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KINETICS OF RELAXATION OF RAT MYOMETRIUM IN CONDITIONS OF INHIBITION OF PLASMA MEMBRANE CALCIUM PUMP AND SYSTEMS OF ACTIVE Ca^{2+} TRANSPORT OF INTRACELLULAR Ca^{2+}-DEPOT

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The processes of regulation of smooth muscle contractile function depend greatly on the efficiency of maintaining Ca^{2+}-homeostasis by systems of active ionic transport of the plasma membrane (calcium pump and Na^+,Ca^{2+}-exchanger) and intracellular stores (sarcoplasmic reticulum and mitochondria). In this work, the effect of calixarene C-716 (macrocyclic compound capable of blocking functioning of the plasma membrane calcium pump) on the kinetics of relaxing K^+-induced contraction of rat myometrium smooth muscles, while inhibiting the systems of active transport of calcium ions of intracellular Ca^{2+}-stores, were investigated. It was established that the application of 100 μM calixarene at the maximum of the phase component of the K^+-induced contractile response did not change the kinetic parameters of the relaxation process (the normalized maximal velocity of relaxation phase V_{nr} and the half-maximal time of relaxation $\tau_{1/2}$). The tonic component of K^+-induced contraction remained without statistically significant changes. Blocking of Ca^{2+}-accumulating function of mitochondria by the protonophore CCCP caused a significant increase in both the parameter V_{nr} and the tonic component of K^+-induced contraction, while the $\tau_{1/2}$ index remained at the control level. Blocking calcium pump of sarcoplasmatic reticulum with thapsigargin (TG) caused a considerable increase in the $\tau_{1/2}$,V_{nr} parameters and the tonic component of the K^+-induced contraction. Combination of TG and CCCP induce further increase in the V_{nr} parameter and the tonic component of K^+-induced contraction. The action of calixarene C-716 (100 μM) at the background of the impact of TG and CCCP was accompanied by further increase in the tonic contraction, V_{nr} remained at the increased level, and the $\tau_{1/2}$ index decreased. Possible mechanisms for changing the mechanokinetic parameters of relaxing K^+-induced contraction of myometrium is discussed. Analysis of the results of our experimental data and the data of other researchers allow predicting that an increase

in the rate of relaxation of myometrium smooth muscles at the action of calixarene C-716 under the conditions of blockage of intracellular Ca^{2+} depots is associated with a significant activation of NO synthesis by constitutive forms of NO-synthases, whereas an increase in the tonic component of K^+-contraction under these conditions is probably due to an elevated concentration of Ca^{2+} ions in the myocytes.

Keywords: myometrium, relaxation, systems of active Ca^{2+} transport, plasma membrane calcium pump, calixarene C-716, kinetic analysis

INTRODUCTION

Plasma membrane calcium ATPase (PMCA) is a constitutive structure of cellular membrane performing a function of high affinity (K_M for Ca^{2+} is 0.2–0.5 μM) system of pumping Ca^{2+} ions from the cytoplasm that ensures long-term maintenance of basal concentration of these cations in the state of dormancy [2]. PMCA belongs to the family of ion-transporting ATPases of P-type (P2B subclass) [15]. There are four known isoforms of PMCA which form a great variety of the enzyme (over thirty kinds) via an alternative splicing. Isoforms 1 and 4 of the PMCA are expressed practically in all tissues of the organism, whereas PMCA2 and PMCA3 were identified in cells of a limited number of tissues, in particular, brain, skeletal muscles and mammary gland. The PMCA2 isoform in a detectable amount is present in plasma membranes of mouse uterine cells [1, 3]. Two isoforms of PMCA are expressed in smooth muscles of rat non-pregnant myometrium: PMCA1b, PMCA4a and PMCA4b [16, 18, 21]. During pregnancy there is enhanced expression of isoform PMCA1 (PMCA1a and PMCA1b) [7, 8].

PMCA inhibitors are divided into 4 groups: inorganic ions (La^{3+}, Al^{3+}, VO_3^- etc), molecules, interacting with aminoacid residues of a macromolecule (carboxyeosin), calmodulin antagonists (calmidasolium and trifluoperazine) and thermodynamically conditioned transport of counter-ions (inhibiting the capability of transporting protons by alkalization of extracellular space) [2, 5, 23]. With an exception of some caloxins, these inhibitors are not specific regarding the PMCA and, which is more important, that reduces their role in pharmacological experiments, are not isoform-specific.

