Calix[4]arene C-90 Selectively Inhibits Ca²⁺,Mg²⁺-ATPase of Myometrium Cell Plasma Membrane

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Abstract—The supramolecular compound calix[4]arene C-90 (5,11,17,23-tetra(trifluoro)methyl(phenylsulfonylimino)-methylamino-25,26,27,28-tetrapropoxycalix[4]arene) is shown to efficiently inhibit the ATP hydrolase activity of Ca²⁺,Mg²⁺-ATPase in the myometrium cell plasma membrane fraction and also in a preparation of the purified enzyme solubilized from this subcellular fraction. The inhibition coefficient $I_{0.5}$ values were 20.2 ± 0.5 and 58.5 ± 6.4 μM for the membrane fraction and the solubilized enzyme, respectively. The inhibitory effect of calix[4]arene C-90 was selective comparatively to other ATPases localized in the plasma membrane: calix[4]arene C-90 did not influence the activities of Na⁺,K⁺-ATPase and "basal" Mg²⁺-ATPase. The inhibitory effect of calix[4]arene C-90 on the Ca²⁺,Mg²⁺-ATPase activity was associated with the cooperative action of four trifluoromethylphenyl sulfonylimine (sulfonylamidine) groups oriented similarly on the upper rim of the calix[4]arene macrocycle (the calix[4]arene "bowl"). The experimental findings seem to be of importance for studies, using calix[4]arene C-90, of membrane mechanisms of regulation of calcium homeostasis in smooth muscle cells and also for investigation of the participation of the plasma membrane Ca²⁺-pump in control of electro- and pharmacomechanical coupling in myocytes.

Calcium ions (Ca²⁺) play a fundamental role in providing electro- and pharmacomechanical coupling in smooth muscles including those of the myometrium [1]. The Ca²⁺ concentration ($[Ca^{2+}]_i$) in relaxed smooth muscle cells (SMC) is about 100 nM, which is approximately 10^4 times lower than in the extracellular medium ($\sim 10^{-3}$ M). Extracellular Ca²⁺ is the most important factor responsible for activation of SMC contraction. On excitement, the $[Ca^{2+}]_i$ value increases up to at least several hundreds nM, but not higher than 1 μM. Such increase in the calcium concentration in myocytes is due to its passive entry into them through calcium channels of the plasma membrane (PM) and also due to ejection of

this cation from the major intracellular calcium depot, the sarcoplasmic reticulum; Ca²⁺ is released also from mitochondria [2, 3]. The relaxation ("termination") of the calcium signal is realized by the functioning of active Ca²⁺-transporting systems, Mg²⁺,ATP-dependent calcium pumps of PM and sarcoplasmic reticulum (Ca²⁺,Mg²⁺-ATPases) (thapsigargin-insensitive and thapsigargin-sensitive, respectively), the Na⁺-Ca²⁺-exchanger of PM, and also Ca²⁺-uniporter of mitochondria [4]. On the whole, changes in $[Ca^{2+}]_i$ are a result of superposition of functioning of the membrane-related systems of passive and active transport of Ca²⁺.

In uterine myocytes the Mg²⁺,ATP-dependent calcium pump of the PM having high affinity for Ca²⁺ (the activation constant $K_{Ca^{2+}}$ by Ca²⁺ is 0.3-0.6 μM) performs the most important role in the control of $[Ca^{2+}]_i$ [5, 6] because the affinity for Ca²⁺ for the Na⁺-Ca²⁺-exchanger of PM ($K_{Ca^{2+}} \approx 7$-50 μM) is significantly lower than that

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Abbreviations: PM, plasma membrane; SMC, smooth muscular cells.
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for the calcium pump. Kinetic analysis of the trans-sar-colemma exchange of Ca^{2+} has shown that the function-ing of this pump on the background of the continuous entry of a slow diffuse calcium inflow into non-excited uterine myocytes (10^{-15}-10^{-14} mol Ca^{2+}/cm^2 per s) is quite sufficient for long-term stable maintenance of $[Ca^{2+}]_i$ in the myoplasm at the level of <10^{-6} M. Thus, the calcium pump of PM is the most important element of the general control mechanism of the basal tonus of the myometrium. If this pump did not exist, then, on taking into account the saturation of intracellular structures with Ca^{2+}, myocytes would acquire a state of a steady contrac-ture after approximately 100 contractions activated by entry into SMC of extracellular Ca^{2+} as a result of a series of depolarizing pulses [7].

