis via cyclocondensation of para-substituted phenols and formaldehyde. Some calixarenes have antiviral, bactericidal, antitumor, and antithrombotic activity and can be effective inhibitors and activators of the enzymatic, receptor, and transport membrane-bound proteins [17-19]. In our previous experiments, we identified a number of calix[4]arenes, namely calix[4]arene C-107, which can modify the enzymatic and transport activity of membrane-bound cation-transport ATP hydrolases and the enzymatic activity of contractile proteins [1, 20].

The aim of this work is to carry out a comparative study of the effect of calix[4]arene C-107 on the enzymatic activity of Mg^{2+}-dependent ATPases of the uterine SMs: ouabain-sensitive Na^+,K^+-ATPase, Ca^{2+}-independent "basal" Mg^{2+}-ATPase of PM, ATPase of the actomyosin complex and SM myosin subfragment-1, as well as ability of this compound to modify the contractile activity of the myometrium.

Materials and Methods

Synthesis of calix[4]arene C-107. Calix[4]-arene C-107 (5.17-di (phosphon-2-pyridylmethyl) amino-11,23-di-tert-butyl-26,28-dihydroxy-25,27-dipropoxycalix[4]arenes) (Fig. 1) was synthesized and characterized using NMR and infrared spectroscopy in the Phosphoranes Chemistry Department of the Institute of Organic Chemistry, NASU (head of the Department - Academician of NASU, prof. V. I. Kalchenko). The synthesis of calix[4]arene C-107 was described previously [20].

Biochemical studies. Biochemical studies were carried out in the Department of Muscle Biochemistry of Palladin Institute of Biochemistry, NASU (head of the Department - Academician of NASU, prof. S. O. Kosterin).

Preparative biochemistry. The PM fraction of uterine SM cells was isolated from the porcine myometrium as described previously [21, 22]. The protein concentration in the membrane fraction was determined by the Bradford assay [23] with Coomassie Brilliant Blue G-250 reagent.

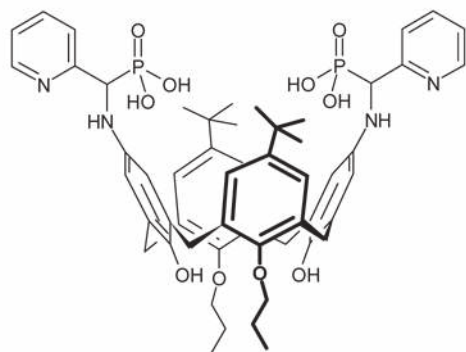

Fig. 1. The structural formula of calix[4]arene C-107

Actomyosin was obtained from SM cells of the porcine uterine by the M. and K. Barany method [24] and A. Weber [25] with some modifications. Subfragment-1 (head, S1) of uterine SM myosin was prepared by cleaving actomyosin by α-chymotrypsin according to Weeds and Taylor with some modifications.

Enzymatic studies. Determination of the activities of membrane-bound ATP hydrolases. The "total" Mg^{2+},Na^{+},K^{+}-ATPase activity was determined in the PM fraction of myometrial cells as described previously [26] at 37 °C in the medium (0.4 ml) containing (mM): 1 ATP, 3 $MgCl_2$, 125 NaCl, 25 KCl, 1 EGTA, 20 Hepes-Tris buffer (pH 7.4), 1 NaN_3 (mitochondrial ATPase inhibitor [27]), 0.1 μM thapsigargin (selective inhibitor of the endo(sarco)plasmic reticulum Ca^{2+},Mg^{2+}-ATPase [27]) and 0.1% digitonin (to permeabilize PM [28]). The amount of protein in the sample of the membrane fraction was 20-30 μg. The incubation time was 4 min. The enzymatic reaction was initiated by adding of the PM suspension (50 μl) to the incubation medium, and terminated by adding 1 ml of the stop solution composed of: 1.5 M sodium acetate, 3.7% formaldehyde, 14% ethanol, 5% three-chloride acetate, pH 4.3 (at 80 °C). The presence of EGTA, Ca^{2+} chelator, in the incubation medium ensures the binding of endogenous Ca ions.

Ca^{2+}-independent "basal" Mg^{2+}-ATPase activity was determined in the same incubation medium but with 1 mM ouabain (a selective inhibitor of Na^{+},K^{+}-ATPase [29]).

The "ouabain-sensitive" Na^{+},K^{+}-ATPase activity was calculated by the difference between the "total" ATPase and Mg^{2+}-ATPase activities.

The incubation medium described above, without PM fraction, was used as a control for non-enzymatic ATP hydrolysis. An aqueous solution of PM fraction was used as a control for the amount of endogenous inorganic phosphate (P_i) in the membrane fraction. Thus, the specific "total" ATPase activity was calculated as the net between the amount of P_i formed in the incubation medium in the presence and absence of PM fragments, and the amount of endogenous P_i in the membrane fraction. The amount of the reaction product P_i was determined by the W. Rathbun, V. Betlach method [30].

