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CATION AND SUBSTRATE SPECIFICITY OF THE SODIUM PUMP AND THE MECHANOKINETICS OF THE "CONTRACTION–RELAXATION" PROCESS IN SMOOTH MUSCLE UNDER THE ACTION OF CALIX[4]ARENE C-1130

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Background. *Plasma membrane Na^+,K^+ATPase plays an important role in maintaining ionic homeostasis and regulating myometrial contractility. Its inhibition may alter uterine smooth muscle responses to uterotonic stimulation.* ***Objective.*** *This study aimed to determine the mechanism by which calix[4]arene C-1130 inhibits Na^+,K^+-ATPase activity and to evaluate its effects on oxytocin-induced myometrial contractions.* ***Methods.*** *Na^+,K^+-ATPase activity was studied in suspensions of perforated plasma membranes isolated from myometrial cells. The kinetic parameters of ATP hydrolysis were determined at different ATP and Mg^{2+} concentrations. The effects of C-1130 on oxytocin-induced isometric and isotonic contractions were examined in myometrial preparations with preserved endometrium.* ***Results.*** *Calix[4]arene C-1130 decreased the maximum rate of Na^+,K^+-ATPase-mediated ATP hydrolysis without significantly affecting the apparent affinity or cooperativity parameters for ATP and Mg^{2+}. At a concentration of 10 μM, C-1130 enhanced oxytocin-induced isometric myometrial contraction and increased the normalized maximal rates of both contraction and relaxation phases. Under isotonic conditions, C-1130 increased the velocity and enhanced the power characteristics of muscle contraction.* ***Conclusions.*** *Calix[4]arene C-1130 inhibits myometrial Na^+,K^+-ATPase through a non-competitive mechanism. The enhancement of oxytocin-induced contractile muscle activity is likely associated with disruption of ionic homeostasis in myocytes caused by inhibition of the plasma membrane Na^+,K^+-ATPase.*

K e y w o r d s: calix[4]arenes, Na^+,K^+-ATPase, plasma membrane, smooth muscle cells, myometrium, ATPase kinetic properties.

Smooth muscle tissue is an integral structural and functional component of the walls of blood vessels, internal organs, and hollow systems, and its contractile activity determines the regulation of vascular tone, peristalsis, bronchial conductivity, and the functioning of the genitourinary system. Disturbances in the mechanisms of smooth muscle contraction underlie the development of a number of pathological conditions, including vascular, metabolic, and ischemic disorders [1-3].

Contraction of smooth muscle cells is ensured by the coordinated functioning of membrane ion-transport systems that maintain ionic homeostasis, generate electrochemical gradients, and determine the intracellular Ca^{2+} level, the key regulator of the contractile process. An important role in these mechanisms is played by the Na^+,K^+-ATPase of the plasma membrane, the activity of which ensures the functioning of Na^+-dependent systems of secondary active transport, in particular Na^+/Ca^{2+} and Na^+/H^+ exchange [4-7].

Impairment of sodium-potassium pump activity leads to shifts in membrane potential, cation imbalance, and disruption of calcium signaling, which directly affects the contractile function of smooth muscles. It has been shown that under conditions of diabetes mellitus and ischemic injury, Na^+,K^+-ATPase activity is significantly reduced, thereby contributing to the development of functional disorders in smooth muscle tissues [8].

In this regard, investigation of the molecular mechanisms regulating Na^+,K^+-ATPase and the search for selective and reversible effectors of this enzyme are considered a promising approach for correcting impaired cation regulation and restoring the contractile function of smooth muscles.

For this reason, cyclic oligomers of phenolic nature, namely calixarenes, are of particular interest. They are characterized by low toxicity [9, 10] and immunogenicity [11, 12], and certain representatives have been described as possessing bactericidal, antiviral, antithrombotic, and antitumor properties. Owing to their membranotropic properties and ability to penetrate into the cell, calixarenes can reversibly modify the activity of protein targets [13, 14], which makes them a promising basis for the creation of selective inhibitors and activators of intracellular biochemical processes associated with the regulation of the contractile activity of smooth muscle cells.

In previous studies performed on preparations of the plasma membrane of uterine myocytes, calix[4]arene C-1130 was shown to effectively inhibit the enzymatic activity of ouabain-sensitive Na^+,K^+-ATPase while having virtually no effect on the activities of Mg^{2+}-ATPase, Ca^{2+}-ATPase, and Ca^{2+},Mg^{2+}-ATPase of the plasma membrane (PM) [15].

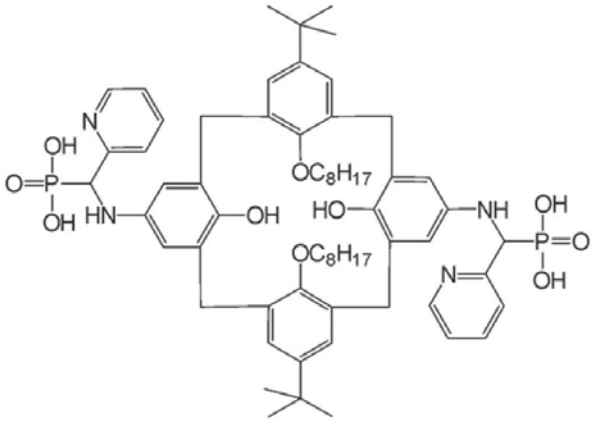
In the present study, we aimed to investigate the effect of calix[4]arene C-1130 on the dependence of Na^+,K -ATPase activity of the plasma membrane of myometrial cells on ATP and Mg ion concentrations, as well as on the contractile activity of the myometrium.

Materials and Methods

The structure and synthesis of calix[4]arene C-1130. Calix[4]arene C-1130 (5,17-bis(dihydroxyphosphoryl-2-pyridylmethyl)amino-11,23-di-tert-butyl-25,27-dioctyloxycalix[4]arene) was synthesized by the method described previously [15].

Preparative biochemistry. The plasma membrane fraction of smooth muscle cells was isolated from pig myometrium as described previously [16, 17]. The protein content in the membrane fraction was determined by the M. Bradford method using the reaction with Coomassie G-250 reagent [18].

The "total" Mg^{2+},Na^+,K^+-ATPase activity was determined in the PM fraction of myometrial cells, as described previously [16], at 37°C in a standard



Calix[4]arene C-1130

medium (volume 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, 0.1 μM thapsigargin, and 0.1% digitonin. The amount of membrane fraction protein in the sample was 20–30 μg. The incubation time was 4 min. The enzymatic reaction was initiated by adding an aliquot (50 μl) of the PM suspension to the incubation medium and terminated by adding 1 ml of the following stop solution to the incubation mixture: 1.5 M sodium acetate, 3.7% formaldehyde, 14% ethanol, 5% TCA, pH 4.3 (at 8°C). The presence of the Ca^{2+} chelator EGTA in the incubation medium ensured binding of endogenous Ca ions present in it.

The "ouabain-sensitive" Na^+,K^+-ATPase activity was calculated as the difference between the values of "total" ATPase activity in the presence and absence of 1 mM ouabain (a selective inhibitor of Na^+,K^+-ATPase [19]).