The elaboration of efficient and selective inhibitors of PMCA is in progress. At present there are two determined groups of perspective compounds-inhibitors which may qualify in terms of selectivity for PMCA. Firstly, a scientific group headed by Ashok Grover synthesized caloxins – small peptides (10–15 aminoacid residues) bound to extracellular part of PMCA [14]. Caloxins act as allosteric modulators affecting functionally-relevant conformational transitions in calcium pump molecule [22]. The efficiency of caloxins reaches the values of K_i at the level of 2.3–86 μM; the last generations of caloxins have expressed specificity regarding PMCA isoforms. Caloxin 1C2 inhibits PMCA4 selectively (K_i = 2.35 μM, whereas for isoforms PMCA1, 2 and 3, this index is one order higher). A second group of synthetic inhibitors is presented by calixarenes – macrocyclic supramolecular compounds that are derivatives of the phenolic aldehyde.

Calixarene C-716 (5,17-di(trifluoro)methyl(phenylsulfonylimino)-methylamino-11,23-di-tret-butyl-25,27-dipropoxycalix[4]arene) has two phenylsulfonylamidine groups at the upper rim of the calixarene bowl in *p*-position. The studies of S. O. Kosterin et al. demonstrated [13, 25] that of C-716 in the concentration of 100 μM decrease Ca^{2+}-ATPase activity of plasma membranes of porcine uterine myocytes by 61.5 % regarding the control. At similar concentration, calixarene C-90 (5,11,17,23-tetra(trifluoro)methyl (phenylsulfonylimino)-methylamino-25,26,27,28-tetrapropoxycalix[4]arene) contains four

phenylsulfonylamidine groups at the upper rim of the calixarene bowl), by inhibiting 75.0 % Ca^{2+}-ATPase activity of porcine uterus preparations of plasma membrane [13, 26].

It is known that the processes of regulating the contractive function of smooth muscles depend greatly on the efficiency of maintaining Ca^{2+}-homeostasis by the intracellular depots – sarcoplasmatic reticulum and mitochondria [6, 20, 27]. To establish the regularities of the participation for some systems of active transport of calcium ions (and, in particular, PMCA) in the regulation of the relaxation process of the uteric smooth muscles, we a studied mechanokinetic effects of blocking these components (Ca^{2+}-pump of sarcoplasmic reticulum, potential-dependent systems of Ca^{2+}-transport of mitochondria and PMCA).

The aim of present work was to study the impact of calixarene C-716 on the kinetics of relaxing K^+-induced contraction of rat myometrium smooth muscles alongside with inhibiting the systems of active transport of calcium ions of the intracellular Ca^{2+}-stores.

MATERIALS AND METHODS

The experiments were conducted using Wistar female rats. All manipulations with animals were conducted according to the International convention for the protection of animals and the Law of Ukraine "On Protection of Animals from Cruelty". Approved at the meeting of the Bioethics Committee of the ESC "Institute of Biology and Medicine" of the Kyiv National Taras Shevchenko University (Protocol N 2, October 20, 2016).The animals were killed by a lethal dose of the propofol narcosis (Sigma).

The contractive activity was studied by a tenzometric method in the isometric mode using the preparations of the longitudinal muscles of uterine horns (average size – 2×10 mm). Smooth muscle stripes were placed in the working chamber of 2 ml with the flowing Krebs solution (the flow rate of 5 ml/min), thermostated at 37 °C; the preparations were provided with a passive tension (5–10 mN) and left for 1 h until contractions with constant parameters appear. The registration of signals was conducted using electric potentiometer H339 and analogue-to-digital transformer.

Krebs solution used for experiments contained (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 hyperkalemic solution, containing K^+ ions in the 80 mM concentration, was prepared by isotonic replacement of the required amount of Na^+ ions in the initial Krebs solution with the equimolar amount of K^+ ions.

Calixarene C-716 was synthesized at the Institute of the Organic Chemistry, NAS of Ukraine (Phosphoranes Chemistry Department), headed by Prof. V. I. Kalchenko.

Calixarene C-716 was preliminarily diluted in the DMSO, and introduced into the solution in the concentration of 10 μM (the final aliquot of the organic solvent solution was 0.25 % from the total volume of this solution) 30 min prior to studying the mechanic activity of the preparations. Control contractions were studied in solutions that contain 0.25 % DMSO.

The analysis of kinetic properties of contractions was done according to the method described previously [4]. Experimental data were processed by variation statistics methods using OriginPro 8 program. The samples were checked in terms of belonging to normally distributed general populations according to Shapiro-Wilk test. The Student's *t*-test was used to determine the reliable differences between the mean values of samplings. In all cases the results were considered reliable on a condition of the probability value of p under 5% ($p < 0.05$). The results were presented as the arithmetic mean ± standard error of the mean value (M±m), n – number of experiments.