The average magnitude of the specific activity of PM Na^{+},K^{+}-ATPase and Ca^{2+}-independent "basal" Mg^{2+}-ATPase were 10.2 ± 0.7 and 18.1 ± 1.2 μmol P_i/mg of protein per 1 h ($M \pm m$; $n = 7$), respectively.

Determination of ATP hydrolase activities of contractile proteins. The ATPase activity of actomyosin and myosin subfragment-1 in porcine myometrium was determined at 37 °C in an incubation medium (total volume 1 ml) containing (mM): Tris-HCl buffer (pH 7.2) – 5, KCl – 100, $CaCl_2$ – 0.01, $MgCl_2$ – 5, ATP – 3 (standard conditions). The actomyosin concentration in the incubation medium was 20 μg/ml, and myosin subfragment-1 concentration ranged from 5 to 15 μg/ml. The incubation time was 2 min. Samples without actomyosin and myosin subfragment-1 were used as a control for the non-enzymatic ATP hydrolysis. The amount of inorganic phosphate P_i cleaved during the ATP hydrolase reaction was determined by the P. S. Chen et al. method [31].

The average magnitude of the specific activity of actomyosin ATPase and subfragment-1 ATPase were 20 ± 4 and 35 ± 8 nmol P_i/mg of protein per 1 min ($M \pm m$; $n = 7$), respectively.

To investigate the effect of calix[4]arene C-107 on the specific enzymatic activity of membrane-bound ATP hydrolases and ATP hydrolases of contractile proteins, the standard incubation mediums (described above) were used, with the addition of the calix[4]arene solution of corresponding concentration. We used a concentrated (1 mmol) solution of calix[4]arene C-107 in DMSO, which was then diluted in water. The magnitude of ATP hydrolase activity determined in the standard incubation medium without calix[4]arene C-107 was taken as 100% ("zero point").

Kinetics of PM Na^{+}, K^{+}-ATPase and ATPase of myometrial actomyosin and myosin subfragment-1. The magnitude of inhibition coefficient I_{50} and Hill coefficient n_H were calculated using concentration dependence curve plotted in logarithmic scale according to the linearized Hill equation [32]:

$$\lg[(V_0-V)/V] = n_H \cdot \lg I_{50} - n_H \cdot \lg I, \quad (1)$$

where V is the specific enzymatic activity, V_0 is the specific enzymatic activity in the absence of effector in the incubation medium, I is the concentration of the inhibitor in the incubation medium.

The magnitude activation coefficient A_{50} and the Hill coefficient were calculated using the linearized Hill plots of the Hill equation [32]:

$$\lg[(V_{max}-V)/(V-V_0)] = n_H \cdot \lg A_{50} - n_H \cdot \lg A, \quad (2)$$

where V_{max} is the maximum specific enzymatic activity in the presence of activator in the incubation medium, A is the activator concentration in the incubation medium.

Registration of smooth muscle contractile activity and studying the effect of calix[4]arene C-107 on this activity. For registration of isometric contractile activity, the sample of the rat longitudinal muscles of the uterine horns and the circular muscles of the large intestine (the mucosa-free circular muscle strips from *caecum*) were used [33]. Muscle strips (average size – 1.5×10 mm) were placed in a chamber (volume 2 ml) of a tensometric device at constant temperature 37 °C with flowing Krebs solution (flow rate – 5 ml/min.). The contractile activity of the samples was analyzed after maintaining for one hour in the chamber at a constant force of 10 mN. The content of Krebs solution (mM): 120.4 NaCl, 5.9 KCl, 15.5 $NaHCO_3$, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.5 $CaCl_2$, 11.5 glucose, pH 7.4. The induced contractile activity was triggered by a high-potassium solution and acetylcholine, an agonist of muscarinic cholinergic receptors. The high-potassium solution (80 mM) was prepared from Krebs solution by replacing the required amount of Na^+ with an equimolar amount of K^+. A concentration of acetylcholine chloride was 10 μM; a concentration of uterotonic hormone oxytocin was 0.1 IU. Calix[4]arene C-107 dissolved in DMSO was added to the Krebs solution at a concentration of 10 μM (final aliquot of DMSO was 0.25% of the total solution volume). Control contractions were studied in solutions containing 0.25% DMSO.

Kinetic analysis of contractile activity. The kinetic analysis of SM contractions in samples was carried out using the calculation of the maximum rate of the contraction phase (V_{nc}) and relaxation phase (V_{nr}) adjusted to contraction amplitude according to the method [34]. This method allows to calculate f_m-independent parameters: adjusted to fm magnitude maximum rates of relaxation and contraction (taking into consideration the fundamental similarity between the profiles of the contractile muscle phases) V_n:

$$V_n = \left| -(1/f_m)(df/dt) = \frac{(n-1)^{\frac{n-1}{n}} \cdot (n+1)^{\frac{n+1}{n}}}{4n\tau} \right|$$

To analyze the mechanokinetic curves, the contraction and relaxation phases were linearized in the coordinates $\{\ln[(f_m-f)/f]; \ln t\}$, where f – the instantaneous (at the time point t) force, f_m – the maximum force, τ – the characteristic time (equal to the time when the instantaneous force reaches the half-maximum force ½ f_m), n – the slope coefficient of the mechanokinetic curve. The time, when f_m is reached, was taken as the starting point of the relaxation phase $t = 0$. Thus, the part of the contractile response after f_m was considered as a relaxation phase, and the part of the mechanokinetic curve before f_m was considered as the contraction phase.