Selective inhibitors of energy-dependent Ca^{2+}-transporting systems are extremely necessary for studies on the role of these systems in the control of cellular functions under normal and pathological conditions. In particular, o-vanadate and eosin are nonspecific inhibitors of the Ca^{2+},Mg^{2+}-ATPase of PM. However, o-vanadate inhibits not only Ca^{2+},Mg^{2+}-ATPase and Na^+,K^+-ATPase of PM, but it also inhibits indirectly the system of Na^+-Ca^{2+}-exchange localized in the same sub-cellular structure (as a result of inhibition of the sodium pump activity and, respectively, a decrease in the trans-membrane sodium gradient). Eosin inhibits Ca^{2+}- and Na^+-transporting ATP-hydrolases of PM and also Ca^{2+}-transporting ATP-hydrolase of the sarco(endo)plasmic reticulum [8]. Relatively selective new inhibitors of the calcium pump of PM, the caloxin peptides, have been described recently [9]. But low molecular weight selec-tive inhibitors of the PM Ca^{2+},Mg^{2+}-ATPase are unknown. However, it is obvious that the search for them is extremely important for clinical practice because non-toxic compounds capable of selective blocking function-ing of the Mg^{2+},ATP-dependent calcium pump of PM are promising for creation of pharmaceuticals regulating the tonus and contractility of the uterus under patholog-ical situations (uterine inertia, uterine hypotonus, uter-ine bleeding, etc.).

In the present work, we present findings indicating that the compound calix[4]arene C-90 can selectively (comparatively to other ATP-hydrolase systems) inhibit Ca^{2+},Mg^{2+}-ATPase of the myometrium cell PM.

MATERIALS AND METHODS

Calix[4]arenes. Calix[4]arenes were synthesized in the Department of Phosphorane Chemistry, Institute of Organic Chemistry, National Academy of Sciences of Ukraine (Chief − Correspondent Member of the National Academy of Sciences of Ukraine Prof. V. I. Kalchenko). Calix[4]arene C-90 was synthesized as described earlier [10]. The structure of the synthesized

calix[4]arenes was confirmed by infrared and NMR spec-troscopy.

The following compounds were used in experiments:
− calix[4]arene C-90 − (5,11,17,23-tetra(trifluo-ro)methyl(phenylsulfonylimino)-methylamino-25,26,27,28-tetrapropoxycalix[4]arene), and also its analogs:
− calix[4]arene C-715 − 5,17-di(trifluoro)acetami-do-11,23-di-*tret*-butyl-25,27-dipropoxycalix[4]arene;
− calix[4]arene C-716 − 5,17-di(trifluoro)meth-yl(phenylsulfonylimino)-methylamino-11,23-di-*tret*-butyl-25,27-dipropoxycalix[4]arene;
− calix[4]arene C-772 − 5,11-di(trifluoro)meth-yl(phenylsulfonylimino)-methylamino-17,23-di-*tret*-butyl-26,27-dipropoxycalix[4]arene;
− calix[4]arene C-150 − 25,27-dipropoxycal-ix[4]arene (the calix[4]arene "bowl" as it is);
− the compound M-1 − N-(4-etoxyphenyl)-N′-(phenylsulfonyl)-trifluoromethylacetimidoamide (a structural element of the calix[4]arene C-90 molecule).

Structural formulas of studied compounds are pre-sented in Fig. 1.

Biochemical studies on the influence of calix[4]arenes on the activity of Ca^{2+},Mg^{2+}-ATPase in uterine SMC PM were performed in the Department of Muscle Biochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine (Chief − Correspondent Member of the National Academy of Sciences of Ukraine Prof. S. A. Kosterin).

The PM fraction of SMC was isolated from swine myometrium as described earlier in [11, 12]. The protein content in the membrane fraction was determined by the Bradford method [13].

The Ca^{2+},Mg^{2+}-ATPase activity was determined in the PM fraction of the myometrium cells at 37°C in 0.4 ml of medium containing 3 mM ATP, 3 mM $MgCl_2$, 0.95 mM $CaCl_2$, 25 mM NaCl, 125 mM KCl, 1 mM EGTA, 20 mM Hepes-Tris buffer (pH 7.4), 1 mM NaN_3 (an inhibitor of mitochondrial ATPase [14]), 1 mM ouabain (an inhibitor of Na^+,K^+-ATPase [15]), 0.1 μM thapsigargin (a selective inhibitor of Ca^{2+},Mg^{2+}-ATPase of endo(sarco)plasmic reticulum [14]), and 0.1% digi-tonin (a factor of PM perforation [16]). The protein con-tent of the membrane fraction in the sample was 20-30 μg. The incubation time was 5 min. The reaction was initiated by addition into the medium of an aliquot (50 μl) of the PM suspension and was stopped by addition to the reaction mixture of 1 ml of "stop" solution with the fol-lowing composition: 1.5 M sodium acetate, 3.7% formaldehyde, 14% ethanol, 5% TCA (pH 4.3 at 8°C). The Ca^{2+},Mg^{2+}-ATPase activity was calculated by the dif-ference between values of the ATPase activity in the absence and in the presence of calcium ions. In our experiments the mean value of the specific enzymatic activity of Ca^{2+},Mg^{2+}-ATPase of the uterine myocyte PM was 3.4 ± 0.3 μmol P_i/mg protein per h (M ± m; $n = 7$).