RESULTS AND DISCUSSION

The application of calixarene C-716 (100 μM) at the maximum of phase component of K^+-induced a contraction of the myometrium smooth muscle preparations did not cause significant changes in the mechanokinetic of the relaxation process (Fig. 1). Kinetic analysis confirmed the absence of the effect from a direct application of C-716 on the indices of normalized maximal velocity of relaxation phase V_{nr} of muscle preparations. In the control, this index was 0.23±0.03 min^{-1}, and at the action of calixarene C716 – 0.26±0.03 min^{-1} (n = 6, p>0.05). Similarly, no changes in the time parameter of half-maximal relaxation were found ($\tau_{1/2}$): it was 1.54±0.28 min in the control and 1.44±0.27 min at the action of C-716 (n = 6, p>0.05). In these conditions, the tonic component of K^+-induced contraction was without statistically significant changes.

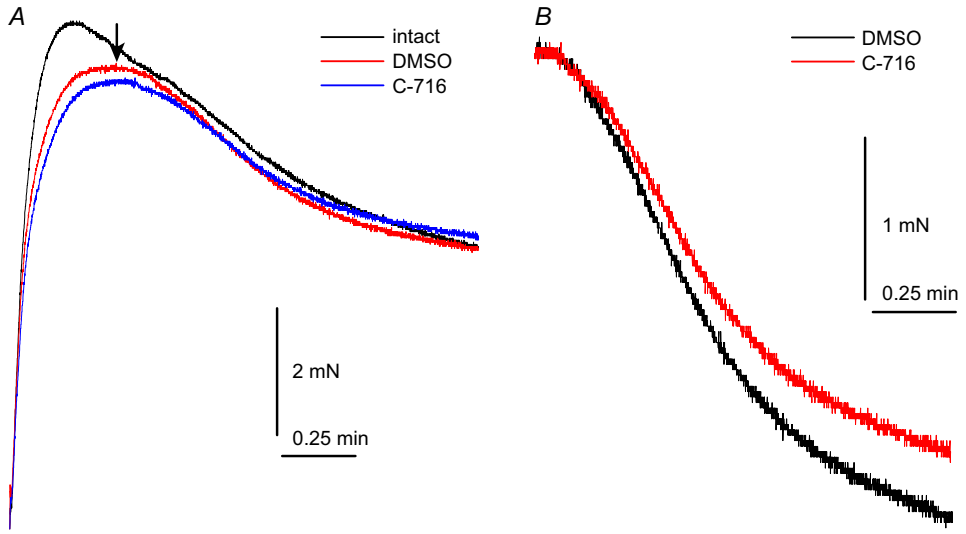

Fig. 1. The effect of calixarene C-716 (100 μM) on the kinetics of relaxation phase of K^+-induced (80 mM) contraction: *A* – action of C-716 compared to the control trends (intact – control, DMSO – application of organic solvent, dimethylsulfoxide 0.25 % in high-potassium (80 mM) solution); *B* – an enlarged fragment of a mechanograms post C-716 addition. The asterisk shows the moment of applying the DMSO and calixarene C-716

Рис. 1. Вплив каліксарену С-716 (100 мкМ) на кінетику фази розслаблення K^+-викликаного (80 мМ) скорочення: *А* – порівняно з контрольними трендами (intact – інтактний контроль, DMSO – аплікація органічного розчинника диметилсульфоксиду 0,25 % у гіперкалієвому (80 мМ) розчині); *В* – збільшений фрагмент механограм після додавання С-716. Стрілка вказує на момент аплікування DMSO та каліксарену С-716

Since mitochondria are among key organelles of smooth muscle tissue that determine the efficiency of contractive activity via potential-dependent formation of Ca^{2+}-microdomains [12, 17], in the first series of experiments, we studied the introduction of these organelles into the relaxation process of the longitudinal myometrium smooth muscles. Blocking Ca^{2+}-reserving function of mitochondria by protonophore CCCP (2 μM, 5 min of previous incubation), caused the activation of spontaneous contractive activity.

As one can see on Fig. 2, the application of CCCP (2 μM) at the maximum of phase contraction evoked by high-potassium solution (80 mM), caused several effects. Firstly, there is initial acceleration of the relaxation process, that goes into a second phase with

low velocity. Secondly, at the effect of CCCP, there is an increase in the tonic component of K^+-induced contraction. CCCP conditioned a considerable increase in the normalized maximal velocity of relaxation, that was 182.4±25.1 % on average regarding the control, accepted as 100% (n = 6, $p<0.05$), while the $\tau_{1/2}$ index remained at the control level.

