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CALIX[4]ARENE C-715 MODULATES MITOCHONDRIAL FUNCTIONING AND CONTRACTILE ACTIVITY OF UTERINE SMOOTH MUSCLE

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Aim. To elucidate the effects of calix[4]arene C-715 on the contractile activity of myometrial strips and the Ca^{2+}-dependent functional activity of mitochondria.

Materials and Methods. Confocal imaging was performed on Wistar rats myocytes using MitoTracker Orange CM-H_2TMRos and FITC-labeled compound C-1308. NADH autofluorescence, energy-dependent Ca^{2+} accumulation, and NO generation in the isolated mitochondria were measured by spectrofluorimetry and flow cytometry. The contractile activity of rat's myometrium was recorded in the isometric mode. The hydrodynamic diameter of myocytes was assessed by laser correlation spectroscopy.

Results. C-1308 accumulates in myocytes and colocalizes with MitoTracker, confirming the ability of C-715 to interact with mitochondria. A mechano-kinetic analysis showed that C-715 at 30 μM enhances the amplitude, frequency, force, temporal, and impulse parameters of spontaneous contractions while reducing the velocity. C-715 at 30 μM inhibited NO synthesis in intact myocytes and mitochondria; at 10–30 μM suppressed NADH oxidation and energy-dependent Ca^{2+} accumulation, which correlate with the contractility increase and the decrease in the hydrodynamic diameter of myocytes.

Conclusions. The action of C-715 is directed at reducing Ca^{2+} transport activity and Ca^{2+}-dependent processes in mitochondria, resulting in an enhancement of myometrial contractility.

Key words: calix[4]arenes, Ca^{2+}, myometrium, contractile activity, mitochondria.

Mitochondria are crucial for both Ca^{2+} signaling and homeostasis, and smooth muscle contraction. At the same time, disruptions in inner mitochondrial membrane Ca^{2+} transport systems can lead to dysfunction, including ROS overproduction and contractile impairments [1]. Given the link between myometrial contractility and labor complications, identifying non-toxic compounds that modulate mitochondrial Ca^{2+}-transport systems and Ca^{2+}-dependent processes is a relevant medical challenge. The basis of the smooth muscle cell contraction-relaxation process lies in the coordinated functioning of systems that regulate changes in Ca^{2+} concentration within the myoplasm [2]. It has been demonstrated that polyphenolic macrocyclic compounds calix[4]arenes effectively modulate

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the activity of ATP-hydrolyzing Ca^{2+}-transporting systems of the plasma membrane, sarcoplasmic reticulum, and mitochondria in uterine smooth muscle (myometrium) [3]. The aforementioned energy-dependent Ca^{2+} transport systems of the plasmalemma and reticulum, as well as the ATPase activity of the myosin head, exhibit low sensitivity to the action of calix[4]arene C-715 (5,17-di(trifluoro)acetamido-11,23-di-*tert*-butyl-26,28-dihydroxy-25,27-dipropoxycalix[4]arene) (Fig. 1), suggesting a specific effect of this compound on mitochondrial Ca^{2+}-transporting systems.