C-90

C-716

C-772

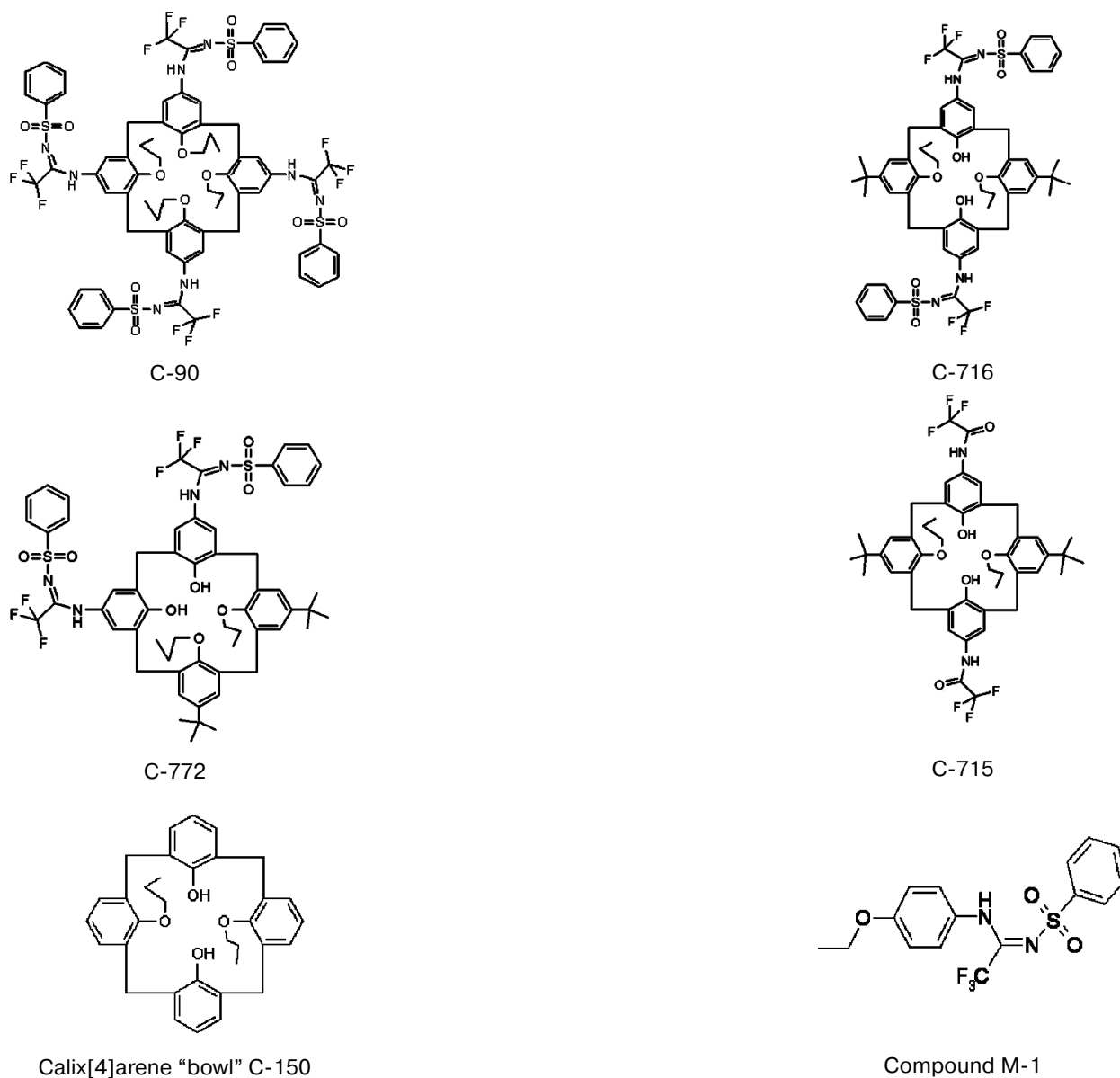C-715

Calix[4]arene "bowl" C-150

Compound M-1

Fig. 1. Structural formulas of the studied calix[4]arenes and of compound M-1.

A purified transporting Ca^{2+},Mg^{2+}-ATPase solubilized with Triton X-100 was prepared from the swine myometrium cell PM using affinity chromatography on calmodulin-Sepharose 4B as described earlier [17]. The activity of the solubilized Ca^{2+},Mg^{2+}-ATPase was determined at 37°C in 0.2 ml of standard incubation medium with the following composition: 50 mM Tris-HCl (pH 7.4), 125 mM KCl, 25 mM NaCl, 3 mM ATP, 3 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.1 mM EGTA, and 1.5-2.0 μg protein. The incubation time was 10 min. According to data of SDS-PAGE, the purified enzyme preparation contained two polypeptides with molecular weights of 130 and 205 kDa and was characterized by Ca^{2+}-dependent autophosphorylation [17]. Based on the mean specific

activity value of the solubilized Ca^{2+},Mg^{2+}-ATPase $(83.8 \pm 7.4$ μmol P_i/mg protein per h; $M \pm m$; $n = 7)$, this method allowed us to reach a 52-fold purification of the enzyme. Enzymatic activity of "basal" Ca^{2+}-independent Mg^{2+}-ATPase was not detected in the preparation of purified Ca^{2+},Mg^{2+}-ATPase.

