UDC 577.332.4 **https://doi.org/10.15407/biotech17.02.041**

CALIX[4]ARENES C-715 AND C-772 AS INSTRUMENTS OF INFLUENCE ON Ca^{2+}-TRANSPORT AND BIOENERGETICS IN MYOMETRIAL MITOCHONDRIA

I.S. FORYS, M.R. PAVLIUK

Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv.

E-mail: illia.forys@ukr.net

Received 2024/03/27
Revised 2024/04/09
Accepted 2024/04/30

Mitochondria play an important role in the processes of Ca^{2+}-signaling and Ca^{2+}-dependent smooth muscle contraction. Disruption of Ca^{2+}-transport functioning systems, localized in the inner mitochondrial membrane (IMM), can lead to Ca^{2+}-overloading of the matrix, hyperpolarization, disruption of Ca^{2+}-dependent metabolism of reactive oxygen species (ROS) and their hyperproduction, which results in mitochondrial dysfunction accompanied by smooth muscle contractile disruption [1, 2]. Since disordering of the myometrium contractile activity is the main cause of labor activity impairment or miscarriages, which constitutes a significant medical and social problem, the search for exogenous non-toxic compounds that would be able to effectively target mitochondrial Ca^{2+}-transport systems becomes relevant.

In recent years, significant attention from medical and biological sciences has been paid to macrocyclic compounds calix[4]arenes due to their ability to act like molecular platforms for the design of specific biologically active compounds [3]. It was demonstrated that at low (μM) concentrations calix[4]arenes were able to influence the functional activity of the myometrium mitochondria and its cells in general. In particular, it was shown that Ca^{2+}-transport systems of the plasma membrane and sarcoplasmic reticulum are low-sensitive to the studied calix[4]arenes — C-715 and C-772 [4], which implies the possibility of their more specific effect on mitochondrial Ca^{2+}-transport systems and Ca^{2+}-dependent processes. An important characteristic of these compounds is the absence of large hydrophobic substituents, which could have made significant changes in the functional activity of membranes. Also, they are neutrally charged, which implies relative easiness of penetration into cells and distribution in intracellular compartments.

Aim. Thus, the goal of the work was to study the effects of calix[4]arenes C-715 and C-772 on Ca^{2+}-transport, the electron transport chain (ETC) activity, and the ROS generation in the mitochondria of uterine smooth muscle.

Methods. Studies were conducted on isolated myometrial mitochondria and cells from non-pregnant Wistar rats. Measurement of changes in NADH autofluorescence, as well as the energy-dependent accumulation of Ca^{2+} (with Ca^{2+}-specific probe Fluo-4 AM) and ROS generation (with ROS-specific probe DCF-DA) in the fraction of isolated mitochondria was carried out using spectrofluorimetry method. The hydrodynamic diameter of mitochondria was measured using the laser correlation spectroscopy method. The concentration of protein in the mitochondrial fraction was determined with the Bradford method.

Results and Discussion. Calix[4]arenes C-715 (5,17-di(trifluoro)acetamido-11,23-di-*tert*-butyl-26,28-dihydroxy-25,27-dipropoxycalix[4]arene) and C-772 (5,11-di(trifluoromethyl(phenylsulfonylimino)methylamino-17,23-di-*tert*-butyl-25,26-dipropoxy-27,28-dihydroxycalix[4]arene) (Fig. 1) have autofluorescence, increasing linearly with concentrations ranging from 0.1 to 30 μM. Their fluorescence signal change indicates potential penetration through myocytes' plasma membrane and interaction with the IMM.