To quantify changes in the kinetics of contractile responses, the normalized maximum rates of the contraction phase (V_{nc} – from the beginning of the increase in muscle tension to maximum) and relaxation phase (V_{nr} – from the maximum to the basal level of muscle tension) were calculated.

Statistical analysis. Statistical analysis of the obtained enzymatic data was performed by Student's *t*-test. Kinetic and statistical calculations were made using MS Excel software. The experimental data of contractile activity were processed with variation statistics method using OriginPro 8 software.

Reagents. The following reagents were used in the experiments: ATP, Hepes, ouabain, thapsigargin, acetylcholine, α-chymotrypsin, serum albumin, ascorbic acid, tris (Sigma, USA), digitonin, glycine (Merck, Germany), acrylamide (Fluka, Switzerland), oxytocin (Gedeon Richter, Hungary), dithiothreitol (Serva, Germany), N,N-methylenebisacrylamide (Acros organics, Belgium) N,N,N,N-tetramethylethylenediamine (Reanal, Hungary). Other reagents were of domestic production, UHP (ultra-high purity) and HP (high purity) grades.

Results and Discussion

Investigation of the effect of calix[4]arene C-107 on the Mg^{2+}-dependent ATP hydrolase activity in the PM fraction of myometrial cells revealed that this compound at a concentration of 100 μM significantly inhibited Na^+,K^+-ATPase activity of PM of myometrial cells to the level of 2.5 ± 0.3% relative to the control magnitude (taken as 100%) ($M \pm m$;

n = 5) (Fig. 2). However, the studied calixarene at the same concentration did not affect the enzymatic activity of the "basal" PM Mg^{2+}-ATPase: the activity was 90.0 ± 0.6% relative to the control (M ± m; n = 5) (Fig. 2). Thus, calix[4]arene C-107 selectively inhibited the activity of the PM Na^+,K^+-ATPase, without affecting the activity of "basal" PM Mg^{2+}-ATPase.

In addition, the studied calix[4]arene at a concentration of 100 μM was shown to activate Mg^{2+}-dependent ATPase of the myometrium actomyosin more than 2-fold (230 ± 12%) (M ± m; n = 5) (Fig. 2) that is completely opposite to its inhibitory effect on the activity of the PM Na^+,K^+-ATPase. Obviously, the opposite effects of calix[4]arene C-107, which molecule contains two aminopyridine groups on the upper rim of the calixarene cup, significantly caused by the different interaction with structurally and functionally different Mg^{2+}-ATPases which also have different regulation and localization. The properties of the surrounding media can also be important, in particular, the PM phospholipid matrix can affect the sensitivity of Na^+,K^+-ATPase to calixarene.

SM actomyosin is a multiprotein complex, which, in addition to myosin and actin, composed of regulatory proteins (tropomyosin, caldesmon), myosin light chain kinase and others. Actomyosin ATPase responsible for ATP hydrolysis is localized in the catalytic domain of subfragment-1 (myosin head). The isolated myosin head is a sufficient functional unit of myosin since it has the full ATPase activity of myosin and the ability to interact with actin. Subfragment-1 has high solubility (unlike myosin) in aqueous low ionic strength solutions and has ATPase activity close to native myosin, therefore, it is a convenient model for studying the effect of calixarenes [35]. That is why our subsequent research were studying calix[4]arene C-107 influence the ATP hydrolysis of contractile proteins using myosin subfragment-1.

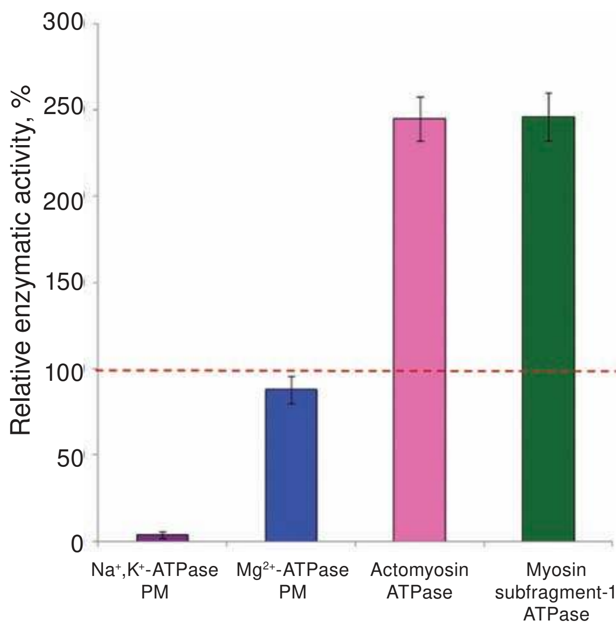

Fig. 2. The "reciprocal" effect of calix[4]arene C-107 (100 μM) on the ATP-hydrolase activity of myometrial cells (M ± m, n = 5). Calix[4]arene C-107 at a concentration of 100 μM inhibits the activity of the PM Na^+,K^+-ATPase and stimulates the activity of the "mechanochemical" (myosin) ATPase. 100% is the magnitude of each enzymatic activity in the absence of calix[4]arene C-107 in the incubation medium