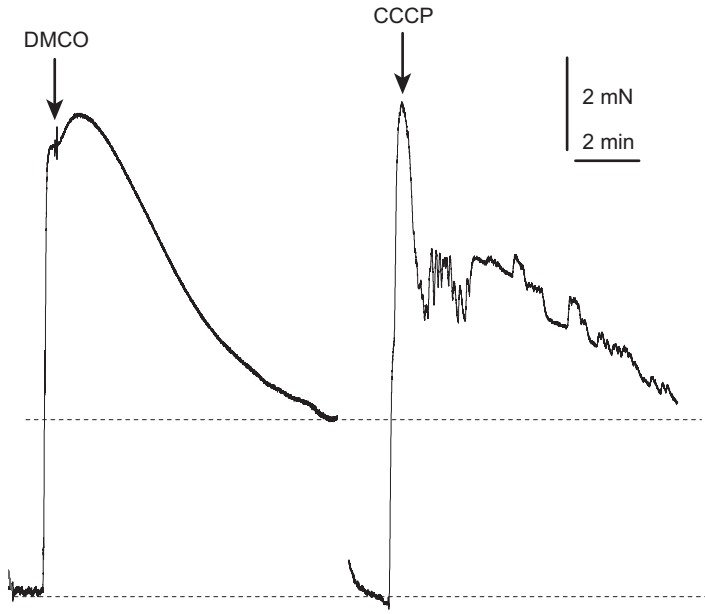

Fig. 2. The modulation of the relaxation phase of K^+-induced (80 mM) contraction of myometrium smooth muscles of rats under the effect of the mitochondrial protonophore CCCP (2 μM, 5 min of previous incubation). The asterisk shows a moment of adding DMSO or CCCP solution

Рис. 2. Модуляція фази розслаблення K^+-викликаного (80 мМ) скорочення гладеньких м'язів міометрія щурів за впливу мітохондріального протонофору CCCP (2 мкМ, тривалість попередньої інкубації 5 хв). Стрілка вказує на момент додавання розчину ДМСО або CCCP

The following series of experiments were address at studying the impact of inhibiting Ca^{2+}-reserving function of a sarcoplasmatic reticulum using such alkaloid as TG. Blocking sarcoplasmatic reticulum calcium pump (SRCA) with TG (1 μM, 30 min of previous incubation) caused a considerable increase in basal tone, a relative decrease in the amplitude, and a significant increase in frequency of the spontaneous contractions.

TG also increased tonic component slowing down the relaxation process for smooth muscle contractions, caused by K^+-depolarization (Fig. 3). TG caused a decrease in parameter $\tau_{1/2}$ to (162.0±13.2) % on average compared to the control, accepted as 100 % ($p<0.05$, n = 6); the index of normalized maximal velocity of relaxation phase tended to increase and amounted to (128.2±11.6)% ($p>0.05$, n = 6).

In order to isolate a contribution of the process of pumping Ca^{2+} ions out of smooth muscle cells via PMCA system, the following series of experiments were focused on combining the inactivation of the ability to accumulate Ca^{2+} simultaneously by both intracellular stores (sarcoplasmatic reticulum and mitochondria), using a combination of the thapsigargin, SRCA blocker (1 μM), and protonophore of mitochondria, CCCP (2 μM). As one can see on Fig. 4, under these conditions, the relaxation process for K^+-induced contraction was faster (by 75 % on average), but regardless of this fact, the tonic component

of high-potassium contraction was considerably increased (three-fold, compared to the control). The use of calixarene C-716 (100 μM) at the background of the impact of TG (1 μM) and CCCP (2 μM) was accompanied with further increase in the tonic contraction (383.3±29.4 % compared to the control, n = 6, p<0.05); the normalized maximal velocity of the relaxation phase remained at the increased level (177.6±15.4 %, n = 6, p<0.05), and the index $\tau_{1/2}$ decreased two-fold on average.

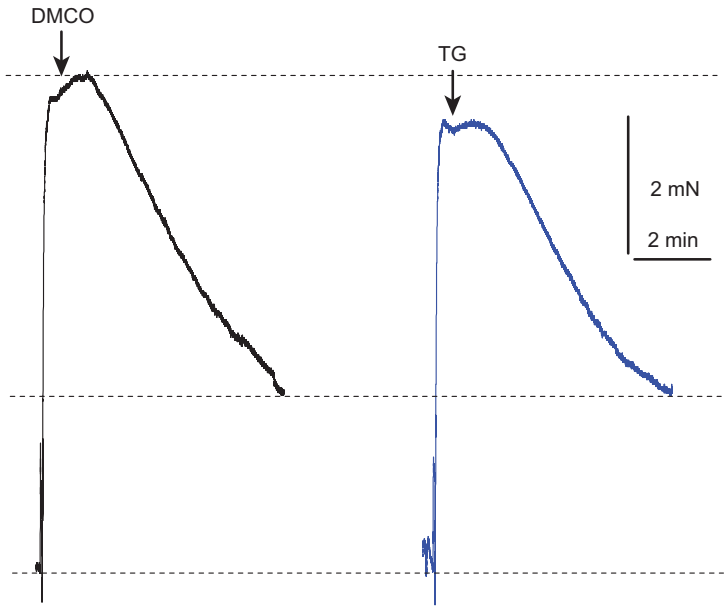

Fig. 3. The modulation of the relaxation phase for K^+-induced (80 mM) contraction of the myometrium smooth muscles of rats at blocking of Ca^{2+}-calcium pump of sarcoplasmatic reticulum with thapsigargin (TG, 1 μM, 20 min of previous incubation). The action of the organic solvent dimethylsulfoxide (DMSO, 0.25 %) was accepted as a control. The asterisk shows the moment of adding DMSO or TG solution