We found that studied calix[4]arenes slow down NADH oxidation in isolated mitochondria, suggesting they may inhibit complex I activity of the ETC (Fig. 2, *A, B*). Additionally, it was revealed that the studied compounds reduced the efficiency of energy-dependent accumulation of Ca^{2+} by

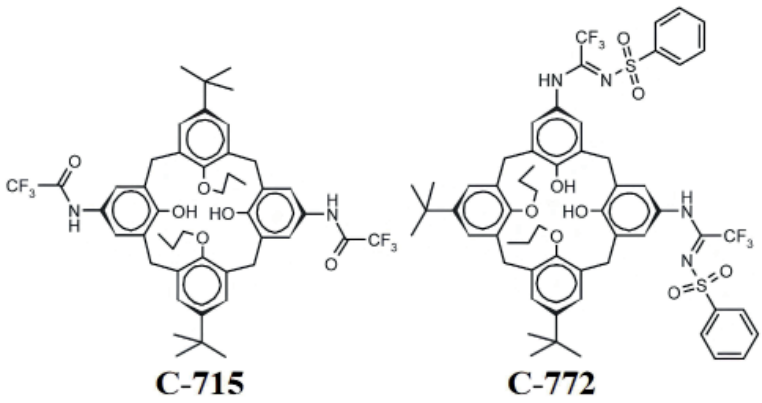

Fig. 1. **Structural formulas of calix[4]arenes C-715 and C-772**

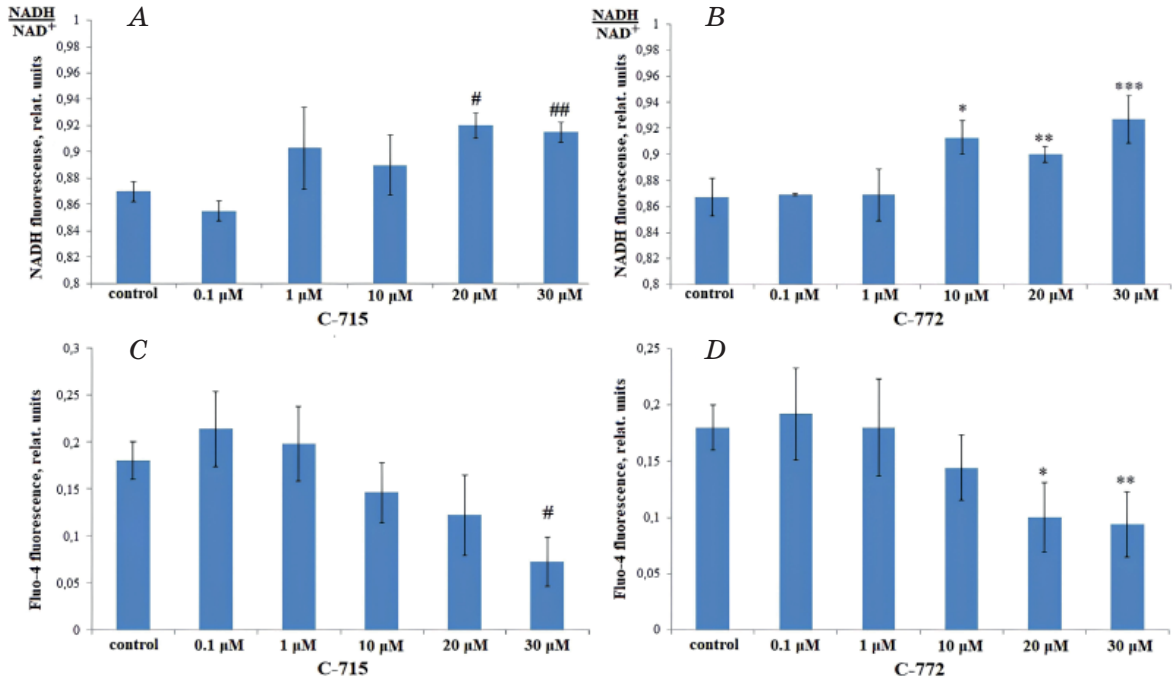

Fig. 2. **Results of statistical processing of calix[4]arenes C-715 and C-772 effect on:**
A, B — NADH oxidation; * $P = 0.05$; ** $P = 0.1$; *** $P < 0.05$; # $P = 0.01$; ## $P < 0.01$, relative to control (M±m, $n = 3$); *C, D* — energy-dependent accumulation of Ca^{2+}; # $P < 0.01$; * $P < 0.05$; ** $P = 0.05$, relative to control (M±m, $n = 5$)

isolated mitochondria with C-715 at 30 μM reducing it by 60%, and C-772 at 20 and 30 μM by 40% (Fig. 2, *C, D*).