To detect "ouabain-sensitive" Na^+,K^+-ATPase activity, the "total" ATPase activity was determined in 0.4 ml of the standard incubation medium containing: 1 mM ATP, 3 mM $MgCl_2$, 125 mM NaCl, 25 mM KCl, 1 mM EGTA, 20 mM Hepes-Tris buffer (pH 7.4), 1 mM NaN_3, 0.1 μM thapsigargin, and 0.1% digitonin. The membrane fraction protein content in the sample was 20-30 μg. The incubation time at 37°C was 4 min. Under

these conditions the "basal" Mg^{2+}-ATPase activity was determined in the same medium but in the presence of 1 mM ouabain. The "ouabain-sensitive" Na^+,K^+-ATPase activity was calculated as the difference between values of the "total" ATPase activity and the "basal" Mg^{2+}-ATPase activity. The mean value of the specific enzymatic activity of the "ouabain-sensitive" Na^+,K^+-ATPase of the uterine myocyte PM was 10.2 ± 0.7 µmol P_i/mg protein per h ($M \pm m$; $n = 7$).

In all experiments the incubation medium without PM fragments or the purified Ca^{2+},Mg^{2+}-ATPase preparation was used as control of non-enzymatic hydrolysis. As control on the endogenous inorganic phosphate (P_i) quantity in the membrane or enzyme preparation, incubation medium was used containing only PM fragments or the purified enzyme preparation in aqueous solution. The amount of the reaction product P_i was determined as described in [18].

The influence of calix[4]arenes and of compound M-1 on the activities of ATPases of PM was studied in the standard incubation medium described above; in all experiments initial concentrated solutions of calix[4]arenes (20 mM) in dimethylsulfoxide were used; at first, these solutions were diluted with water to final concentration of calix[4]arenes of 1 mM, and then aliquots of the diluted solutions of these compounds were introduced directly into the incubation medium. The final maximal concentration of dimethylsulfoxide in the incubation medium was 0.5%. We have shown earlier that dimethylsulfoxide in the concentration of 1% in the incubation medium does not influence the activity of the calcium pumps of PM, sarcoplasmic reticulum, and mitochondria of the myometrium cells [8]. The model compound M-1 was easily soluble directly in water.

In all experiments, the specific ATPase activity determined under standard conditions in the reaction medium without calix[4]arenes, compound M-1, or dimethylsulfoxide was taken as 100% ("the null point").

The concentration dependences of the action of calix[4]arenes on the activity of Ca^{2+},Mg^{2+}-ATPase were used to determine values of the inhibition coefficients $I_{0.5}$ calculated using linearized Hill plots corresponding to the empiric equation: $\log[(A_0 - A)/A] = -n_H \log I_{0.5} + n_H \log[I]$, where A_0 and A are specific enzymatic activities in the absence ("the null point") and in the presence of calix[4]arenes in the incubation medium in the concentration I. In the case of linearized plots, the typical value of the correlation coefficient r was 0.97-0.99.

Results of the studies were treated statistically with Student's t-test. Kinetic and statistical calculations were performed using the MS Excel software.

Reagents used were as follows: ATP, Hepes, ouabain, and thapsigargin (Sigma, USA); digitonin (Serva, Germany); Tris-hydroxymethyl-aminomethane (Reanal, Hungary); EGTA (Fluka, Switzerland). Other reagents

were of domestic production of analytical and chemical purity.

RESULTS AND DISCUSSION

In our experiments the mean value of the Ca^{2+},Mg^{2+}-ATPase activity in the PM fraction was 3.4 ± 0.3 µmol P_i/mg protein per h ($M \pm m$; $n = 7$), and the activity of a preparation of the purified solubilized Ca^{2+},Mg^{2+}-ATPase was 83.8 ± 7.4 µmol P_i/mg protein per h ($M \pm m$; $n = 7$) (in the last case "basal" Mg^{2+}-ATPase activity was not detectable).

Our studies on the effect of calix[4]arenes on Mg^{2+}-dependent ATP-hydrolase activities in the PM fraction of the myometrium cells have shown that calix[4]arene C-90 at the concentration of 100 µM inhibits the activity of Ca^{2+},Mg^{2+}-ATPase to $25.1 \pm 0.5\%$ with relation to the control value taken as 100%. This calix[4]arene inhibited the activity of solubilized Ca^{2+},Mg^{2+}-ATPase to $32.2 \pm 2.3\%$. However, calix[4]arene C-90 (100 µM) had virtually no influence on the activities of Na^+,K^+-ATPase and "basal" Mg^{2+}-ATPase, their residual activities in the presence of this compound were, respectively, 94.2 ± 0.6 and $107.7 \pm 1.0\%$ in relation to the control values (Fig. 2).