Рис. 3. Модуляція фази розслаблення K^+-викликаного (80 мМ) скорочення гладеньких м'язів міометрія щурів за блокування Ca^{2+}-кальцієвої помпи саркоплазматичного ретикулуму тапсигаргіном (TG, 1 мкМ, тривалість попередньої інкубації 20 хв). За контроль прийнято дію органічного розчинника диметилсульфоксиду (DMSO, 0,25 %). Стрілка вказує на момент додавання розчину ДМСО або TG

The work in restoring the role of the PMCA in maintaining Ca^{2+}-homeostasis and contractive activity of the visceral smooth muscles was done by L. Liu et al. [10, 11] using the models of ablation of genes coding isoforms PMCA1 and PMCA4 (*Pmca1*$^{+/-}$, *Pmca4*$^{+/-}$, *Pmca4*$^{-/-}$, and *Pmca1*$^{+/-}$*Pmca4*$^{-/-}$). The studies on a contractive activity and Ca^{2+}-signals, induced by depolarization of plasma membrane and carbacholine, the agonist of muscarinic receptors in smooth muscles of urine bladder of mice with the mentioned knockout of PMCA isoforms demonstrated that sensitive indices for a selective knockout of these enzymes are not just a relaxation phase, but also (and even more so) a contraction phase [10]. It is noteworthy that half-time of relaxation of muscle preparations after washing-out hyperkalemic solution in case of *Pmca1*$^{+/-}$ and *Pmca1*$^{+/-}$*Pmca4*$^{-/-}$ was 84.1 % and 94.2 % on average respectively compared to intact controls. The following effects were observed while inhibiting the functioning of other systems of Ca^{2+} transport: inhibiting sarcoplasmatic reticulum Ca^{2+}-pump was not accompanied with reliable changes in $\tau_{1/2}$ of relaxation of smooth muscle preparations after washing hyperkalemic solution (the average value

was 122.5 % compared to intact objects) [11]. As myometrium smooth muscle cells express similar isoforms of PMCA, one can use the material of the studies [10, 11] for deeper understanding of processes in the myometrium tissue at the impact of a macrocyclic compound calixarene C-716.

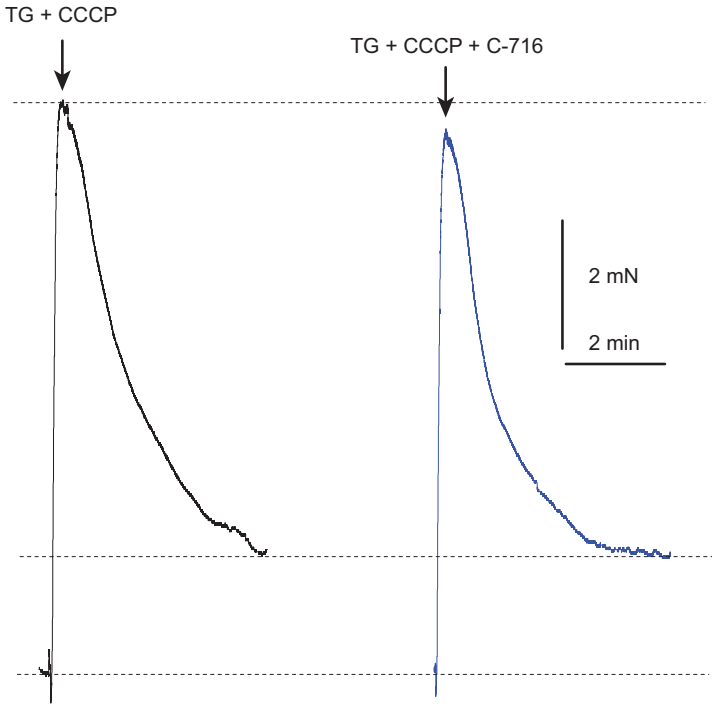

Fig. 4. The modulation of the relaxation phase for K^+-induced (80 mM) contraction of the myometrium smooth muscles of rats at the blocking of Ca^{2+}-calcium pump of a sarcoplasmatic reticulum with thapsigargin (TG, 1 μM, 20 min of previous incubation) and mitochondrial protonophore CCCP (2 μM, 5 min of previous incubation) and cumulative effect of TG, CCCP and calixarene C-716 (100 μM). The asterisk shows the moment of adding substances solutions