The specific enzymatic activity of Na^+,K^+-ATPase in the sarcolemma of pig myometrium is 10.2 ± 0.7 μmol P_i/mg protein per 1 h (n = 7) [16].

The amount of the reaction product P_i was determined by the method of W. Rathbun and V. Betlach [20].

In experiments studying the effect of different ATP concentrations (0.01–1 mM) and calix[4]arene C-1130 (10–100 nM) on Na^+,K^+-ATPase activity, the standard incubation medium described above was used, to which an aliquot of ATP solution at the appropriate concentration was added. In these experiments, the $MgCl_2$ concentration in the standard incubation medium was constant and amounted to 3 mM.

In experiments studying the effect of different Mg^{2+} ion concentrations (0.01–3 mM) and ca-

lix[4]arene C-1130 (10–100 nM) on Na^+,K^+-ATPase activity, the standard incubation medium described above was used, to which an aliquot of $MgCl_2$ solution at the appropriate concentration was added. The ATP concentration in the standard incubation medium was 1 mM. In all experiments, a concentrated (1 mM) solution of calix[4]arene C-1130 in DMSO was used and then diluted with water to the required final concentration.

The kinetic parameters of the action of ATP and Mg^{2+} ions on Na^+,K^+-ATPase enzymatic activity were calculated using concentration dependences plotted in double logarithmic coordinates according to the linearized Hill equation: $\lg[(V_{max}-V)/V] = n_H \cdot \lg K - n_H \cdot \lg S$, where V is the specific enzymatic activity, V_{max} is the maximum specific enzymatic activity, K is the apparent Michaelis constant or the apparent activation constant for Mg ions, and S is the substrate concentration or activator ion concentration in the incubation medium. The values of the apparent inhibition constant K_i of calix[4]arene C-1130 and the Hill coefficient were calculated using linearized Hill plots according to the equation: $\lg[(V_0-V)/V] = n_H \cdot \lg I - n_H \cdot \lg K_i$, where V is the specific enzymatic activity in the absence of an inhibitor, and I is the inhibitor concentration in the incubation medium. In the case of such plots, the typical value of the standard deviation of the approximation coefficient was 0.9–0.99. Kinetic and statistical calculations were performed using MS Excel software.

Functional tensometric experiments. Contractile activity was studied tensometrically in isometric and isotonic modes on preparations (average size 2×10 mm) of longitudinal smooth muscles with preserved endometrium from rat uterine tubes.

During isolation of the muscle preparations and in the experiments, Krebs solution was used (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; the pH of the solution was 7.4.

The analysis of the kinetic regularities of oxytocin-induced (0.1 IU) contractions was performed according to the Kosterin–Burdyha analysis method with calculation of normalized maximal velocities of the contraction and relaxation phases (V_{nC} and V_{nR}, respectively) [21]. Isotonic contractions were additionally analyzed according to the empirical multiparametric mechanokinetic analysis method developed earlier by us, with calculation of time parameters (τ_0, τ_C, and τ_R), shortening parameters (ΔL_{max}, ΔL_C, and ΔL_R), velocity parameters (V_C and V_R), and energetic parameters: work (maximum A_{max} and at the inflection point of the contraction phase $A_{\tau C}$) and power (mean during the contraction phase Nmean and maximum N_{max}) [22]. In all cases, the indices "C" indicate the contraction phase, whereas the indices "R" indicate the relaxation phase.

Statistical analysis. Experimental data were processed by methods of variation statistics using Origin 2018 and Excel software. The Shapiro–Wilk test was used to verify whether the samples belonged to normally distributed populations. A paired *t*-test was used to determine significant differences between sample means. Differences were considered significant at a probability value p of less than 5% ($P < 0.05$). The reliability of linear function approximation of the data (linearization) was analyzed using Fisher's F-test; in all cases, the coefficients of determination (R^2) were not lower than 0.96. The results are presented as mean ± standard error of the mean, where n is the number of experiments.

Reagents. The following reagents were used in the study: ATP, Hepes, ouabain, acetylcholine, thapsigargin (Sigma, USA), Tris(hydroxymethyl) aminomethane (Reanal, Hungary), digitonin (Merck, Germany), EGTA (Fluka, Switzerland), oxytocin (Gedeon Richter, Hungary). Other reagents were of domestic production and of analytical and chemically pure grade.

Results and Discussion

In previous studies, we showed that the synthetic compound calix[4]arene C-1130 (5,17-bis(dihydroxyphosphoryl-2-pyridylmethyl)amino-11,23-di-tert-butyl-25,27-dioctyloxycalix[4]arene) highly effectively inhibited the enzymatic activity of Na^+,K^+-ATPase in the plasma membrane of uterine myocytes, with an inhibition constant $I_{0.5}$ of 38 ± 5 nM and 98% inhibition at a concentration of 100 μM relative to control [15]. At the same time, at this concentration, C-1130 had virtually no effect on the enzymatic activities of Ca^{2+},Mg^{2+}-ATPase, "basal" Mg^{2+}-ATPase, or Ca^{2+}-ATPase of the plasma membrane [15].

Therefore, for further kinetic interpretation of the effect of calix[4]arene C-1130 on the enzymatic activity of Na^+,K^+-ATPase of the myometrial plasma membrane, we investigated its action on the nature of the concentration dependences of this activity on ATP and Mg ions.

An increase in ATP concentration in the incubation medium within the range from 0.01 to

1 mM led to an increase in the enzymatic activity of Na^+,K^+-ATPase (Fig. 1, control) under conditions of fixed $MgCl_2$ concentration (3 mM) in the incubation medium. Using the Hill method, the apparent Michaelis constant K_m for the nucleoside triphosphate ATP and the Hill coefficient n_H were calculated; these values were 206.7 ± 8.1 μM and 1.33 ± 0.16, respectively ($M \pm m$; $n = 5$) (Fig. 2). The K_m value for ATP of Na^+,K^+-ATPase obtained by us correlates with literature data. The K_m value for the ATP hydrolysis reaction in rat nervous tissue is 260 μM [23].

We then investigated the effect of calix[4]arene C-1130 on the kinetic parameters characterizing the affinity of the enzyme for ATP. The effect of five concentrations of calix[4]arene C-1130 (10, 25, 50, 75, and 100 nM, respectively) on the ATP concentration dependence was studied. In all cases, a decrease in Na^+,K^+-ATPase activity of varying magnitude was observed, while the dependence of enzymatic activity on ATP retained a pattern similar to the corresponding control dependence obtained in the absence of calix[4]arene C-1130; however, the plateau level of activity decreased with increasing calixarene concentration (Fig. 1). The calculated mean values of the apparent Michaelis constant K_m and the Hill coefficients n_H in the presence of different concentrations of calix[4]arene C-1130 did not differ significantly from the control values of these kinetic parameters in the absence of the effector in the incubation medium (Fig. 2). The value of the Hill coefficient n_H indicates a positive cooperative effect in the dependence of Na^+,K^+-ATPase enzymatic activity on ATP concentration, which remained nearly unchanged in the presence of different concentrations of calix[4]arene C-1130 (Fig. 2).