Moderate inhibition of NADH oxidation might lower the electrochemical potential of the IMM, leading to reduced efficiency of the Ca^{2+} accumulation in the matrix and decreasing the intensity of Ca^{2+}-dependent processes. This includes the activity of Ca^{2+}-dependent dehydrogenases in the Krebs cycle and pyruvate dehydrogenase complex.

ROS generation in mitochondria is also a Ca^{2+}-dependent-process, which is closely coupled with the ETC activity [2]. We found that studied calix[4]arenes decrease the level of ROS generation by mitochondria. The more hydrophobic C-772 shows an effect at 0.1 and 1 μM with a shorter exposure, while the effect of C-715 increases with concentration, significantly decreasing ROS generation at 30 μM. Since complex I of ETC plays a leading role in ROS generation, and assuming that studied calix[4]arenes suppress its activity, this effect can be considered as protective on mitochondria.

The studied compounds moderately increase mitochondria size depending on concentration. C-715 at 20 and 30 μM increases hydrodynamic diameter by 30%, and C-772

at 30 μM by 20% ; at other concentrations the effect is negligible. Alterations in ETC activity affect mitochondrial osmotic balance, causing a moderate volume increase. The results of hydrodynamic diameter measurements support the notion that the compounds do not induce mitochondrial dysfunction.

Conclusions. Studied calix[4]arenes slow down the oxidation of NADH in isolated mitochondria, which represents an inhibitory effect on the ETC functioning, in particular its complex I. Additionally, selected compounds reduce both the energy-dependent accumulation of Ca^{2+} and ROS generation by isolated mitochondria. Such an effect on ROS biosynthesis could be considered as protective on organelles. Moderate increase in mitochondria hydrodynamic diameter suggests that the studied compounds do not cause mitochondrial dysfunction. Researched calix[4]arenes can be used in experimental practice to influence the mitochondrial functional activity.

Key words: calix[4]arene, Ca^{2+}, bioenergetics, mitochondria, myometrium.

Authors' contribution. ISF performed: preparation of biological objects, spectrofluorimetric studies, statistical processing of results, and wrote the article; MRP prepared biological solutions, and carried out measurements of the hydrodynamic diameter of mitochondria.

Funding source. This work was supported by funding from the National Academy of Sciences of Ukraine "Creation of modern calixarene regulators of biochemical processes for medicine and biotechnology" (State registration number 0123U100894).

Acknowledgments. Authors gratefully acknowledge Dr. Yu.V. Danylovych and Dr. H.V. Danylovych for valuable advice and recommendations during the research and discussion of scientific results; PhD R.V. Rodik for chemical synthesis of calix[4]arenes; PhD O.Yu. Chunikhin for technical support of works on a laser correlation spectrometer.

REFERENCES

1. *Rizzuto R., Giorgi C., Romagnoli A., Pinton P.* Ca^{2+} signaling, mitochondria and cell death. *Curr. Mol. Med*. 2008, 8(2): 119–130. https://doi.org/10.2174/156652408783769571
2. *Feissner R. F., Skalska J., Gaum W. E., Sheu S. S.* Crosstalk signaling between mitochondrial Ca^{2+} and ROS. *Front. Biosci. (Landmark Ed)*. 2009, 14(4): 1197–218. https://doi.org/10.2741/3303
3. *Rodik R. V., Boyko V. I., Kalchenko V. I.* Calixarenes in bio-medical researches. *Curr. Med. Chem.* 2009, 16(13): 1630–1655. https://doi.org/10.2174/092986709788186219
4. *Veklich T.O.* The comparative study of the inhibitory influence of calix[4]arene 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. https://doi.org/10.15407/ubj88.02.005