Рис. 4. Модуляція фази розслаблення K^+-викликаного (80 мМ) скорочення гладеньких м'язів міометрія щурів за дії блокатора Ca^{2+}-кальцієвої помпи саркоплазматичного ретикулуму тапсигаргіну (TG, 1 мкМ, тривалість попередньої інкубації 20 хв) і мітохондріального протонофору CCCP (2 мкМ, тривалість попередньої інкубації 5 хв) та сукупної дії TG, CCCP і каліксарену С-716 (100 мкМ). Стрілка вказує на момент додавання розчинів речовин

It is known that active PMCA molecules block the function of the constitutive isoforms of the nitric oxide synthase [9, 19]. In our previous study it was found that blocking the synthesis of nitric oxide in rat uteric smooth muscles eliminates the effects of calixaren C-90 on their contractile function [24]. It is reasonable to predict that accelerating the relaxation of the smooth muscles of myometrium by the action of the C-716 under conditions of blocking functioning of the intracellular Ca^{2+}-stores is associated with a significant activation of synthesis of the nitric oxide by constitutive (Ca^{2+}-dependent) isoforms of NO-synthase in tissues of the uterus.

CONCLUSIONS

The application of calixarene C-716 at the maximum of phase-wise K^+-induced contraction in conditions of blocking Ca^{2+}-reserving function of intracellular calcium

stores that allow isolating the contribution of plasma membrane into Ca^{2+}-transporting processes causes acceleration of the velocity of relaxing myometrium smooth muscles at the background of an increased tonic component of contraction.

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1. *Brini M., Calì T., Ottolini D., Carafoli E.* The plasma membrane calcium pump in health and disease. **FEBS J**, 2013; 280 (21): 5385–5397.
[DOI: 10.1111/febs.12193; PMID: 23413890; Google Scholar]
2. *Brini M., Carafoli E.* Calcium Pumps in Health and Disease. **Physiol. Rev**, 2009; 89: 1341–1378.
[DOI: 10.1152/physrev.00032.2008; PMID: 19789383; Google Scholar]
3. *Brini M., Carafoli E.* The Plasma Membrane Ca^{2+} ATPase and the Plasma Membrane Sodium Calcium Exchanger Cooperate in the Regulation of Cell Calcium. **Cold Spring Harb. Perspect Biol**, 2011; 3 (2): a004168.
[DOI: 10.1101/cshperspect.a004168; PMID: 21421919; Google Scholar]
4. *BurdygaTh.V., Kosterin S.A.* Kinetic analysis of smooth muscle relaxation. **Gen. Physiol. Biophys**, 1991; 10: 589–598.
[PMID: 1802780; Google Scholar]
5. *de Sautu M., Saffioti N.A., Ferreira-Gomes M.S., Rossi R.C., Rossi J.P.F.C., Mangialavori I.C.* Aluminum inhibits the plasma membrane and sarcoplasmic reticulum Ca^{2+}-ATPases by different mechanisms. **Biochim. Biophys. Acta**, 2018; 1860 (8): 1580–1588.
[DOI: 10.1016/j.bbamem.2018.05.014; PMID: 29859139; Google Scholar]
6. *Gravina F.S., Parkington H.C., Kerr K.P., de Oliveira R.B., Jobling P., Coleman H.A., Sandow S.L., Davies M.M., Imtiaz M.S., van Helden D.F.* Role of mitochondria in contraction and pacemaking in the mouse uterus. **Br. J. Pharmacol**, 2010; 161 (6): 1375–1390.
[DOI: 10.1111/j.1476-5381.2010.00949.x; PMID: 20942856; Google Scholar]
7. *Khan I., Tabb T.N., Garfield R. E.,Grover A.K.* Ca pump messenger RNA expression in pregnant rat uterus. **Biochem. Int**, 1992; 27(1): 189–196.
[PMID: 1320884; Google Scholar]
8. *Kim H.J., Lee G.S., Ji Y.K., Choi K.C., Jeung E.B.* Differential expression of uterine calcium transporter 1 and plasma membrane Ca^{2+} ATPase 1b during rat estrous cycle. **Am. J. Physiol. Endocrinol. Metab**, 2006; 291: E234–E241.
[DOI: 10.1152/ajpendo.00434.2005; PMID: 16825604; Google Scholar]
9. *Lewis S., Little R., Baudoin F., Prehar S., Neyses L., Cartwright E.J., Austin C*. Acute inhibition of PMCA4, but not global ablation, reduces blood pressure and arterial contractility via a nNOS-dependent mechanism. **J. Cell. Mol. Med**, 2018; 22(2): 861–872.
[DOI: 10.1111/jcmm.13371; PMID: 29193716; Google Scholar]
10. *Liu L., Ishida Y., Okunade G., Pyne-Geithman G.J., Shull G.E., Paul R.J*. Distinctrolesof PMCA isoformsin Ca^{2+} homeostasis of bladder smooth muscle: evidence from PMCA gene-ablated mice. **Am. J. Physiol. Cell Physiol**, 2007; 292: C423–C431.
[DOI: 10.1152/ajpcell.00313.2006; PMID: 16956963; Google Scholar].
11. *Liu L., Ishida Y., Okunade G., Shull G.E., Paul R.J.* Role of plasma membrane Ca^{2+}-ATPase in contraction-relaxation processes of the bladder: evidence from PMCA gene-ablated mice. **Am. J. Physiol. Cell. Physiol**, 2006; 290(4): C1239–C1247.
[DOI: 10.1152/ajpcell.00440.2005; PMID: 16291816; Google Scholar]
12. *McCarron J.G., Chalmers S., Bradley K.N., MacMillan D., Muir T.C.* Ca^{2+}microdomains in smooth muscle. **Cell Calcium**, 2006; 40(5–6): 461–493.
[DOI: 10.1016/j.ceca.2006.08.010; PMID: 17069885; Google Scholar]