Calix[4]arene C-107 (100 μM) increased the ATPase activity of the myometrial myosin subfragment-1 to 246 ± 16%, relative to control value (M ± m; n = 5) (Fig. 2). These findings indicate a similar activating effect of calix[4]arene C-107 on the myometrial actomyosin complex ATPase and myosin subfragment-1, and allow us to suggest that the target of the calix[4]arene C-107 action is specifically the myosin subfragment-1.

Studying the effect of various concentrations of calix[4]arene C-107 (10^{-8}–10^{-4} M) on the ATPase activity in myometrial PM, revealed that calix[4]arene C-107 inhibited PM Na^+,K^+-ATPase activity in whole concentration range. The calculated inhibition coefficient I_{50} for calix[4]arene C-107 was 54 ± 6 nM, the Hill coefficient n_H was 0.40 ± 0.08 (M ± m; n = 5). Moreover, this compound in the same concentration range had practically no effect on the activity of the "basal" PM Mg^{2+}-ATPase (Fig. 3). Thus, calix[4]arene C-107 not only selectively (relative to other PM ATPases) inhibits transport Mg^{2+}-dependent Na^+,K^+-ATPase but also with high efficiency. This probably leads to the calix[4]arene-induced dissipation of the trans-sarcolemma sodium and potassium gradients.

Calix[4]arene C-107 (10^{-8}–10^{-4} M) was also shown to less efficiently activate ATPase of the actomyosin complex (the activation coefficient A_{50} is 9.6 ± 0.7 μm, the Hill coefficient n_H is 1.7 ± 0.1) and myosin subfragment-1 of myometrium (the activation coefficient A_{50} – 25 ± 4 μM, the Hill coefficient n_H – 2.7 ± 0.4) (M ± m; n = 5) (Fig. 3). Since the target of the calix[4]arene attack on the ATPase of the contractile complex is precisely myosin subfragment-1, that probably causes calix[4]arene C-107-induced interaction of contractile proteins of the uterine SM.

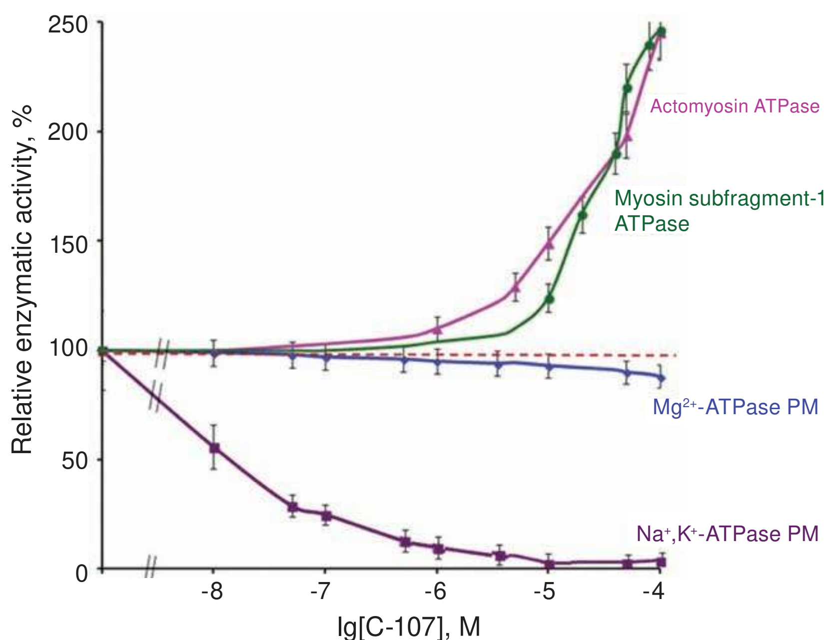

Fig. 3. The effect of the calix[4]arene C-107 on the ATP hydrolase activity of myometrium cells depending on concentration (M ± m, n = 5). 100% is the magnitude of each enzymatic activity in the absence of calix[4]arene C-107 in the incubation medium

Thus, the comparative study of the calix[4]arene C-107 effect on the activity of functionally different Mg^{2+}-dependent ATP-hydrolase systems of the uterine SM showed that this compound affects the activity of membrane-bound transport ATPases and ATPases of contractile proteins oppositely, but ouabain-sensitive PM Na^+,K^+-ATPase is significantly more sensitive to calix[4]arene C-107 action compared to myometrial myosin ATPase. Therefore, we suggested that investigated compound can also affect the myometrium contractile activity in several ways depending on the concentration. Moreover, it was shown earlier that calix[4]arene C-107 inhibited the energy-dependent Ca^{2+}-accumulation in the myometrium mitochondria [36], that can also lead to elevation of Ca^{2+} concentration and also muscle tone.