Thus, calix[4]arene C-1130, while causing inhibition of Na^+,K^+-ATPase activity relative to control, practically does not alter the apparent affinity of the enzyme for ATP, nor the cooperativity of the enzymatic reaction with respect to ATP. Obviously, in this case, inhibition by calix[4]arene C-1130 occurs due to a decrease in the enzyme turnover number, that is, the V_{max} of the ATPase reaction.

We also calculated the kinetic parameters of calix[4]arene C-1130 action in the presence of different concentrations of the ATPase reaction substrate ATP (Figs. 3, 4).

It was shown that the use of different ATP concentrations (Fig. 3) has little effect on the inhibition constant K_i and the Hill coefficient n_H for calix[4]arene C-1130 (Fig. 4).

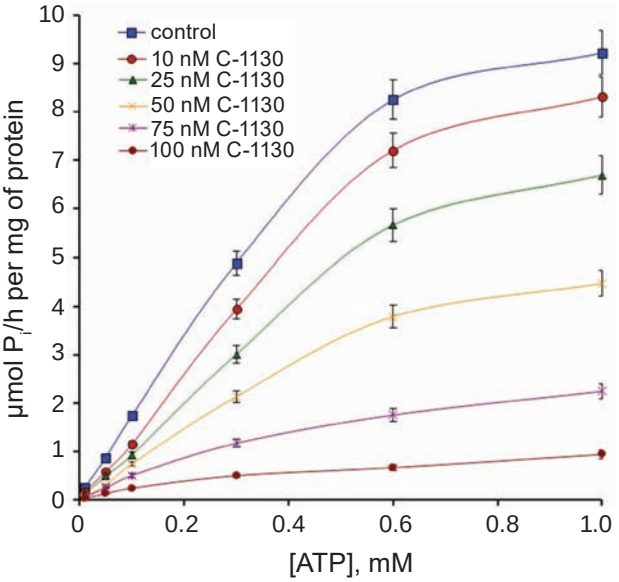


Fig. 1. Effect of calix[4]arene C-1130 at different concentrations on the dependence of the specific enzymatic activity of Na^+,K^+-ATPase in the plasma membrane fraction of myometrial cells on ATP concentration ($M \pm m$; $n = 5$). $MgCl_2$ concentration: 3 mM

Thus, the inhibitory effect of calix[4]arene C-1130 on the specific enzymatic activity of Na^+,K^+-ATPase does not depend on the amount of ATP in the incubation medium, indicating the absence of competition between ATP and calix[4]arene C-1130. Therefore, it may be assumed that the substrate-binding center of Na^+,K^+-ATPase and the hypothetical interaction site of calix[4]arene C-1130 do not overlap on the enzyme surface.

Magnesium is one of the key cofactors of cellular metabolism because it is capable of binding to oxygen-containing groups of biomolecules and thereby influencing their spatial organization and reactivity. In enzymatic processes, Mg^{2+} often plays not an "auxiliary" but a structural role: it ensures the proper geometry of enzyme–substrate interaction and supports the formation of the catalytic environment in the active center. Its involvement is particularly emphasized in reactions involving ATP: in most cases, ATP functions not as a free molecule, but as a complex with Mg^{2+}. Coordination of phosphate groups by magnesium reduces their electrostatic repulsion, "draws together" and orients the reaction centers, and thereby facilitates phosphate group transfer, in particular the attack on the terminal phosphate of ATP [24].

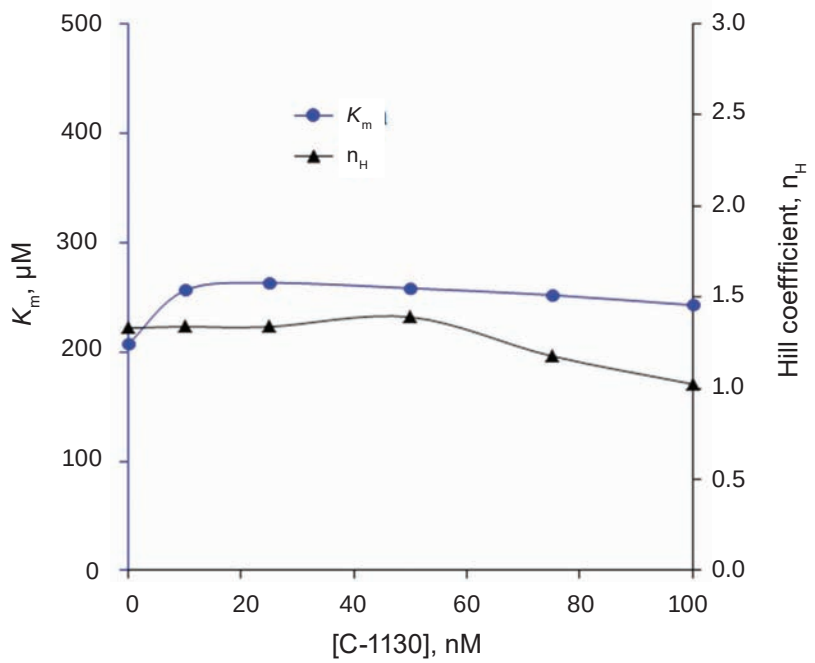


Fig. 2. Effect of calix[4]arene C-1130 on the kinetic parameters (Michaelis constant K_m and Hill coefficient n_H) of ATP action on Na^+,K^+-ATPase activity in the plasma membrane fraction of myometrial cells (M ± m; n = 5). [C-1130] = 0 mM – control. $MgCl_2$ concentration: 3 mM

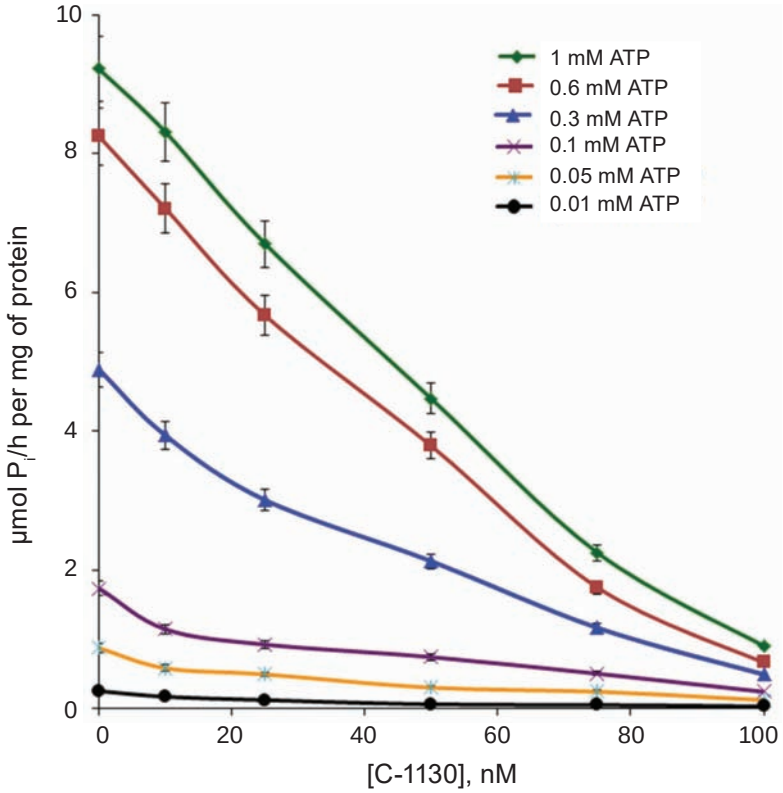


Fig. 3. Effect of ATP at different concentrations on the dependence of the specific enzymatic activity of Na^+,K^+-ATPase in the plasma membrane fraction of myometrial cells on calix[4]arene C-1130 concentration (M ± m; n = 5). $MgCl_2$ concentration: 3 mM