Thus, 100 µM calix[4]arene C-90 efficiently inhibited the Ca^{2+},Mg^{2+}-ATPase activity in both the PM fraction of the uterine myocytes and the preparation of puri-

Kinetic parameters of inhibitory effect of calix[4]arene C-90 and its structural analogs calix[4]arenes C-716, C-772, C-715 as well as of the calix[4]arene "bowl" C-150 and of the model compound M-1 on specific enzymatic activity of Ca^{2+},Mg^{2+}-ATPase of plasma membrane of myometrium cells ($M \pm m$; $n = 5$)

Effectors	Inhibition coefficient, $I_{0.5}$, µM	Hill coefficient, n_H	Inhibition (%) at 100 µM effector concentration
C-90	20.2 ± 0.5	0.55 ± 0.02	74.9 ± 0.5
C-716	53.4 ± 3.5	0.52 ± 0.02	61.5 ± 1.8
C-772	168.3 ± 21.1	0.51 ± 0.04	45.6 ± 0.5
C-715	–	–	30.7 ± 0.8
C-150	–	–	13.0 ± 1.7
M-1	–	–	9.3 ± 2.4

Note: While investigating the concentration dependences of the influence of calix[4]arenes on specific enzymatic activity of Ca^{2+},Mg^{2+}-ATPase, values of the inhibition coefficients $I_{0.5}$ and of Hill coefficients were calculated using linearized Hill plots (values of correlation coefficients were 0.98-0.99).
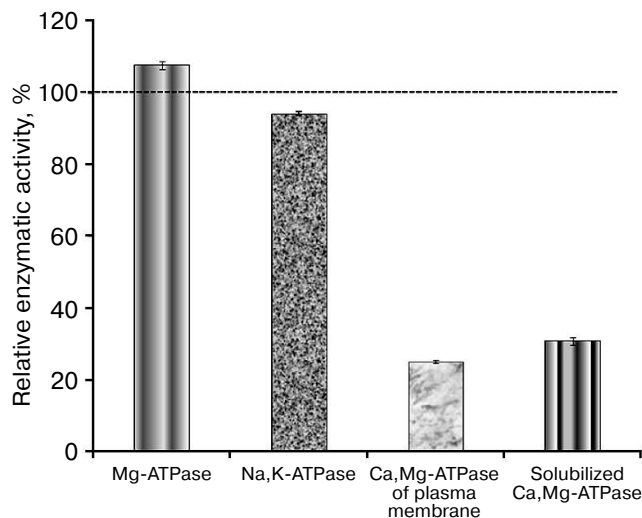
Fig. 2. Calix[4]arene C-90 (100 μM) inhibits Ca^{2+}, Mg^{2+}-ATPase activities of the plasma membrane fraction of myometrium cells and of the preparation of purified solubilized Ca^{2+}, Mg^{2+}-ATPase (M ± m, $n = 5$). Values of the specific enzymatic activities in the absence of calix[4]arene C-90 in the incubation medium are taken as 100%.

fied solubilized Ca^{2+}, Mg^{2+}-ATPase. It had almost no influence on the activities of other ATP-hydrolases of the PM fraction. Consequently, calix[4]arene C-90 selectively suppressed the Ca^{2+}, Mg^{2+}-ATPase activity of the PM

fraction without influence on the activities of Na^+, K^+-ATPase and Mg^{2+}-ATPase of the PM (we have shown also in independent experiments that calix[4]arene C-90 (100 μM) does not influence the accumulation of calcium ions in the myometrium mitochondria).

Then we studied the concentration dependence of the inhibitory effect of calix[4]arene C-90 (10^{-7}-10^{-4} M) on the Ca^{2+}, Mg^{2+}-ATPase activity in the PM fraction (Fig. 3a). Calix[4]arene C-90 dose-dependently suppressed the Ca^{2+}, Mg^{2+}-ATPase activity, the inhibition coefficient $I_{0.5}$ value was 20.2 ± 0.5 μM and the Hill coefficient n_H value was 0.55 ± 0.02 (M ± m; $n = 5$) (table). Calix[4]arene C-90 also dose-dependently inhibited the ATP-hydrolase activity of the purified solubilized Ca^{2+}, Mg^{2+}-ATPase preparation (Fig. 3b), the $I_{0.5}$ value was 58.5 ± 6.4 μM and the n_H value was 0.67 ± 0.05 (M ± m; $n = 5$).