Our experiments showed that calix[4]arene C-107 (10 μM) enhanced spontaneous contractile activity of the myometrium longitudinal SM, but did not significantly change the maximum tension of contractions activated by the uterotonic hormones oxytocin [37]: the contraction amplitude was 108.9%, on average, relative to the control taken as 100% $P > 0.05$, $n = 4$). Moreover, calix[4]arene C-107 caused significant changes the kinetics of oxytocin-induced contractions, namely, increased the adjusted maximum rate of the contraction phase (V_{nc}) on average 71%, ($P < 0.05$, $n = 4$), and decreased the adjusted maximum rate of the relaxation phase (V_{nr}) on average 31%, ($P < 0.05$, $n = 4$) compared to the control (Fig. 4). Calix[4]arene C-107 increased the tonic component of oxytocin-induced contractions significantly on average 29.4%, ($P < 0.05$, $n = 4$) compared to the control.

It can be assumed that the effect of calix[4]arene C-107 on the force and kinetic parameters of oxytocin-induced contractile responses of myometrial SM can be explained by its influence the enzymatic activity of Na^+,K^+-ATPase (as a consequence, resulting in a disruption of transmembrane ion gradient and membrane potential), as well as by the ability of calix[4]arene C-107 to activate the SM contractile proteins.

Our experiments also showed that calix[4]arene C-107 (10 μM) suppressed the maximum force of annular SM *caecum* contraction activated by high-potassium solution (model of electromechanical activation of excitation-contraction coupling) and by neurotransmitter acetylcholine (model of pharmacomechanical activation of excitation-contraction coupling) (Fig. 4).

Calix[4]arene C-107 significantly reduced (on average 22% ($P < 0.05$, $n = 6$) compared to the control) the adjusted maximum velocity V_{nr} in case of *caecum* muscle contractions activated by high-potassium solution, and reduced V_{nr} even greater in case