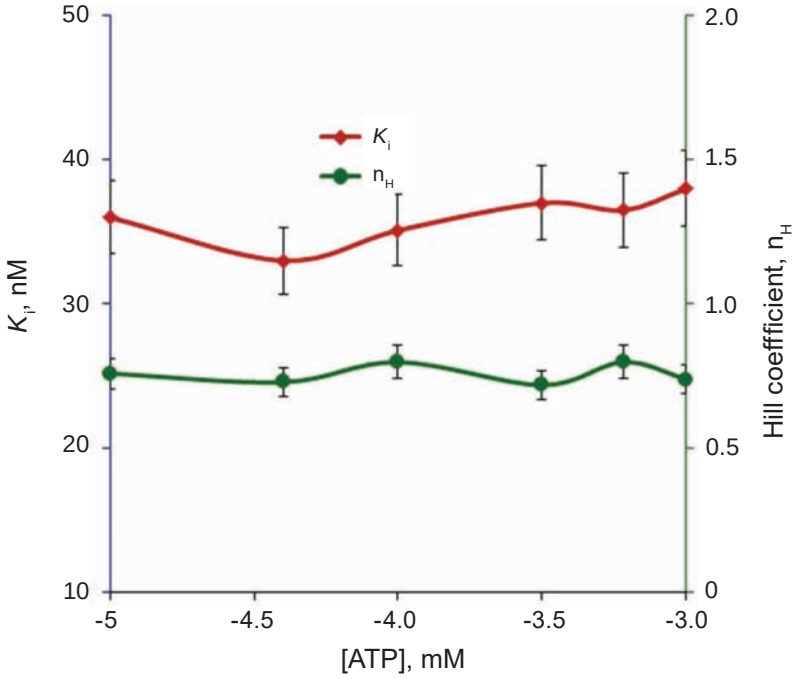


Fig. 4. Effect of ATP on the kinetic parameters (the inhibition constant K_i and the Hill coefficient n_H) of calix[4]arene C-1130 action on Na^+,K^+-ATPase activity in the plasma membrane fraction of myometrial cells ($M \pm m$; $n = 5$). $MgCl_2$ concentration: 3 mM

The enzymatic activity of Na^+K^+-ATPase in the plasma membrane of the myometrium increases with increasing $MgCl_2$ concentration from 0.01 to 3 mM under conditions of fixed ATP concentration (1 mM) in the incubation medium (Fig. 5, control, without C-1130).

The value of the apparent activation constant of Na^+,K^+-ATPase, K_{Mg}, is 179 ± 5.1 μM, and the value of the Hill coefficient n_H is 1.03 ± 0.05 ($M \pm m$; $n = 5$) (Fig. 6, control). It should be noted that the concentration of free Mg^{2+} ions in the incubation medium may differ substantially and does not depend linearly on the $MgCl_2$ concentration; therefore, the presented kinetic parameters were calculated specifically for $MgCl_2$ rather than for Mg^{2+}.

We investigated the effect of calix[4]arene C-1130 on the kinetic parameters of $MgCl_2$ action. The concentration dependence on $MgCl_2$ was studied in the presence of different concentrations of calix[4]arene C-1130 (Fig. 5).

In all cases, upon addition of calix[4]arene C-1130 to the incubation medium, a decrease in Na^+,K^+-ATPase activity of varying magnitude was

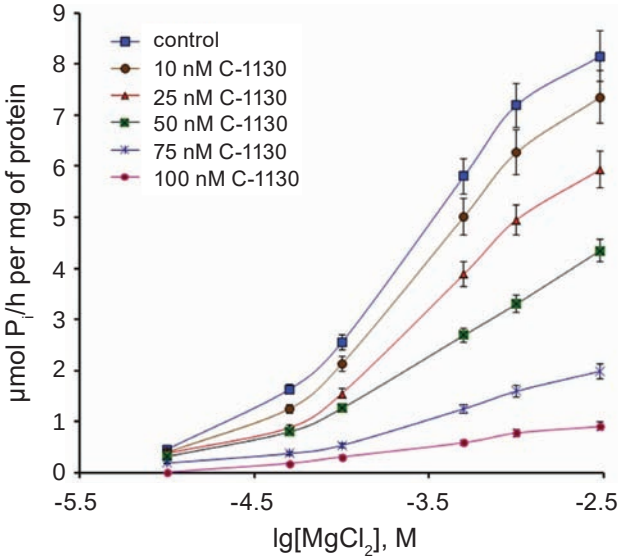


Fig. 5. Effect of calix[4]arene C-1130 at different concentrations on the dependence of the specific enzymatic activity of Na^+,K^+-ATPase in the plasma membrane fraction of myometrial cells on $MgCl_2$ concentration ($M \pm m$; $n = 5$). ATP concentration: 1 mM

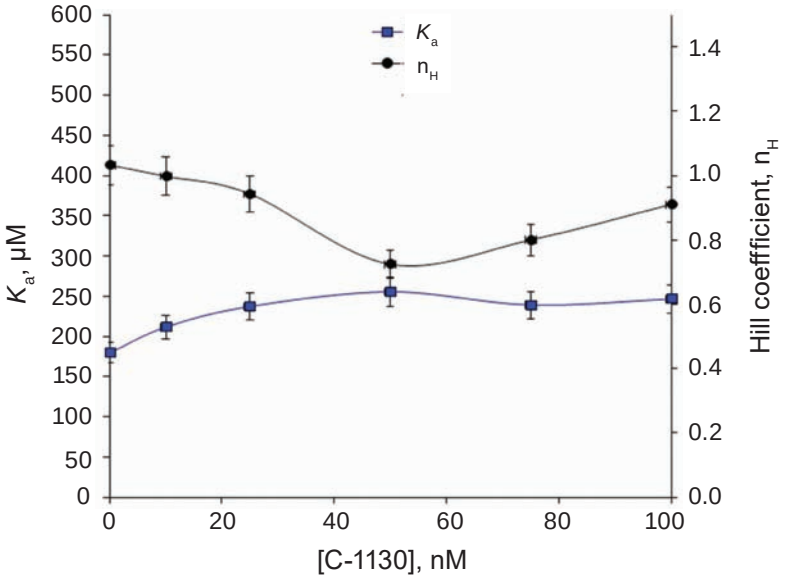


Fig. 6. Effect of calix[4]arene C-1130 on the kinetic parameters (the activation constant K_a and the Hill coefficient n_H) of $MgCl_2$ action on Na^+,K^+-ATPase activity in the plasma membrane fraction of myometrial cells ($M \pm m$; $n = 5$). [C-1130] = 0 mM – control. ATP concentration: 1 mM