13. *NikonishinaYu.V., Mazur Yu.Yu*. Inhibitoral influence of structural analysis of calix[4]arene C-90 on Ca^{2+}, Mg^{2+}-ATPase of plasmatic membrane. **Biology: from molecule to biosphere**: Materials of the Xth International Conference of Young Scientists (December 2–4, 2015, Kharkiv, Ukraine). – Kharkiv: FOP Shapovalova T.M., 2015. – 256 p. (In Ukrainian).
14. *Pande J., Szewczyk M.M., Grover A.K.* Allosteric inhibitors of plasma membrane Ca^{2+} pumps: Invention and applications of caloxins. **World J. Biol. Chem**, 2011; 2(3): 39–47.
[DOI: 10.4331/wjbc.v2.i3.39; PMID: 21537489; Google Scholar].
15. *Pehlivanoğlu B., Bayrak S., Doğan M.* A close look at the contraction and relaxation of the myometrium; the role of calcium. **J. Turk. Ger. Gynecol. Assoc**, 2013; 14(4): 230–234.
[DOI: 10.5152/jtgga.2013.67763; PMID: 24592112; Google Scholar]
16. *Penniston J., Enyedi A.* Modulation of the plasma membrane Ca^{2+} pump. **J. Membr. Biol,** 1998; 165: 101–109.
[PMID: 9744998; Google Scholar]
17. *Poburko D., Liao C.H., Lemos V.S., Lin E., Maruyama Y., Cole W.C., van Breemen C*. Transient receptor potential channel 6-mediated, localized cytosolic [Na^+] transients drive Na^+/Ca^{2+} exchanger-mediated Ca^{2+} entry in purinergically stimulated aorta smooth muscle cells. **Circ. Res**, 2007; 101(10): 1030–1038.
[DOI: 10.1161/CIRCRESAHA.107.155531; PMID: 17872462; Google Scholar]
18. *Sanborn B.M.* Hormonal signaling and signal pathway crosstalk in the control of myometrial calcium dynamics. **Semin. Cell Dev Biol**, 2007; 18(3): 305–314.
[DOI: 10.1016/j.semcdb.2007.05.007;PMID: 17627855; Google Scholar]
19. *Schuh K., Uldrijan S., Telkamp M., Röthlein N., Neyses L.* The plasma membrane calmodulin–dependent calcium pump: a major regulator of nitric oxide synthasel. **J. Cell Biol**, 2001; 155(2): 201–205.
[DOI: 10.1083/jcb.200104131; PMID: 11591728; Google Scholar]
20. *Shmigol A.V., Eisner D.A., Wray S*. The role of the sarcoplasmic reticulum as a Ca^{2+} sink in rat uterine smooth muscle cells. **J. Physiol**, 1999; 520(1): 153–163.
[PMID: 10517808; Google Scholar]
21. *Shull G.E., Okunade G., Liu L.H., Kozel P., Periasamy M., Lorenz J.N., Prasad V.* Physiological functions of plasma membrane and intracellular Ca^{2+} pumps revealed by analysis of null mutants. **Ann N Y Acad Sci**, 2003; 986: 453–460.
[PMID: 12763865; Google Scholar]
22. *Strehler E.E.*Plasma Membrane Calcium ATPases as Novel Candidates for Therapeutic Agent Development. **J. Pharm. Pharm. Sci**, 2013; 16(2): 190–206.
[PMID: 23958189; Google Scholar]
23. *Szewczyk M.M., Pande J., Grover A.K.*Caloxins: a novel class of selective plasma membrane Ca^{2+} pump inhibitors obtained using biotechnology. **Pflugers Arch**, 2008; 456(2): 255–266.
[DOI: 10.1007/s00424-007-0348-6; PMID: 17909851; Google Scholar]
24. *Tsymbalyuk O.V., Kosterin S.O.* Effect of calixaren C-90 on contractile activity of smooth muscles of myometrium of rats. **Studia Biologica**, 2013; 7(3): 5–20. (In Ukrainian).
[DOI: https://doi.org/10.30970/sbi.0703.298; Google Scholar]
25. *Veklich T.A., Shkrabak A.A., Slinchenko N.N., Mazur I.I., Rodik R.V., Boyko V.I., Kalchenko V.I., Kosterin S.A*. Calix[4]arene C-90 Selective lyInhibits Ca^{2+},Mg^{2+}-ATPase of Myometrium Cell Plasma Membrane. **Biokhimiya**, 2014; 79(5): 532–540.
[DOI: 10.1134/S0006297914050058; PMID: 24954592; Google Scholar]
26. *Veklich T.O.* The inhibitory influence of calix[4]Arene of C-90 on the activity of Ca^{2+},Mg^{2+}-ATPases in plasma membrane and sarcoplasmic reticulum in myometrium cells. **Ukr. Biochem J,** 2016; 88 (2): 5–15.
[DOI: 10.15407/ubj88.02.005; PMID: 29227596; Google Scholar]
27. *Wray S., Burdyga T., Noble D., Noble K., Borysova L., Arrowsmith S*. Progress in understanding electro-mechanical signalling in the myometrium. **Acta Physiol (Oxf)**, 2015; 213(2): 417–431.
[DOI: 10.1111/apha.12431; PMID: 25439280; Google Scholar]

