

KINETIC PROPERTIES OF Na^+, K^+ -ATPase OF SPERMATOZOA FROM FERTILE AND INFERTILE MEN UNDER EFFECT OF CALIX[4]ARENE C-107

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The calix[4]arene C-107 (5,17-diamino(2-pyridyl)methylphosphono-11,23-di-tert-butyl-26,28-dihydroxy-25,27-dipropoxy-calix[4]arene) effects on the kinetic properties of Na^+, K^+ -ATPase in spermatozoa of fertile (normozoospermia) and infertility men (oligozoospermia, and asthenozoospermia) were studied. It was shown that in spermatozoa of healthy men calix[4]arene C-107 inhibited Na^+, K^+ -ATPase activity and decreased the maximum reaction rate of ATP hydrolase reaction without affecting the coefficient of (half-) activation by ATP and Hill coefficient n_H . In oligo- and asthenozoospermic samples of spermatozoa almost a 2-fold decrease of cooperativity coefficient n_H of ATPase inhibition with calyx[4]aren C-107 was observed. In normozoospermic samples of spermatozoa the K_{MgCl_2} for Na^+, K^+ -ATPase was decreased at calix[4]arene C-107 high concentrations (≥ 50 nM) in the incubation medium in contrast to oligozoospermic samples of spermatozoa where K_{MgCl_2} was increased only at high calix[4]arene C-107 concentration (100 nM). The increase of the K_{MgCl_2} in the entire range of investigated calix[4]arene concentrations and the decrease of cooperativity coefficient n_H of MgCl_2 activating effect were detected in asthenozoospermic samples of Na^+, K^+ -ATPase.

Key words: calix[4]arene C-107, Na^+, K^+ -ATPase, male infertility, pathospermia.

Calix[4]arenes attracted more and more attention in recent years. They are macrocyclic oligomers formed by cyclic condensation of n-substituted phenols and formaldehyde. Calix[4]arenes are low molecular, low-toxic supramolecular compounds capable of altering the activity of ion-transporting systems [1, 2]. Due to their ability to form supramolecular complexes with biologically important molecules and ions they affect biochemical processes. Calix[4]arene matrices have low toxicity and sufficiency conformational mobility in solution which provides a great advantage for their application in biomedical studies as enzyme effectors, receptor simulators, organic polymer stabilizers, DNA microarray platform, etc [3-5]. Therefore calix[4]arenes are considered as promising molecular platforms for the creation of physiologically active compounds [6].

Approximately 50% of the infertility cases are related to male factor. The most common ab-

normal parameters are low motility (asthenozoospermia), low sperm count (oligozoospermia), or a combination of these abnormalities (oligoasthenozoospermia) [7, 8]. From the standpoint of modern biomembranology, many diseases are associated with changes in the structure and function of biomembranes. Membrane binding proteins, including the integral ATP-dependent transport enzyme, play an important role in proper functioning of the membranes. Therefore, it is necessary to further the study of properties of membrane-bound ion transport systems in the normal and at pathological states.

Na^+, K^+ -ATPase (Na^+, K^+ -activated, Mg^{2+} -dependent ATP-hydrolase, EC 3.6.1.37) is an electrogenic Ca^{2+} -independent transport system which reacts quickly to changes in intracellular and extracellular concentration of Na^+ and K^+ ions and maintains the transmembrane gradient of these ions and resting membrane potential needed for normal cell functioning [9]. The activity of many

other biochemical processes depends on the activity of this enzyme. Specifically, Na^+, K^+ -ATPase transmits signals to the nucleus of the cell by protein-protein interactions with sensor proteins [10]. Na^+, K^+ -ATPase is present in male germ cells and differentiated spermatozoa [11]. A unique $\alpha 4$ subunit was identified in the structure of Na^+, K^+ -ATPase of spermatozoa [12]. In addition to $\alpha 4$ subunit, $\alpha 1$ Na, K -ATPase isoform, which is ubiquitously present in all tissues, is also expressed in spermatozoa [11]. Na^+, K^+ -ATPase plays a crucial role in sperm physiology [13]. Many studies showed a significant relation between Na^+, K^+ -ATPase activity and sperm motility [9, 14]. Obviously, the search for effective inhibitors and activators of Na^+, K^+ -ATPase is important from both fundamental and practical point of view. Specifically, such studies may be useful for the development of potential pharmacological agents for the modulation of sperm motility.