To determine the role of chemical groups of the calix[4]arene C-90 molecule in the inhibition of the activity of Ca^{2+}, Mg^{2+}-ATPase of the PM fraction, two model compounds were studied: the calix[4]arene "bowl" calix[4]arene C-150 and the compound M-1. Figure 1 shows that calix[4]arene C-150 is an unmodified calix[4]arene scaffold. The compound M-1 contains one phenolic fragment and a sulfonylamidine group analogous to those in the calix[4]arene C-90 molecule (Fig. 1).
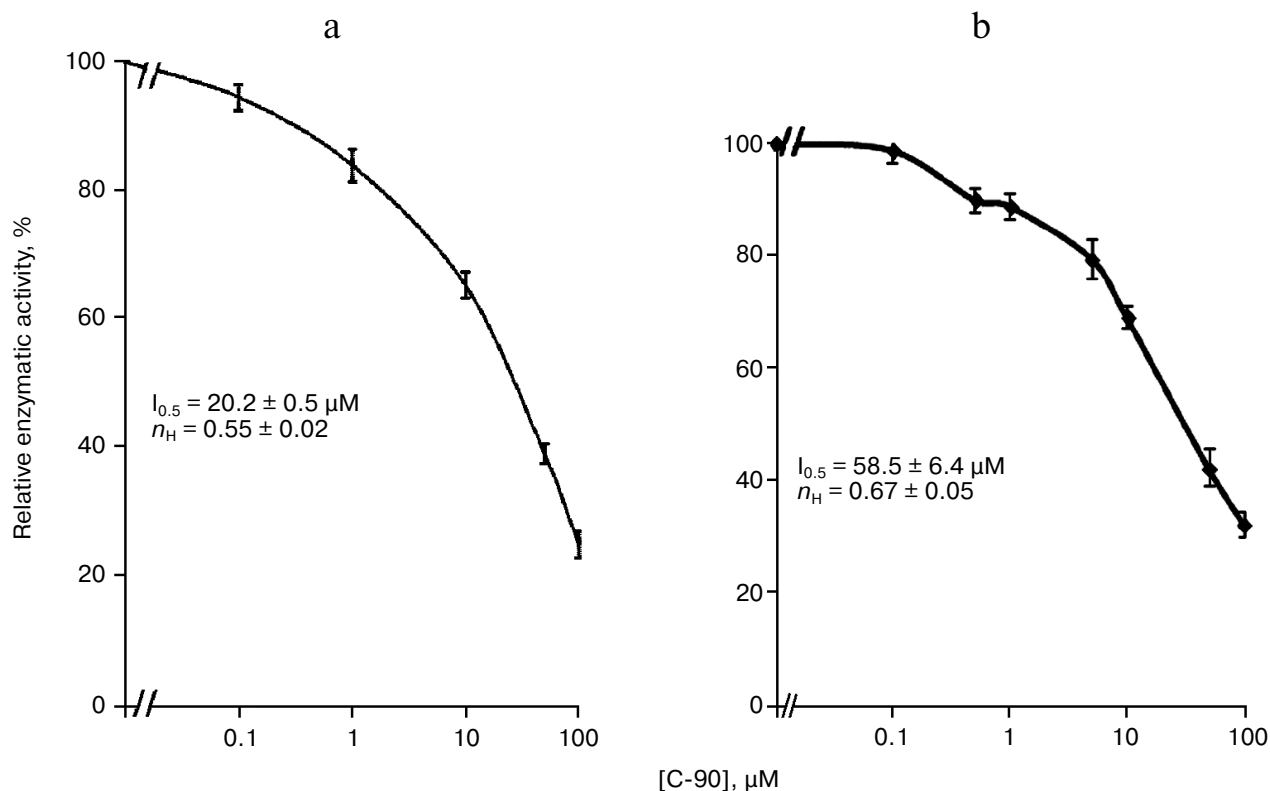

a

b

$I_{0.5}$ = 20.2 ± 0.5 μM
n_H = 0.55 ± 0.02

$I_{0.5}$ = 58.5 ± 6.4 μM
n_H = 0.67 ± 0.05

[C-90], μM

Fig. 3. Concentration dependences of the inhibitory effect of calix[4]arene C-90 on the enzymatic activities of Ca^{2+}, Mg^{2+}-ATPase of the plasma membrane fraction of myometrium cells (a) and of the purified solubilized Ca^{2+}, Mg^{2+}-ATPase preparation (b) (M ± m; $n = 5$). Value of the specific enzymatic activity in the absence of calix[4]arene C-90 in the incubation medium is taken as 100%.
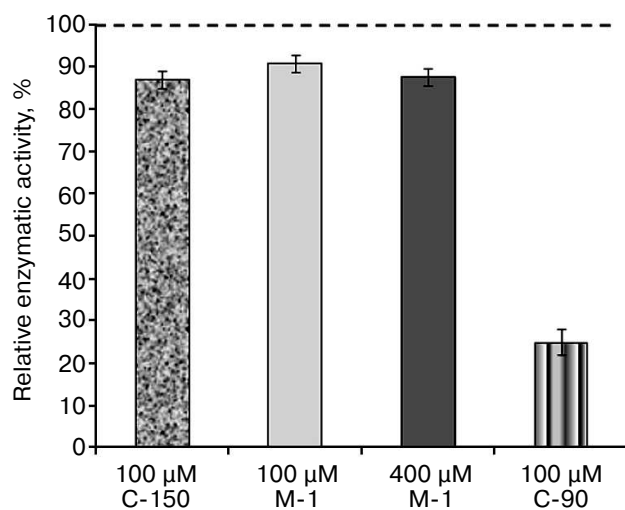
Fig. 4. Enzymatic activity of Ca^{2+},Mg^{2+}-ATPase in the plasma membrane fraction of myometrium cells in the presence of calix[4]arenes C-90 and C-150 and also of the model compound M-1 (M $\pm$ m; $n = 5$). The specific enzymatic activity value in the absence of calix[4]arenes C-90 and C-150 and also of compound M-1 in the incubation medium is taken as 100%.