observed. It was demonstrated that the activation constant for magnesium chloride slightly increased to 255 ± 14 μM with increasing concentration of calix[4]arene C-1130 up to 50 nM; with a further increase in calix[4]arene concentration, K_{Mg} decreased to almost the control level at a calix[4]arene C-1130 concentration of 100 nM (Fig. 6). At the same time, the value of the Hill coefficient n_H remained practically unchanged in the presence of different concentrations of calix[4]arene C-1130 (n_H = 1.03 ± 0.05 – 0.91 ± 0.03).

We also showed that the use of different $MgCl_2$ concentrations (Fig. 7) does not significantly affect the inhibition constant K_i or the Hill coefficient of calix[4]arene C-1130 (Fig. 8). That is, the inhibitory effect of calix[4]arene C-1130 on the specific enzymatic activity of Na^+,K^+-ATPase does not depend on the amount of Mg ions in the incubation medium, indicating the absence of competition between Mg^{2+} and calix[4]arene C-1130.

Thus, the obtained results indicate that the highly effective inhibitory action of calix[4]arene C-1130 on Na^+,K^+-ATPase is noncompetitive with respect to ATP and Mg ions and is associated with a decrease in the enzyme turnover number in its presence.

However, taking into account the multicomponent composition of the incubation medium (ATP^{4-}, $MgATP^{2-}$, Mg^{2+}, Na^+, K^+, and calix[4]arene C-1130), a more in-depth investigation of the mechanism of action of calix[4]arene C-1130 on the sodium pump requires further research.

Previously, we established that calix[4]arene C-1130 substantially activates the spontaneous contractile activity of rat uterine smooth muscle preparations [15], probably as a result of disturbance of ionic homeostasis in myocytes caused by blockade of Na^+,K^+-ATPase of the plasma membrane. Therefore, it was important to test the effect of C-1130 on contractions of myometrial preparations during activation of contractions by oxytocin, the principal uterotonic hormone.

Against the background of calix[4]arene C-1130 action (10 μM), an increase was observed in the phasic component of contractions of myometrial preparations activated by oxytocin application (0.1 IU) (on average to 129.7 ± 4.8%, $n = 5$, $P < 0.01$), whereas the tonic component of these contractions remained at the control level (92.4 ± 6.7%, $n = 5$, $P > 0.05$) (Fig. 9, *A*).

Subsequently, isometric contractions in control and in the presence of calix[4]arene C-1130 were analyzed using the Kosterin–Burdyha mechanokinetic analysis method [21]. It was established that under the action of C-1130, the normalized maximal velocity of the contraction phase (V_{nC}) increased significantly, reaching 132.3 ± 5.4% of control ($n = 5$, $P < 0.01$), whereas the corresponding parameter of

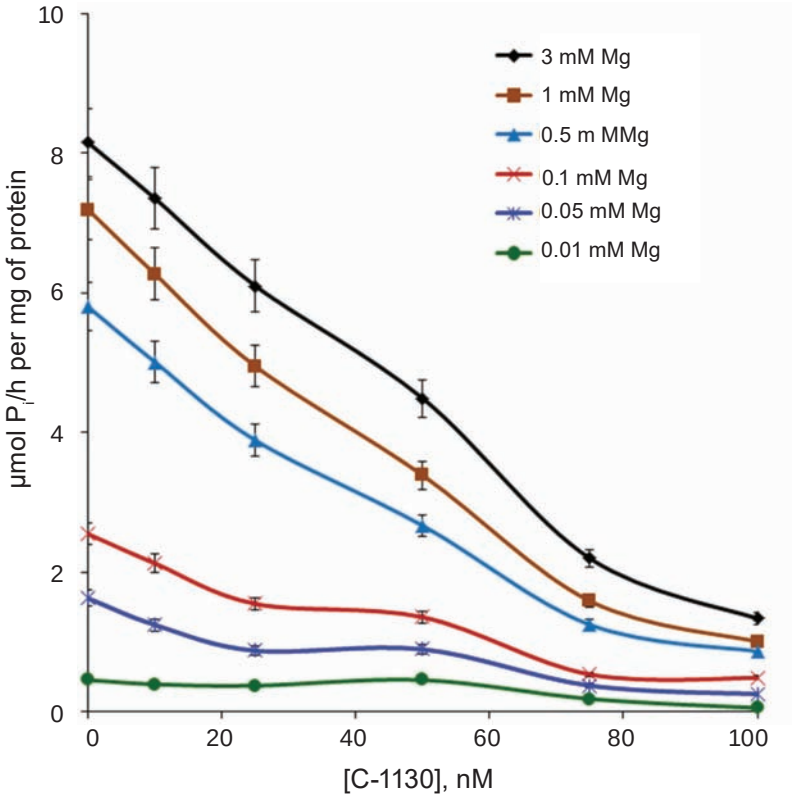


Fig. 7. Effect of $MgCl_2$ at different concentrations on the dependence of the specific enzymatic activity of Na^+,K^+-ATPase in the plasma membrane fraction of myometrial cells on calix[4]arene C-1130 concentration (M ± m; n = 5). ATP concentration: 1 mM

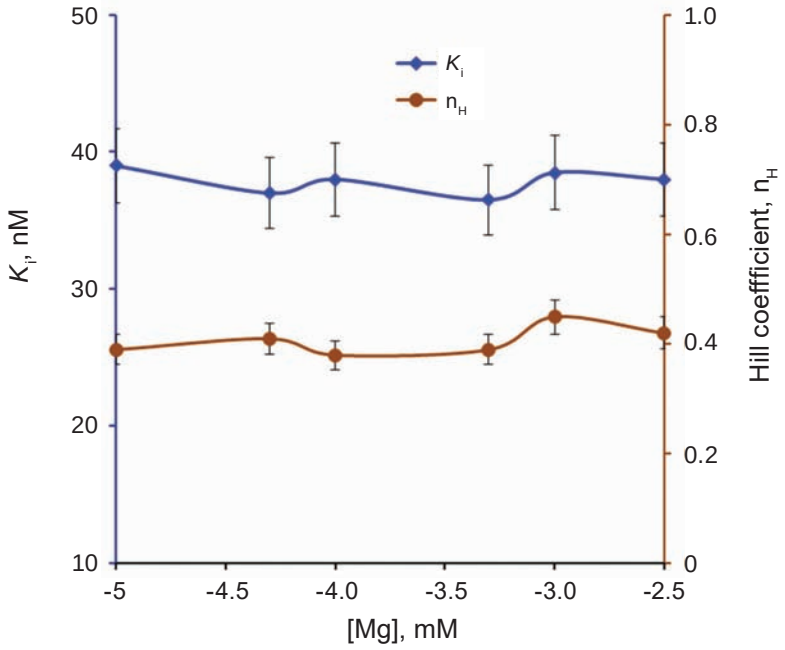


Fig. 8. Effect of $MgCl_2$ on the kinetic parameters (the inhibition constant K_i and the Hill coefficient n_H) of calix[4]arene C-1130 action on Na^+,K^+-ATPase activity in the plasma membrane fraction of myometrial cells (M ± m; n = 5). ATP concentration: 1 mM

the relaxation phase increased almost twofold, averaging 278.9 ± 11.6% (n = 5, P < 0.001) (Fig. 10).