In previous studies, it was shown that calix[4]arene C-107 (5,17-diamino(2-pyridyl)methylphosphono-11,23-di-tert-butyl-26,28-dihydroxy-25,27-dipropoxy-calix[4]arene) in 100 μM concentration inhibited Na^+, K^+ -ATPase activity in human spermatozoa almost completely (more effectively than ouabain: $I_{0.5}$ for calix[4]arene C-107 – 32.6 ± 2.9 nM), without affecting the activity of basal Mg^{2+} -ATPase [15]. The aim of present work was to evaluate the calix[4]arene C-107 effect on the kinetic properties of Na^+, K^+ -ATPase activity in spermatozoa of fertile (normozoospermia) and infertility men (oligozoospermia, and asthenozoospermia).

Materials and Methods

Structure and synthesis of calix[4]arene C-107. Calix[4]arene C-107 ((5,17-diamino(2-pyridyl)methylphosphono-11,23-di-tert-butyl-26,28-dihydroxy-25,27-dipropoxy-calix[4]arene) (Fig. 1) was syn-

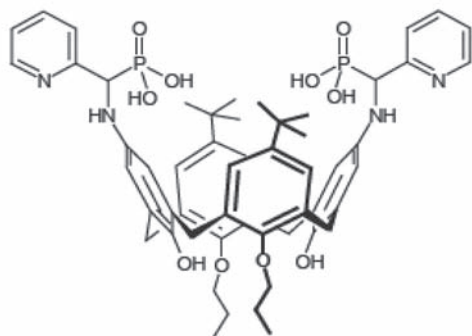


Fig. 1. Structural formulas of calix[4]arene C-107

thesized and characterized using nuclear magnetic resonance and infrared spectroscopy in the Phosphoranes Chemistry Department of the Institute of Organic Chemistry, NAS of Ukraine.

Donors and semen sample preparation. Human semen was obtained from 10 healthy volunteers and 14 pathozoospermic men undergoing routine semen analysis for couple infertility at Lviv Regional Clinical Hospital (Ukraine). Approval for the study was taken from the ethics committee of Danylo Halytsky Lviv National Medical University (Ethical Committee Approval, protocol No 6 from March 29, 2017). Terms of sample selection meet the requirements of the principles of Convention of Europe Council on human rights, Helsinki Declaration on protection of human rights and biomedicine and the laws of Ukraine. All patients and healthy donors were matched by age and gave written informed consent to participate in research. Exclusion criteria: subjects currently on any medication or antioxidant supplementation were not included. In addition, subjects with infertility over 10 years, azoospermia, testicular varicocele, genital infection, chronic illness and serious systemic diseases, smokers and alcoholic men were excluded from the study because of their well-known high seminal reactive oxygen species levels and decreased antioxidant activity which may affect calcium level. Samples were obtained by masturbation after 3–4 days sexual abstinence and processed immediately upon liquefaction. The classical semen parameters of spermatozoa concentration, motility, and morphology were examined according to World Health Organization criteria (2010).

Biochemical study. Biochemical studies were carried out in the Department of Medical Biology of Danylo Halytsky Lviv National Medical University. Sperm cells were washed from semen plasma by 3 times centrifugation at 3000 $\times g$ for 10 min in media which contained (mM): 120 NaCl, 30 KCl, 30 HEPES (pH 7.4). The protein concentration in the samples was determined by Lowry method using a kit to determine its concentration (Simko Ltd). Determination of ATPases activities was carried out in permeabilized spermatozoa. The detergent saponin in a final concentration of 0.5% was added to sperm suspension for permeabilization of sperm membranes.

Enzymatic studies. The total Na^+, K^+ -ATPase activity was assayed with the following incubation medium (mM): 120 NaCl, 30 KCl, 5 MgCl_2 , 3 ATP, 1 EGTA, 0.01 thapsigargin (specific inhibitor of SERCA), 1 NaN_3 (specific inhibitor of mitochondrial

ATPase), 20 Hepes-Tris (pH 7.4; at 37 °C) [16]. The protein concentration did not exceed 50 mg/ml. The reaction was started by addition of an aliquot of permeabilized sperm cells. After a 5 min incubation, 1 ml of a stop solution containing (mM) 1.5 M sodium acetate, 3.7% formaldehyde, 14% ethanol, 5% trichloroacetic acid (pH 4.3) acid was added. P_i was determined by the Fiske-Subbarow method using the assay kit Simko Ltd (Ukraine). Ouabain-sensitive Na^+, K^+ -ATPase activity was determined as the difference of ATP hydrolysis in the absence and presence of 1 mM ouabain, which is known to completely block the Na^+, K^+ -ATPase. Activity was expressed as nmoles of P_i released /mg of protein per min. The effect of calix[4]arene C-107 at different concentrations (10-100 nM) on Na^+, K^+ -ATPase was studied using the standard incubation medium (as described above) to which the solution of calix[4]arene was added.