At 100 μM concentration, calix[4]arene C-150 insignificantly (by $13.0 \pm 1.8\%$) decreased the Ca^{2+},Mg^{2+}-ATPase activity in the PM fraction of the myometrium cells (M $\pm$ m; $n = 5$). The model compound M-1 at 100 μM concentration also suppressed Ca^{2+},Mg^{2+}-ATPase slightly, by $9.3 \pm 2.4\%$, and at 400 μM concentration (the C-90 molecule includes four M-1 fragments) it inhibited the Ca^{2+},Mg^{2+}-ATPase activity by $12.5 \pm 1.7\%$ (M $\pm$ m; $n = 5$) (Fig. 4 and the table). However, calix[4]arene C-90 at 100 μM concentration inhibited efficiently (as compared to calix[4]arene C-150 and compound M-1) the Ca^{2+},Mg^{2+}-ATPase activity in both the PM fraction of the uterine myocytes (by 75% with respect to the control) and the preparation of purified Ca^{2+},Mg^{2+}-ATPase solubilized from PM (by 68% with respect to the control) (Figs. 2 and 3). Consequently, the inhibitory effect of calix[4]arene C-90 on the Ca^{2+},Mg^{2+}-ATPase activity from the PM fraction (Figs. 2-4) is associated, first of all, with the cooperative influence of four sulfonylamidine groups spatially organized on the upper rim of the calix[4]arene scaffold and not with the action of the tetraphenol macrocycle as it is (the calix[4]arene "bowl" – calix[4]arene C-150), or with a possible effect of the separate sulfonylamidine residue (fragment M-1).

In the subsequent experiments we studied the influence of calix[4]arene C-90 structural analogs, that is calix[4]arenes C-715, C-716, and C-772 (Fig. 1), on the Ca^{2+},Mg^{2+}-ATPase activity of PM. Obviously, the main structural features of these compounds are specific just for the upper rim of the calix[4]arene "bowl". Thus, calix[4]arene C-716, in contrast to calix[4]arene C-90, contains only two phenylsulfonylamidine groups situated on the opposite phenolic residues of the calix[4]arene "bowl", whereas on two other phenolic cycles *tret*-butyl residues are located instead of phenylsulfonylamidine groups. Calix[4]arene C-772 also has only two phenylsulfonylamidine groups, in contrast to calix[4]arene C-90, but they are located on the neighboring phenolic residues of the calix[4]arene "bowl", whereas on the two other phenolic cycles, as in the case of calix[4]arene C-716, *tret*-butyl residues are located instead of phenylsulfonylamidine groups. Finally, calix[4]arene C-715 has the simplest structure: compared to calix[4]arene C-716, it lacks phenylsulfonyl residues. That is, trifluoromethylcarbonylamino groups are located on its upper rim instead of trifluoromethyl(phenylsulfonylimino)methylamino groups (Fig. 1). Our studies revealed that calix[4]arenes used at 100 μM concentration suppressed the activity of Ca^{2+},Mg^{2+}-ATPase of PM, on average, by 20% (C-715), by 45% (C-772), and by 61% (C-716), whereas calix[4]arene C-90 suppressed the Ca^{2+},Mg^{2+}-ATPase activity by 75% (as compared to the control value) (Fig. 5). None of these calix[4]arenes influenced the activities of Na^+,K^+-ATPase and "basal" Mg^{2+}-ATPase. In our subsequent experiments, we studied concentration (10^{-7} to 10^{-4} M) dependences of action of the above-mentioned calix[4]arenes on the activity of Ca^{2+},Mg^{2+}-ATPase of PM. All these compounds were shown to dose-dependently suppress this activity (Fig. 6). The $I_{0.5}$ values were 168.3 ± 21.1 and 53.4 ± 3.5 μM, respectively, for calix[4]arenes C-772 and C-716, where-
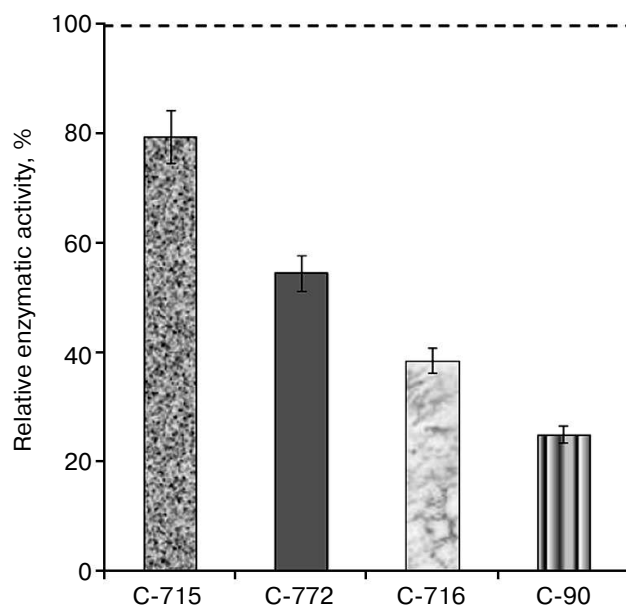