КІНЕТИКА РОЗСЛАБЛЕННЯ ГЛАДЕНЬКИХ М'ЯЗІВ МІОМЕТРІЯ ЩУРІВ ЗА УМОВ ПРИГНІЧЕННЯ Ca^{2+}-ПОМПИ ПЛАЗМАТИЧНОЇ МЕМБРАНИ ТА СИСТЕМ АКТИВНОГО ТРАНСПОРТУ ІОНІВ Ca^{2+} ВНУТРІШНЬОКЛІТИННИХ Ca^{2+}-ДЕПО

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Процеси регуляції скорочувальної функції гладеньких м'язів значною мірою залежать від ефективності підтримання Ca^{2+}-гомеостазу системами активного іонного транспорту плазматичної мембрани (кальцієвої помпи і Na^+,Ca^{2+}-обмінника) та внутрішньоклітинних депо (саркоплазматичного ретикулуму та мітохондрій). У роботі нами було досліджено вплив каліксарену С-716 (макроциклічної сполуки зі здатністю блокувати роботу кальцієвої помпи плазматичної мембрани) на кінетику розслаблення K^+-індукованого напруження гладеньких м'язів міометрія за умов пригнічення систем активного транспорту іонів кальцію внутрішньоклітинних Ca^{2+}-депо. Дія каліксарену С-716 (100 мкМ) на максимумі фазної складової K^+-індукованої скорочувальної відповіді не супроводжувалося змінами показників нормованої максимальної швидкості фази розслаблення (V_{nr}) та часу напівмаксимального розслаблення ($\tau_{1/2}$). Варто зазначити, що амплітуда тонічної складової K^+-скорочення за умов дії каліксарену С-716 не мала статистично значущих змін. Пригнічення здатності мітохондрій до накопичення іонів Ca^{2+} за дії протонофору СССР супроводжувалося суттєвим зростанням параметра V_{nr} на тлі збільшеної тонічної складової K^+-індукованого скорочення. За дії блокатора Ca^{2+}-помпи саркоплазматичного ретикулуму тапсигаргіну спостерігали збільшення тонічної складової на тлі збільшених значень параметрів $\tau_{1/2}$ і V_{nr}. За сукупного застосування протонофору СССР і блокатора тапсигаргіну спостерігали суттєве зростання параметра V_{nr} на тлі подальшого збільшення тонічного компонента K^+-скорочення. Аплікування 100 мкМ каліксарену С-716 за умов сукупної дії тапсигаргіну і СССР спричиняло подальше зростання тонічного скорочення на тлі підвищеної швидкості V_{nr} і суттєвого зменшення $\tau_{1/2}$. У роботі обговорено можливі механізми зміни механокінетичних параметрів процесу розслаблення K^+-індукованого напруження гладеньких м'язів міометрія. За сукупністю результатів власних експериментальних даних і даних інших дослідників ми можемо передбачити, що збільшення швидкості процесу розслаблення гладеньких м'язів міометрія під дією каліксарену С-716 (за попереднього блокування внутрішньоклітинних Ca^{2+}-депо) пов'язано зі суттєвою активацією синтезу оксиду азоту конститутивними формами NO-синтаз, тоді як підвищення тонічної складової K^+-скорочення за цих умов, ймовірно, обумовлюється підвищеною концентрацією іонів Ca^{2+} в міоцитах.

Ключові слова: міометрій, розслаблення, системи активного транспорту іонів Ca^{2+}, кальцієва помпа плазматичної мембрани, каліксарен С-716, кінетичний аналіз

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