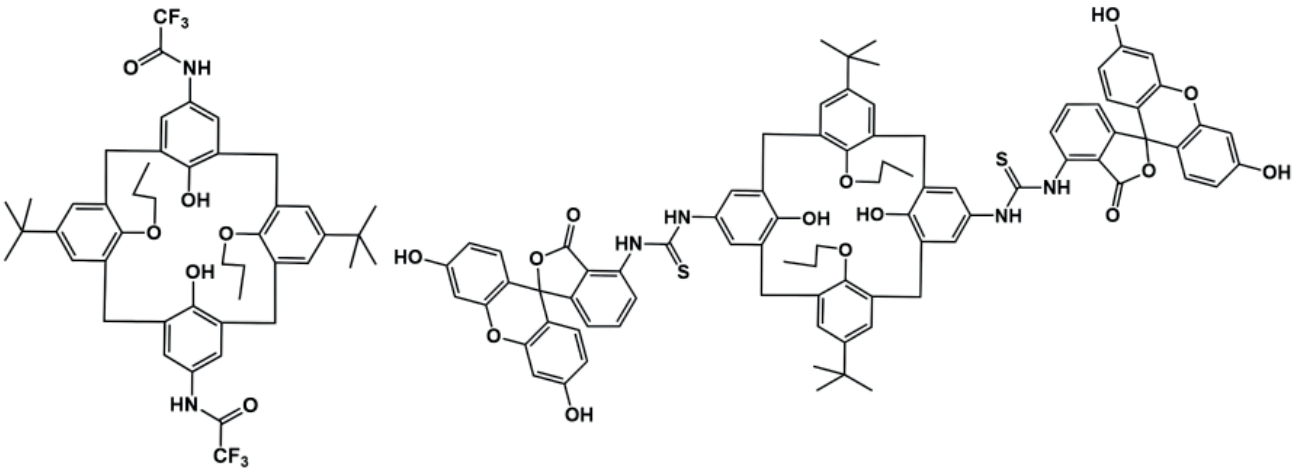

Fig. 1. **Structural formulas of calix[4]arenes C-715 and C-1308**

Aim. The study purposed to elucidate the effects of calix[4]arene C-715 on the contractile activity of myometrial strips and the Ca^{2+}-dependent functional activity of isolated mitochondria.

Methods. Studies were conducted on isolated myometrial mitochondria, cell suspensions, and strips from non-pregnant Wistar rats. Confocal imaging was performed on freshly isolated myocytes using MitoTracker Orange CM-H_2TMRos and FITC-labeled analog of calix[4]arene C-715 — compound C-1308 (Fig. 1). Measurement of changes in NADH autofluorescence, as well as the energy-dependent Ca^{2+} accumulation (using the Ca^{2+}-specific probe Fluo-4 AM), and NO generation (using the NO-specific probe DAF-FM) in the isolated mitochondria was carried out by spectrofluorimetry and flow cytometry (for NO production in myocytes). The spontaneous contractile activity of longitudinal smooth muscle preparations of rat uterus was recorded in the isometric mode under a constant load of 10 mN. Changes in the hydrodynamic diameter of myocytes in suspension were assessed using laser (photon) correlation spectroscopy. Data are presented as means ± SE based on the numbers of determinations. Differences between data sets from fluorometric experiments were analyzed using unpaired Student's *t*-tests in Microsoft Excel.

Results and Discussion. It was demonstrated that the FITC-labeled analog of calix[4]arene C-715 effectively accumulates in myocytes and colocalizes with MitoTracker Orange CM-H_2TMRos, confirming the ability of compound C-715 to interact with mitochondria (Fig. 2).

It was shown that calix[4]arene C-715 at 30 μM, both the amplitude and frequency increased while a decrease in basal tension was observed (Fig. 3). A comprehensive mechano-kinetic analysis [4] revealed that C-715 at 30 μM significantly increased the force at which the maximum velocity of the relaxation phase occurred, whereas a similar parameter for the contraction phase only showed a tendency to increase. Additionally, C-715 at 30 μM increased certain temporal and impulse parameters of myometrial contractile activity while reducing the velocity of spontaneous contractions relative to the control.