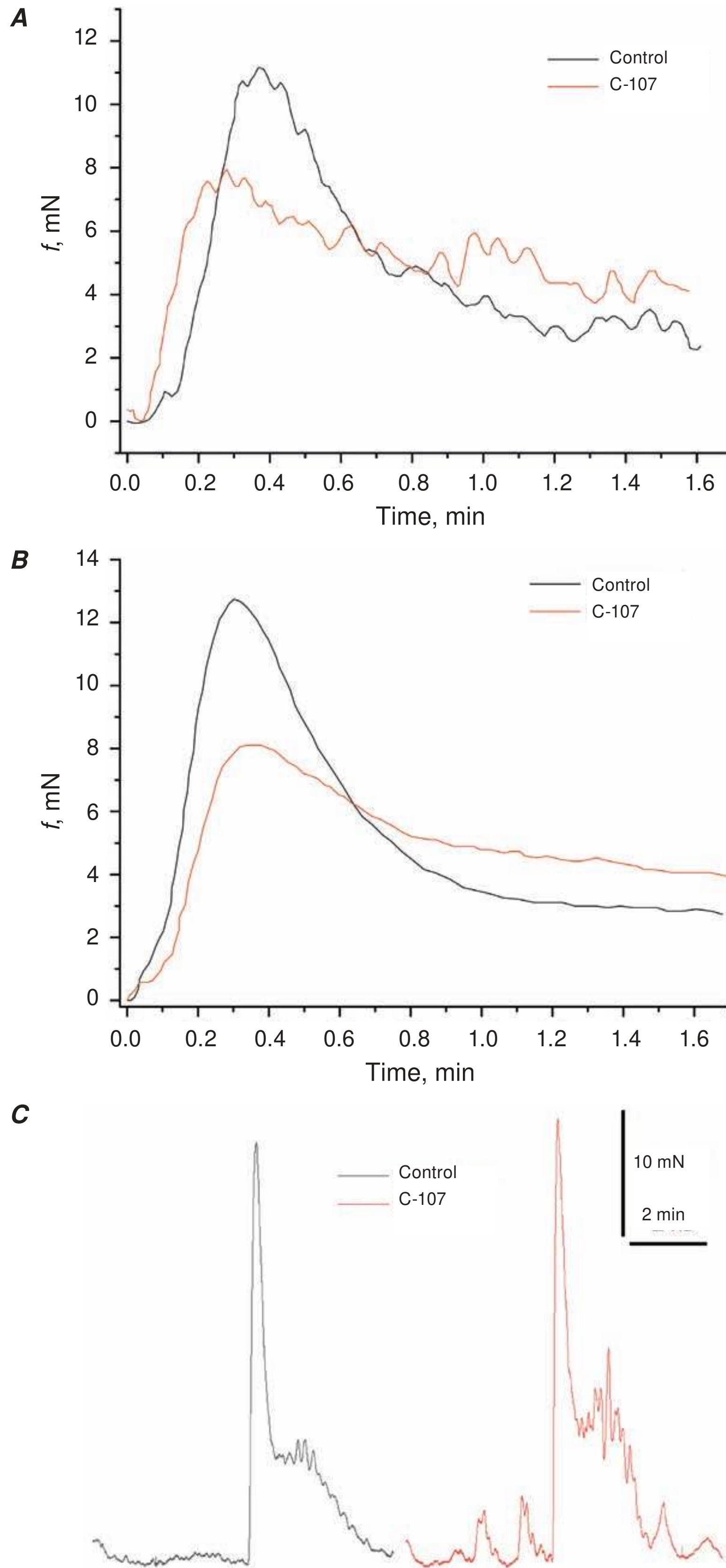

*Fig. 4. The effect of calix[4]arene C-107 (10 μM) on high-potassium- (80 mM) (**A**) and acetylcholine-induced (10 μM) (**B**) contraction of caecum circular SM, as well as oxytocin-induced (0.1 IU) (**C**) contraction of the longitudinal SM of rat myometrium. Typical mechanograms are given*

of acetylcholine-induced contractions (on average 40% ($P < 0.05$, $n = 5$) compared to the control). It was also found that calix[4]arene C-107 caused a significant increase the tonic component of contractions induced by high-potassium solution (on average 130% ($P < 0.05$, $n = 6$) compared to the control) and acetylcholine (on average 144% ($P < 0.05$, $n = 6$) compared to the control), as well as it maintained such increased tension for a long time.

Calix[4]arene C-107 was shown to retain the ability to cause the above effects under the condition of it chronic intramuscular application *in vivo* [38]. It is also very important that chronic application of calix[4]arene C-107 did not lead to the death of animals and was not accompanied by visible toxic effects [39].

Thus, the physiological phenomenon of the stimulation effect of calix[4]arene C-107 on the tonic component of SM contractions can be explained as follows. First, inhibition of PM Mg^{2+}-dependent Na^+,K^+-ATPase by calix[4]arene C-107 (Fig. 2, 3) leads to the dissipation of the trans-sarcolemmal sodium gradient. This, in turn, leads to the decreasing of the PM Na^+/Ca^{2+}-exchanger activity and, consequently, to a slower Ca^{2+} exclusion from myocytes. Second, activation of Mg^{2+}-dependent ATPase of the actomyosin complex (Fig. 2, 3) by the studied calix[4]arene (Fig. 2, 3) can leads to an increase of the actin and myosin interaction. The combination of these two effects: inhibition of the activity of PM Mg^{2+}-dependent Na^+,K^+-ATPase and stimulation of the activity of Mg^{2+}-dependent ATPase of the actomyosin complex results in the physiological effect observed in the experiment, namely, the increase of the tonic component of contractions induced by oxytocin, high-potassium solution and acetylcholine, as well as sustained increased muscle tone (Fig. 4).

The data obtained suggest that calix[4]arene C-107 can be a promising agent to enhance basal tone and/or activate the contractile activity of hollow visceral organs, which structure and function based on SM (intestines, uterus) in case of their hypotonia and functional disorders.

This work was financially supported by the grants with state registration numbers: 0115U003638, 0115U003639, 0117U006348.

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

ІНГІБУВАННЯ Na^+,K^+-АТРази ТА АКТИВАЦІЯ МІОЗИНОВОЇ АТРази КАЛІКС[4]АРЕНОМ С-107 СТИМУЛЮЄ ТОНІЧНУ СКЛАДОВУ СКОРОТЛИВОЇ АКТИВНОСТІ ІЗОЛЬОВАНИХ ГЛАДЕНЬКИХ М'ЯЗІВ

Т. О. Векліч[1], Р. Д. Лабинцева[1], О. А. Шкрабак[1], О. В. Цимбалюк[2], Р. В. Родік[3], В. І. Кальченко[3], С. О. Костерін[1]

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

Метою дослідження було показати, що синтетична сполука калікс[4]арен С-107 виявляє кілька унікальних біохімічних та фізіологічних ефектів на гладенькі м'язи і є перспективною для медичного застосування. Зокрема, цей калікс[4]арен є ефективним інгібітором Na^+,K^+-АТРази міометрія ($I_{50} = 54 \pm 6$ нМ), селективним відносно інших АТР-гідролаз плазматичної мембрани та одночасно активатором ензиматичної активності міозинової АТРази гладеньких м'язів ($A_{50} = 9{,}6 \pm 0{,}7$ мкМ). Такі реципрокні біохімічні ефекти спрямовані на одну фізіологічну відповідь, а саме стимулювання скоротливої активності гладеньких м'язів, що було продемонстровано на різних ізольованих гладеньких м'язах тензометричним методом. А саме: калікс[4]арен С-107 стимулює підвищення тонічної складової спричинених окситоцином скорочень міометрія, а також зумовлених гіперкалієвим розчином та ацетилхоліном скорочень м'яза *caecum* товстого кишечника, та утримання цього підвищеного тонусу тривалий час. Отже, калікс[4]арен С-107 є перспективною речовиною для посилення базального тонусу та/або активації скорочувальної активності гладеньком'язових органів у разі їхніх гіпотонічних дисфункційних порушень.