Fig. 5. Enzymatic activity of Ca^{2+},Mg^{2+}-ATPase in the plasma membrane fraction of myometrium cells in the presence of calix[4]arene C-90 and of its structural analogs: calix[4]arenes C-716, C-772, and C-715 (M $\pm$ m; $n = 5$). The specific enzymatic activity value in the absence of calix[4]arenes in the incubation medium is taken as 100%.
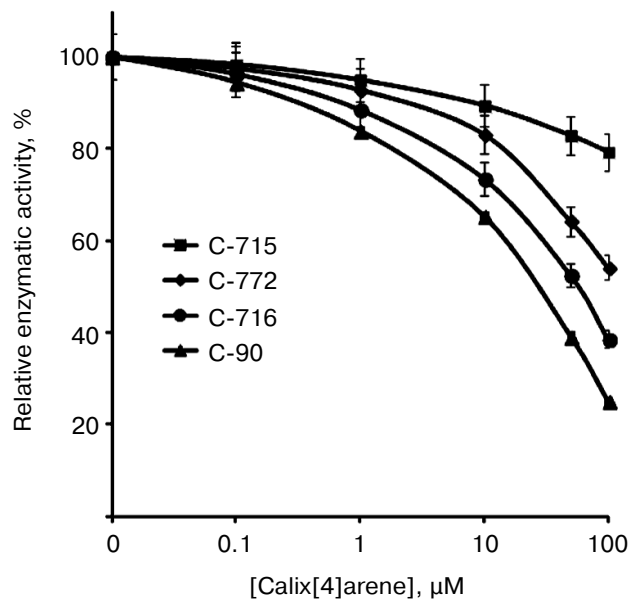
Fig. 6. Concentration dependences of the inhibitory effect of calix[4]arene C-90 and of its structural analogs on the activity of Ca^{2+},Mg^{2+}-ATPase in the plasma membrane fraction of myometrium cells (M $\pm$ m; $n = 5$). The specific enzymatic activity in the absence of calix[4]arenes in the incubation medium is taken as 100%.

as for calix[4]arene C-90 the $I_{0.5}$ value was 20.2 $\pm$ 0.5 µM. It is impossible to correctly determine the inhibition coefficient $I_{0.5}$ for calix[4]arene C-715 because of its weak inhibitory influence on the activity of Ca^{2+},Mg^{2+}-ATPase (Figs. 5 and 6 and the table).

Thus, to efficiently inhibit the Ca^{2+},Mg^{2+}-ATPase activity of PM (Figs. 2-5), the calix[4]arene molecule has to have four phenylsulfonylamidine groups (calix[4]arene C-90) on the upper rim of the macrocycle (Fig. 1). However, it should be noted that the inhibitory effects of the studied calix[4]arenes (Fig. 1) on the Ca^{2+},Mg^{2+}-ATPase activity of PM also depended on the number and mutual location of phenylsulfonylamidine groups on the upper rim of the calix[4]arene "bowl" (Figs. 5 and 6).

The activity of Ca^{2+},Mg^{2+}-ATPase of PM is a biochemical manifestation of functioning of the Mg^{2+},ATP-dependent calcium pump that pumps calcium ions from the myoplasm into the extracellular medium [1, 2, 5, 6, 19, 20]. Considering the kinetic parameters of Ca^{2+},Mg^{2+}-ATPase of PM and the inhibitory effect of calix[4]arene C-90 on its activity that we determined earlier, we created (in a stable regimen) a model of the influence of this inhibitor on the "basal" concentration of calcium ions ($[Ca^{2+}]_i$) in an unexcited uterine myocyte. According to results of the modeling, the inhibitory effect of calix[4]arene C-90 on Ca^{2+},Mg^{2+}-ATPase of PM has to lead to an increase in $[Ca^{2+}]_i$, and the $[Ca^{2+}]_i$ dependence on the C-90 concentration is biphasic: within the calix[4]arene concentration range of 1-25 µM, this com-

pound promotes a moderate increase in $[Ca^{2+}]_i$ in unexcited myocytes (approximately to 150-200 nM), whereas at concentration of C-90 > 25 µM the $[Ca^{2+}]_i$ value increases exponentially with an increase in the calix[4]arene concentration [21]. In this regard, it should be noted that in preliminary experiments performed in our laboratory by L. G. Babich and S. G. Shlykov with the Ca^{2+}-sensitive fluorescent probe Fluo-4AM, it was found that the Ca^{2+},Mg^{2+}-ATPase inhibitor calix[4]arene C-90 (100 µM) induced an increase in the "basal" $[Ca^{2+}]_i$ and decelerated the relaxation of the oxytocin-induced (100 nM) calcium signal in uterine myocytes.

We suppose that our experimental data on the selective and sufficiently high affinity ($I_{0.5} \approx 20$ µM) inhibitory effect of calix[4]arene C-90 on the activity of transport Ca^{2+},Mg^{2+}-ATPase of PM can be used for further elucidation of mechanisms regulating Ca^{2+} concentration in SMC and of the role of this pump in control of electromechanical coupling in myocytes.

Taking into account the nontoxic nature of calixarenes [22], we think that our data are promising for use in practice of calix[4]arene C-90 as a "soft" stimulator of uterine contractility.

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