A series of biochemical and biophysical studies were conducted to elucidate the underlying mechanisms of these effects. In particular, it was shown that calix[4]arene C-715 at 30 μM inhibited NO synthesis in intact myocytes and isolated mitochondria. Additionally, C-715 (10–30 μM) suppressed NADH oxidation in mitochondria and reduced the efficiency of energy-dependent Ca^{2+} accumulation in these subcellular structures. These effects may explain the stimulatory effect of the studied compound on myometrial strips' contractile activity. In

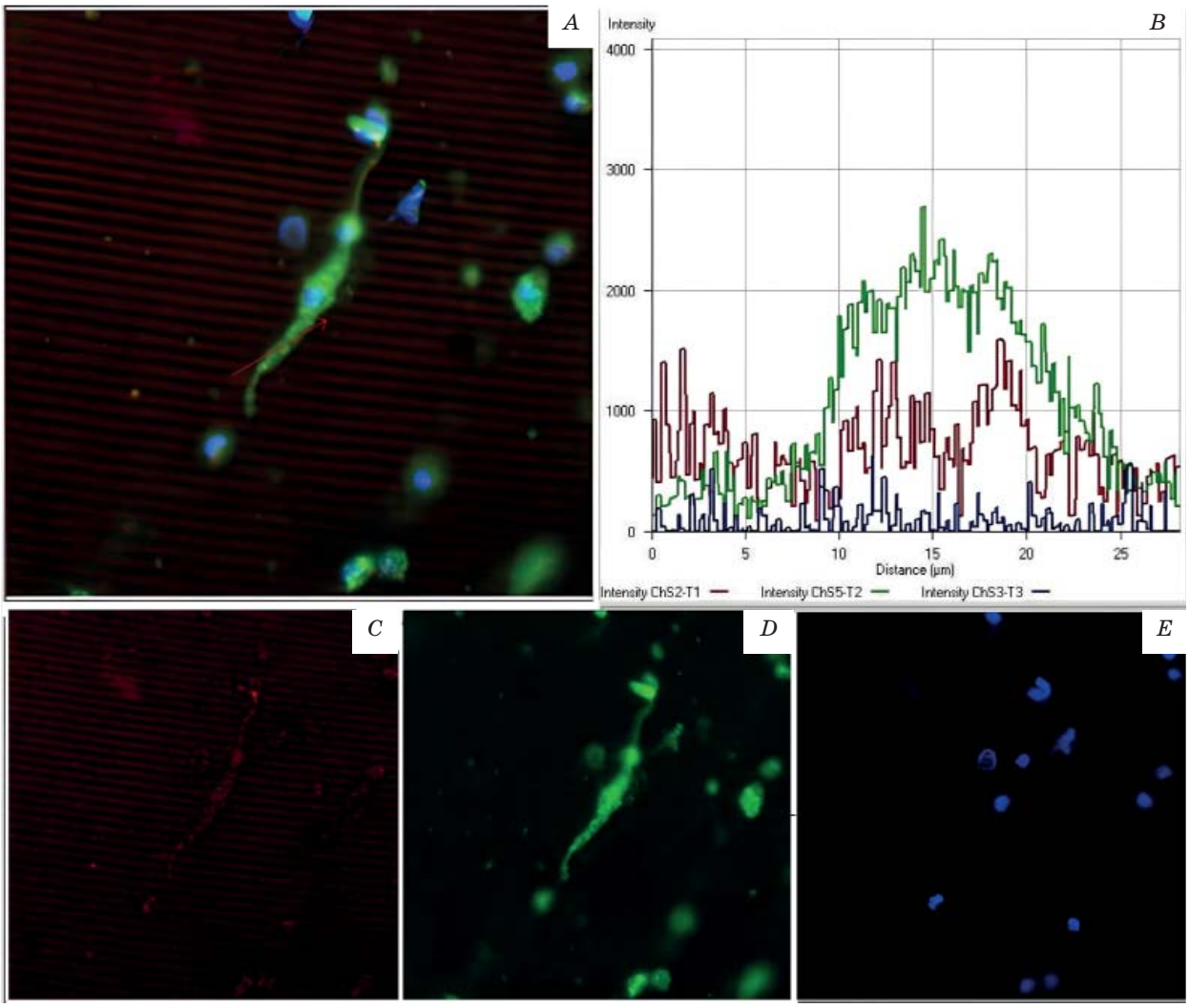

Fig. 2. **Evidence of calix[4]arenes' penetration in uterine smooth muscle cell**
Example of colocalization of fluorescent probes FITC-labeled calix[4]arene C-1308 (10 µM, green) and MitoTracker Orange CM-H2TMRos (200 nM, red) in myocytes (*A, B*). The computer analysis of the fluorescent dyes distribution profiles (*C–E*). Blue color — specific to the nucleus dye Hoechst 33342 (50 nM)