Ключові слова: Na^+,K^+-АТРаза, Mg^{2+}-АТРаза, субфрагмент-1 міозину, плазматична мембрана, гладеньком'язові клітини, міометрій, калікс[4]арени.

References

1. Kosterin SO, Babich LG, Shlykov SG, Danylovych IuV, Veklich TO, Mazur YuYu. Biochemical properties and regulation of smooth muscle cell Ca^{2+}-transporting systems. K.: Science opinion, 2016. 210 p.
2. Hertelendy F, Zakar T. Regulation of myometrial smooth muscle functions. Curr Pharm Des. 2004; 10(20): 2499-2517.
3. Otaibi MA. The physiological mechanism of uterine contraction with emphasis on calcium ion. Calcium Signaling. 2014; 1(2): 70-75.
4. Kosterin SO. Calcium transport in smooth muscles. K.: Science opinion, 1990. 216 p.
5. Veklich TO, Mazur IuIu, Kosterin SO. Mg^{2+},ATP-depenent plasma membrane calcium pump of smooth muscle cells. II. Regulation of acrivity. *Ukr Biochem J.* 2015; 87(2): 5-25. (In Ukrainian).
6. Burdyga T, Paul RJ. Chapter 86 – Calcium Homeostasis and Signaling in Smooth Muscle Muscle. *Muscle.* 2012; 2: 1155-1171.
7. Geering K. FXYD proteins: new regulators of Na-K-ATPase. *Am J Physiol Renal Physiol.* 2006; 290(2): F241-F250.
8. dos Santos L, Xavier FE, Vassallo DV, Rossoni LV. Cyclooxygenase pathway is involved in the vascular reactivity and inhibition of the Na^+, K^+-ATPase activity in the tail artery from L-NAME-treated rats. *Life Sci.* 2003; 74(5): 613-627.
9. Li D, Qu X, Hou K, Zhang Y, Dong Q, Teng Y, Zhang J, Liu Y. PI3K/Akt is involved in bufalin-induced apoptosis in gastric cancer cells. *Anticancer Drugs.* 2009; 20(1): 59-64.
10. Tsymbalyuk OV, Kosterin SO. Na^+,K^+-ATPase, endogenous cardiotonic steroids and their transducing role. *Ukr Biokhim Zhurn.* 2012; 84(1): 5-17. (In Ukrainian).
11. Han KQ, Huang G, Gu W, Su YH, Huang XQ, Ling CQ. Anti-tumor activities and apoptosis-regulated mechanisms of bufalin on the orthotopic transplantation tumor model of human hepatocellular carcinoma in nude mice. *World J Gastroenterol.* 2007; 13(24): 3374-3379.
12. Levitsky DI. Actomyosin systems of biological motility. *Biochemistry (Mosc).* 2004; 69(11): 1177-1189.
13. Preller M, Manstein DJ. Myosin Motors: Structural Aspects and Functionality. In book: Reference Module in Life Sciences. 2017.
14. Woodcock NA, Taylor CW, Thornton S. Effect of an oxytocin receptor antagonist and rho kinase inhibitor on the $[Ca^{++}]_i$ sensitivity of human myometrium. *Am J Obstet Gynecol.* 2004; 190(1): 222-228.
15. Brown HK, Speechley KN, Macnab J, Natale R, Campbell MK. Biological determinants of spontaneous late preterm and early term birth: a retrospective cohort study. *BJOG.* 2015; 122(4): 491-499.
16. Doyle J, Silber A. Preterm labor: role of the nurse practitioner. *Nurse Pract.* 2015; 40(3): 49-54.
17. Rodik RV, Boyko VI, Kalchenko VI. Calixarenes in bio-medical researches. *Curr Med Chem.* 2009; 16(13): 1630-1655.
18. Komisarenko SV, Kosterin SO, Lugovskoy EV, Kalchenko VI. Calixarene methylene bisphosphonic acids as promising effectors of biochemical processes. *Ukr Biochem J.* 2013; 85(6): 106-128.
19. Rodik R, Boiko V, Danylyuk O, Suwinska K, Tsymbal I, Slinchenko N, Babich L, Shlykov S, Kosterin S, Lipkowski J, Kalchenko V. Calix[4]arenesulfonylamidines. Synthesis, structure and influence on $Mg^{2+,}$ATP-dependent calcium pumps. *Tetrahedron Lett.* 2005; 46(43): 7459-7462.
20. Veklich TO, Kosterin SO, Rodik RV, Cherenok SO, Boyko VI, Kalchenko VI. Effect of calixarene-phosphonic acid on Na^+,K^+-ATPase activity in plasma membranes of the smooth-muscle cells. *Ukr Biokhim Zhurn.* 2006; 78(1): 70-86. (In Ukrainian).
21. Veklich TO, Kosterin SO. Comparative study of properties of Na^+,K^+-ATPase and Mg^{2+}-ATPase of the myometrium plasma membrane. *Ukr Biokhim Zhurn.* 2005; 77(2): 66-75. (In Ukrainian).
22. Kondraytuk TP, Bychenok SF, Prishchepa LA, Babich LG, Kursky MD, Osipenko AA. Isolation and characteristics of the fraction of plasma membranes in pig myometrium. *Ukr Biokhim Zhurn.* 1986; 58(4): 50-56. (In Russian).
23. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976; 72(1-2): 248-254.
24. Bárány M, Bárány K, Gaetjens E, Bailin G. Chicken gizzard myosin. *Arch Biochem Biophys.* 1966; 113(1): 205-222.
25. Weber A. The ultracentrifugal separation of L-myosin and actin in an actomyosin sol under