In the case of the isotonic mode of recording muscle functional activity, the amplitude of the phasic component of oxytocin-induced contractions did not undergo significant changes under the action of C-1130; however, as in the case of isometric contractions, in the presence of this compound a much more rapid attainment of the amplitude was observed (Fig. 9, *B*). Nevertheless, the effect of C-1130 on the time required to reach the amplitude value did not significantly affect the parameter of the normalized maximal velocity of the contraction phase, V_{nC} (Fig. 11), whereas the corresponding velocity of the relaxation phase, Vnr, increased on average to 204.2 ± 16.3% (P < 0.05, n = 4).

Subsequently, isotonic oxytocin-activated contractions of myometrial preparations were analyzed in greater detail using the multiparametric mechanokinetic analysis method with calculation of not only mechanokinetic but also energetic parameters [22]. It was established that calix[4]arene C-1130 did not affect the amplitude parameters (Fig. 12, *A*), but caused a decrease in the time parameters of the contraction and relaxation phases (τ_C and τ_R) (Fig. 12, *B*). Also, as in the case of amplitude-normalized velocity parameters, the value of the contraction phase velocity (V_C) remained at the control level, whereas the relaxation phase velocity (V_R) was significantly higher than the control value (199.8 ± 24.7%, P < 0.05, n = 4) (Fig. 12, *B*).

The effects of C-1130 on the energetic parameters of muscle contraction in rat uterine preparations were further analyzed. It was established that under Na^+,K^+-ATPase blockade, the parameters of mean and maximal muscle work remained at the control level (Fig. 13, *A*), whereas both power parameters increased significantly: maximal N_{max} to 166.2 ± 5.3% and mean N_m to 156.2 ± 12.2% (in both cases, P < 0.05, n = 4) (Fig. 13, *B*).

Analyzing the obtained results, it may be assumed that since, under the action of C-1130, the time required to reach the amplitude and the contraction power increase significantly, this calix[4]arene induces activation of Ca^{2+} influx into the myoplasm. It may also be assumed that under these conditions, the additional amount of calcium may enter due to disturbance of ionic homeostasis and, as a consequence, the transition of the Na^+,Ca^{2+} exchanger into the reverse mode [25-27].

At the same time, under these conditions, we observed a significant increase in the absolute and normalized maximal velocities of the relaxation phase of both isometric and isotonic oxytocin-induced contractions of myometrial preparations under the action of C-1130. We may therefore consider the cellular mechanisms that could account for the acceleration of muscle relaxation under these conditions.

It is known that the duration of smooth muscle relaxation is determined by the additive contribution of several systems functioning during this period:

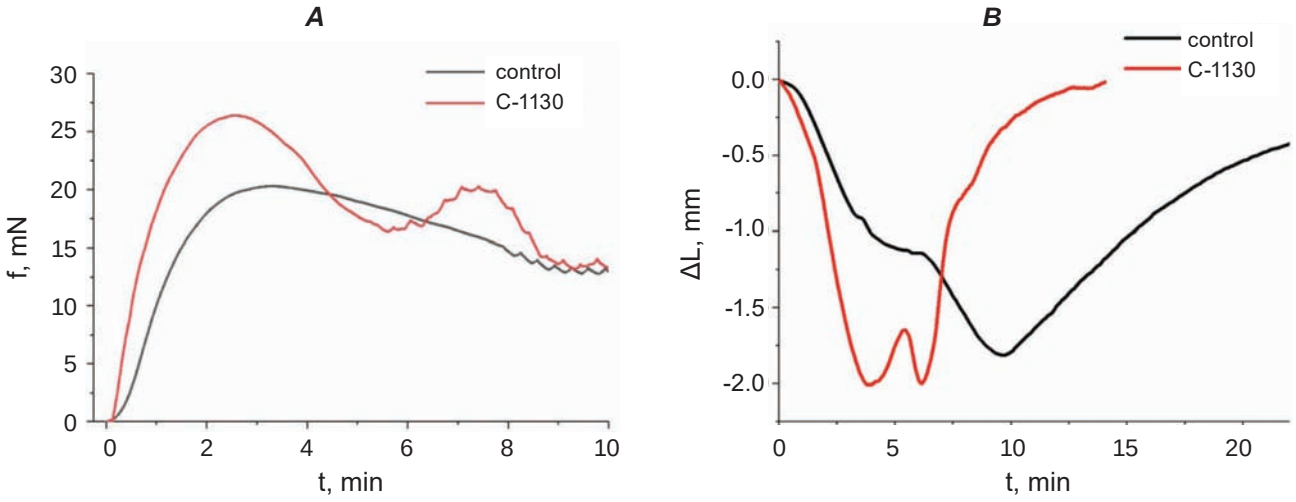


Fig. 9. Isometric (A) and isotonic (B) contractions of rat myometrium induced by oxytocin application (0.1 IU) in control and under the action of calix[4]arene C-1130 (10 μM; duration of incubation with C-1130 before the start of recording: 15 min). Representative mechanograms are shown

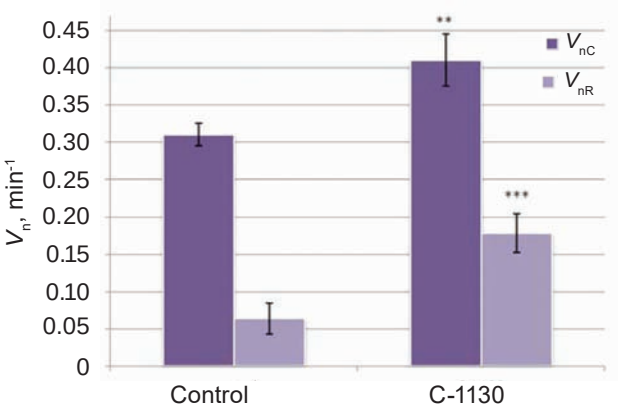


*Fig. 10. Parameters of the normalized maximal velocities of the contraction (V_{nC}) and relaxation (V_{nR}) phases of oxytocin-induced (0.1 IU) isometric contractions of rat myometrium in control and after preincubation of the preparations in the presence of calix[4]arene C-1130 (10 μM, duration of preincubation 15 min; n = 5).**P < 0.01, ***P < 0.001 – the difference is significant relative to control*