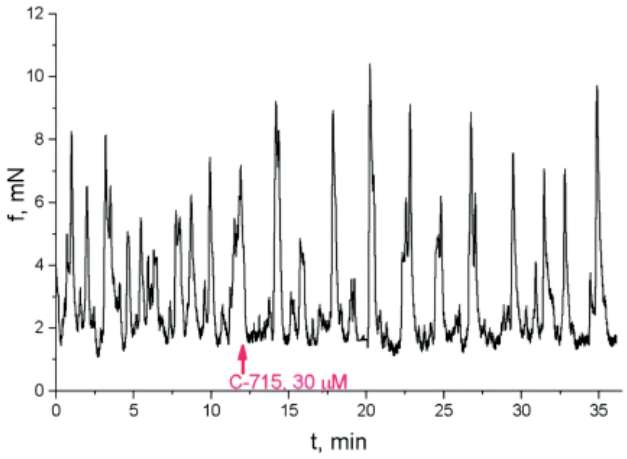

Fig. 3. **Typical mechanogram of the modulation of spontaneous contractions in the longitudinal smooth muscles of rat fallopian tubes and under the action of calix[4]arene C-715 (30 µM)**
The arrow indicates the moment of the compound adding to the normal Krebs solution

particular, inhibiting the electron transport chain under the influence of C-715 results in a decrease in the electrical potential on the inner mitochondrial membrane and a corresponding suppression of Ca^{2+} accumulation. These data also correlate with our results on the decrease in myocytes' hydrodynamic diameter (characteristic size) obtained by photon correlation spectroscopy. At 30 μM, this decrease reaches 30%, comparable to the similar effect of oxytocin at 10 μM.

Conclusions. Thus, the action of calix[4]arene C-715 on uterine smooth muscle cells is directed at reducing Ca^{2+} transport activity and Ca^{2+}-dependent processes in mitochondria, particularly electron transport chain activity and NO synthesis, which enhances myometrial contractile activity. The obtained data suggest the potential use of the studied calix[4]arene for targeted modulation of mitochondrial functional activity and, prospectively, as a uterotonic agent.

Authors' contribution

ISF performed both preparation of biological objects and solutions, executed spectrofluorimetric studies, and wrote the article; OVT carried out contractile activity studies including mechanokinetic analysis; RVR synthesized the studied compounds; MVR performed flow cytometry and laser correlation spectroscopy experiments; HVD carried out confocal imaging and statistical processing of results; YuVD guided the conception of the research and analyzed the results.

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REFERENCES

1. Matuz-Mares, D., González-Andrade, M., Araiza-Villanueva, M. G., Vilchis-Landeros, M. M., Vázquez-Meza, H.(2022). Mitochondrial calcium: effects of its imbalance in disease. *Antioxidants*, 11(5), 801. https://doi.org/10.3390/antiox11050801
2. Wray, S., Prendergast, C. (2019). The myometrium: from excitation to contractions and labour. *Adv. Exp. Med. Biol.,* 1124, 233–263. https://doi.org/10.1007/978-981-13-5895-1_10
3. Kosterin, S. O., Kalchenko, V. I., Veklich, T. O., Babich, L. G., Shlykov, S. G. (2019). Calixarenes as modulators of ATP-hydrolyzing systems of smooth muscles. Kyiv. 256 p.
4. Kosterin, S. O., Tsymbalyuk, O. V. (2022). Mechanokinetics of visceral smooth muscle and its modulation by nanomaterials. Kyiv. 232–247.