the influence of ATP. *Biochim Biophys Acta.* 1956; 19(2): 345-351.
26. Veklich TO, Kosterin SO. Comparative study of properties of Na^+,K^+-ATPase and Mg^{2+}-ATPase of the myometrium plasma membrane. *Ukr Biokhim Zhurn.* 2005; 77(2): 66-75. (In Ukrainian).
27. Flynn ER, Bradley KN, Muir TC, McCarron JG. Functionally separate intracellular Ca^{2+} stores in smooth muscle. *J Biol Chem.* 2001; 276(39): 36411-36418.
28. Veklich TO, Kosterin SO, Shynlova OP. Cationic specificity of a Ca^{2+}-accumulating system in smooth muscle cell mitochondria. *Ukr Biokhim Zhurn.* 2002; 74(1): 42-48. (In Ukrainian).
29. Valente RC, Capella LS, Monteiro RQ, Rumjanek VM, Lopes AG, Capella MA. Mechanisms of ouabain toxicity. *FASEB J.* 2003; 17(12): 1700-1702.
30. Rathbun WB, Betlach MV. Estimation of enzymically produced orthophosphate in the presence of cysteine and adenosine triphosphate. *Anal Biochem.* 1969;28(1):436-45.
31. Chen PS, Toribara TY, Warner H. Microdetermination of phosphorus. *Anal Chem.* 1956; 28(11): 1756-1758.
32. Kurganov VI. Allosteric enzymes. M.: Nauka, 1978. 248 p.
33. Tsymbaliuk OV, Naumenko AM, Skoryk MA, Nyporko OYu, Davidovska TL, Skryshevsky VA. Histamine- and nicotine-stimulated modulations of mechanic activity of smooth muscles in gastrointestinal tract at the impact of nanosized TiO_2 material. *Biopolym Cell.* 2016; 32(2): 140-149.
34. Burdyga ThV, Kosterin SA. Kinetic analysis of smooth muscle relaxation. *Gen Physiol Biophys.* 1991; 10(6): 589-598.
35. Iwane AH, Kitamura K, Tokunaga M, Yanagida T. Myosin subfragment-1 is fully equipped with factors essential for motor function. *Biochem Biophys Res Commun.* 1997; 230(1): 76-80.
36. Danylovych HV, Danylovych YuV, Rodik RV, Kalchenko VI, Chunikhin AJu. Calix[4]arene as modulators of energy-dependent Ca^{2+}-accumulation and functioning of the electron transport chain in smooth muscle mitochondria. *Fiziol Zh.* 2016; 62(5): 27-36. (In Ukrainian).
37. Viero C, Shibuya I, Kitamura N, Verkhratsky A, Fujihara H, Katoh A, Ueta Y, Zingg HH, Chvatal A, Sykova E, Dayanithi G. REVIEW: Oxytocin: Crossing the bridge between basic science and pharmacotherapy. *CNS Neurosci Ther.* 2010; 16(5): e138-e156.
38. Olson ML, Sandison ME, Chalmers S, McCarron JG. Microdomains of muscarinic acetylcholine and Ins(1,4,5)P_3 receptors create 'Ins(1,4,5)P_3 junctions' and sites of Ca^{2+} wave initiation in smooth muscle. *J Cell Sci.* 2012; 125(Pt 22): 5315-5328.
39. Murthy KS. Signaling for contraction and relaxation in smooth muscle of the gut. *Annu Rev Physiol.* 2006; 68: 345-374.
40. Tsymbalyuk OV, Rodik RV, Kalchenko VI, Kosterin SO. The mekhanokinetical parameters of contractile activity of rat caecum smooth muscles under the conditions of calixarene C107 chronic action *in vivo*. *Physics Alive.* 2010; 18(1): 47-51. (In Ukrainian).
41. Tsymbalyuk OV, Kosterin SO, Rodik RV, Kalchenko VI. Comparative study of in vitro and *in vivo* effect of ouabain and calixarene C107 on Na^+,K^+-ATPase activity in plasma membranes of rat hepatocytes. *Ukr Biokhim Zhurn.* 2010; 82(4): 78-85. (In Ukrainian).