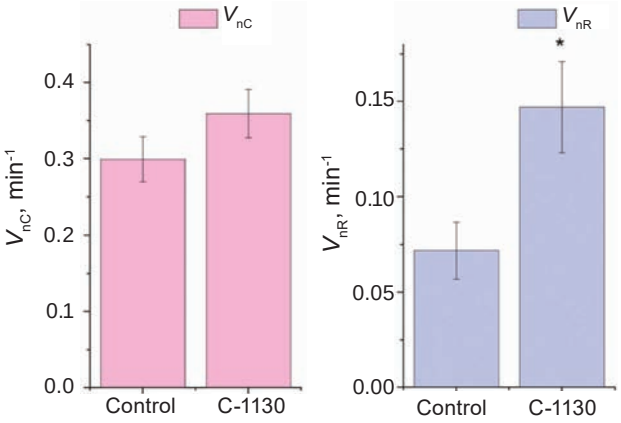


*Fig. 11. Parameters of the normalized maximal velocities of the contraction (V_{nC}) and relaxation (V_{nR}) phases of oxytocin-induced (0.1 IU) isotonic contractions of rat myometrium in control and after preincubation of the preparations in the presence of calix[4]arene C-1130 (10 μM, duration of preincubation 15 min; n = 4)*P < 0.05, the difference is significant relative to control*

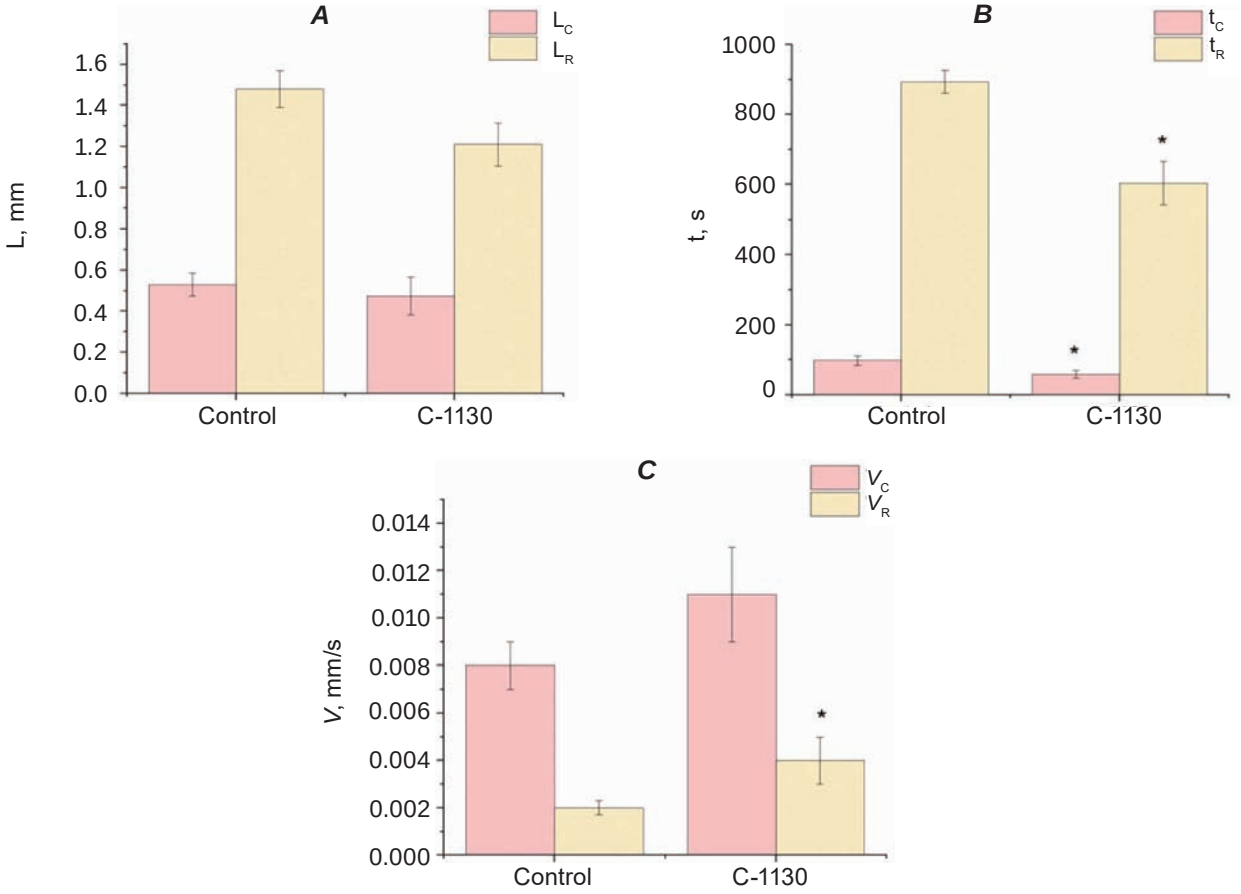


*Fig. 12. Mechanokinetic parameters of isotonic oxytocin-induced (0.1 IU) contractions of rat myometrium in control and after preliminary exposure to calix[4]arene C-1130 (10 μM, for 30 min): **A** – length parameters (L_C and L_R); **B** – time parameters (τ_C and τ_R); **C** – velocity parameters (V_C and V_R); n = 4, *P < 0.05*

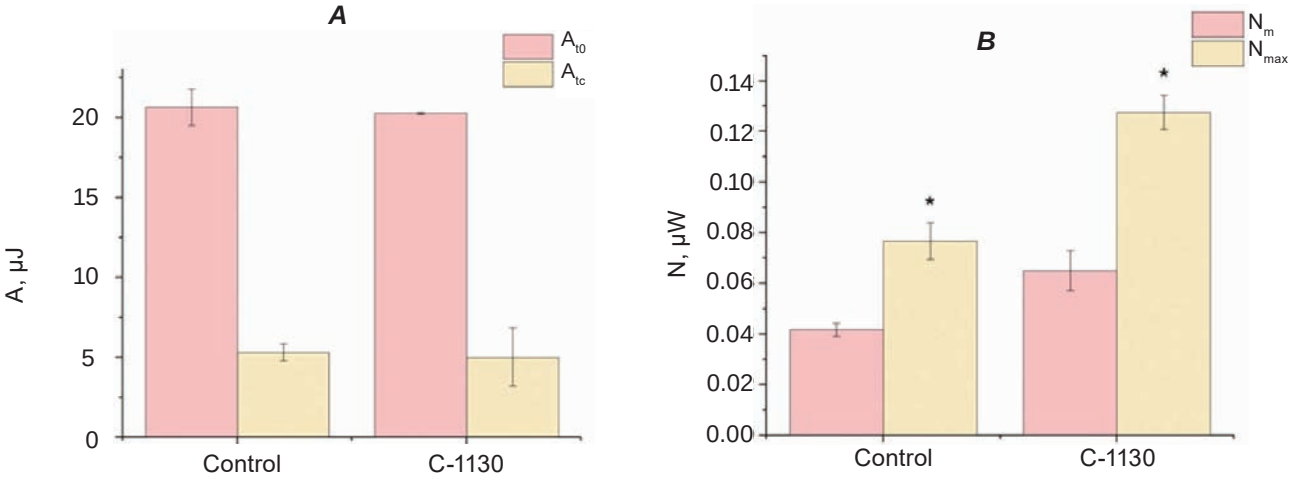


Fig. 13. Energetic parameters of isotonic oxytocin-induced (0.1 IU) contractions of rat myometrium in control and after preliminary exposure to calix[4]arene C-1130 (10 μM, for 30 min): ***A*** *– work parameters (maximum (A_{tc}) and mean (A_{t0}));* ***B*** *– power parameters (maximum (N_{max}) and mean (N_m)); n = 4, *P < 0.05*

active extrusion of Ca^{2+} ions from myocytes, mechanisms regulating the Ca^{2+} sensitivity of contractile proteins, and K^+ efflux through various channels, mainly Ca^{2+}-activated large-conductance K^+ channels (BKCa) and voltage-gated K^+ channels [28-31]. BKCa channels are known to play a major physiological role in the induction of relaxation in response to depolarization and the action of uterotonics on the myometrium, contributing approximately 35% to the repolarizing K^+ current in the non-pregnant uterus [28]. Small-conductance Ca^{2+}-activated K^+ channels (SKCa) are also involved in repolarization processes and in limiting the duration of contraction in the myometrium, although their significance is particularly pronounced during pregnancy and labor [31].

Studies on rat mesenteric artery preparations have shown that the enhancement of methoxamine-induced contraction caused by blockade of Na^+,K^+-ATPase with ouabain was potentiated by the BKCa blocker iberiotoxin and suppressed by inhibition of Src kinase and the Na^+/Ca^{2+} exchanger [32]. Studies on vascular smooth muscle preparations have also demonstrated that the functioning of Ca^{2+}-activated K^+ channels is important for myocyte relaxation when Ca^{2+} enters the cytoplasm through the Na^+/Ca^{2+} exchanger [33, 34].

In addition, both blockade of Na^+,K^+-ATPase, as shown using ouabain, and application of the uterotonic oxytocin lead to stimulation of Src kinase, which, by phosphorylating key tyrosine residues in the structure of these channels, enhances BKCa currents [33, 35]. Src kinase also activates other K^+ channels [36, 37], which may affect the kinetics of relaxation processes. Therefore, the substantial acceleration of relaxation observed in our study in oxytocin-activated contractions of myometrial preparations under the action of calix[4]arene C-1130 may likely be caused by activation of outward K^+ currents in the plasma membrane of myocytes.

Limitations of the study. This study was performed using isolated plasma membrane fractions from porcine myometrium and *ex vivo* rat myometrial preparations. Therefore, the obtained results may not fully reproduce the responses of intact uterine tissue *in vivo* and cannot be directly extrapolated to human myometrium. The number of independent experiments in the functional studies was relatively limited. In addition, membrane potential, intracellular Na^+ and Ca^{2+} concentrations, Na^+/Ca^{2+} exchanger activity, Src kinase activation, and K^+ channel currents were not measured directly. Therefore, the proposed mechanisms underlying the effects of calix[4]arene C-1130 on myometrial contraction and relaxation remain hypothetical. The exact binding site of C-1130 on Na^+,K^+-ATPase and its interaction with individual conformational states of the enzyme were also not determined. Further studies using direct electrophysiological and ion-imaging approaches, additional experimental models, and *in vivo* conditions are required.

It is assumed that the obtained experimental data, acquired using calix[4]arene C-1130, an inhibitor of Na^+,K^+-ATPase, may be of important significance for clarifying the membrane mechanisms of

cation exchange in smooth muscles, in particular during the study of the role of the plasma membrane in providing electro- and pharmacomechanical coupling in them, as well as in the regulation of ionic homeostasis in smooth muscle cells.

Author contributions. T.O. Veklich: conceptualization, methodology, investigation, formal analysis, data curation, and writing - original draft. O.V. Tsymbalyuk: methodology, investigation, formal analysis, visualization, and writing - original draft. R.V. Rodik: synthesis and characterization of calix[4]arene C-1130, resources, and writing - review and editing. O.V. Maliuk: investigation, formal analysis, visualization, and writing - review and editing. V.I. Kalchenko: supervision of the synthesis and characterization of calix[4]arene C-1130, resources, and writing - review and editing. S.O. Kosterin: conceptualization, methodology, supervision, project administration, and writing - review and editing. All authors have read and approved the final version of the manuscript.

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

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КАТІОННА ТА СУБСТРАТНА СПЕЦИФІЧНІСТЬ НАТРІЄВОГО НАСОСА ТА МЕХАНОКІНЕТИКА ПРОЦЕСУ «СКОРОЧЕННЯ–РЕЛАКСАЦІЯ» У ГЛАДКИХ М'ЯЗАХ ПІД ДІЄЮ КАЛІКС[4]АРЕНУ С-1130

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Вступ. Na^+,K^+-АТРаза плазматичної мембрани відіграє важливу роль у підтриманні іонного гомеостазу та регуляції скоротливої активності міометрія. Її інгібування може змінювати реакцію гладеньких м'язів матки на утеротонічну стимуляцію. **Мета.** З'ясувати механізм інгібування Na^+,K^+-АТРази калікс[4]ареном С-1130 та оцінити його вплив на окситоцин-індуковані скорочення міометрія. **Методи.** Активність Na^+,K^+-АТРази досліджували в суспензіях перфорованих плазматичних мембран, виділених із клітин міометрія. Кінетичні параметри гідролізу АТР визначали за різних концентрацій АТР і Mg^{2+}. Вплив С-1130 на індуковані окситоцином ізометричні та ізотонічні скорочення досліджували на багатоклітинних препаратах міометрія зі збереженим ендометрієм. **Результати.** Калікс[4]арен С-1130 знижував максимальну швидкість гідролізу АТР, каталізованого Na^+,K^+-АТРазою та істотно не впливав на уявну спорідненість ензиму і параметри кооперативності щодо АТР і Mg^{2+}. Отримані результати свідчать про неконкурентний механізм інгібування Na^+,K^+-АТРази. У концентрації 10 мкМ С-1130 посилював окситоцин-індуковані ізометричні скорочення та збільшував нормовані максимальні швидкості фаз скорочення і розслаблення. За ізотонічних умов С-1130 збільшував швидкість укорочення та підвищував параметри потужності скорочення м'язів матки. **Висновки**. Калікс[4]арен С-1130 інгібує Na^+,K^+-АТРазу міометрія за неконкурентним механізмом і посилює окситоцин-індуковану скоротливу активність. Імовірно, ці ефекти пов'язані з порушенням іонного гомеостазу міоцитів унаслідок інгібування Na^+,K^+-АТРази плазматичної мембрани.

Ключові слова: калікс[4]арени, Na^+,K^+-АТРаза, плазматична мембрана, гладеньком'язові клітини, міометрій, кінетичні властивості АТРази.

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