Kinetic calculations. For studying of the calix[4]arene effect on enzyme activity, the kinetic parameters were calculated using linearized Hill plots of the equation $\lg[(A_{\max} - A)/A] = n_H \lg K - n_H \lg[S]$, where A is specific enzyme activity, A_{\max} is maximum specific enzymatic activity, K is coefficient of (half-)activation by ATP (K_{ATP}) or $MgCl_2$ ($K_{Mg^{2+}}$), S is the concentration of the substrate or ion activator in the incubation medium, n_H is Hill coefficient. The maximum reaction rate of ATP hydrolysis (V_{\max}) reaction was calculated in the Hanes-Woolf plot $\{[S]/V$

as a function of $[S]\}$. The typical value of the mean-square deviation of the approximation coefficient was 0.85-0.99.

Statistical analysis. Data are expressed as means \pm standard error of the numbers of determinations. Differences between paired sets of fluorimetric experiments were analysed using paired Student's t -tests in Microsoft Excel. Differences were considered significant at $P < 0.05$ as the minimum significance level.

Reagents. The following reagents were used in the present study: ATP, ouabain, thapsigargin, EGTA (Sigma, USA), saponin (from Quillaja Saponaria Molina pract.; Acros organics, Belgium). Other reagents of domestic production were of reagent grade or laboratory grade.

Results and Discussion

In our previous study [17], control samples (fertile men with normozoospermia) showed activity of Na^+, K^+ -ATPase about 46.3 ± 4.2 nmol P_i /min per mg protein. In patients with oligo-, astheno- and oligoastheno-azoospermia the Na^+, K^+ -ATPase activity was 22.4 ± 3.5 , 23.6 ± 2.6 and 21.8 ± 3.8 nmol P_i /min per mg protein correspondingly, that was two fold lower vs. control group. In previous experiments with calix[4]arenes, it was shown that C-107 calixarene-phosphonic acid in 100 μ M concentration inhibited sperm Na^+, K^+ -ATPase more effectively than ouabain [15]. Therefore, for further kinetic interpretation of

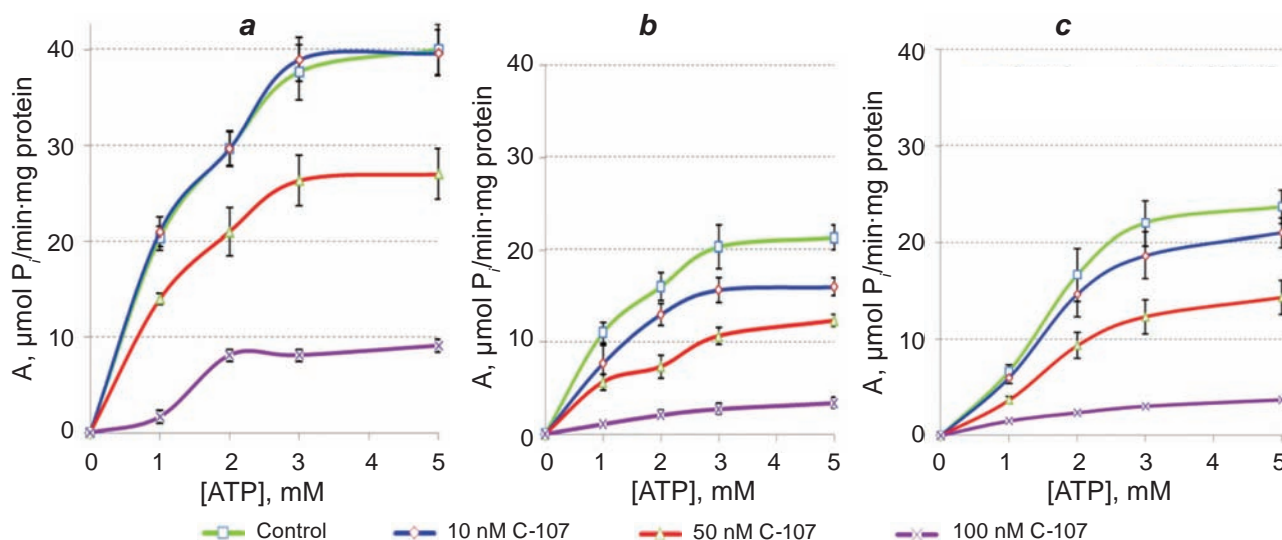


Fig. 2. Calix[4]arene C-107 effect on the dependence of Na^+, K^+ -ATPase activity of spermatozoa of normozoospermic (a), oligozoospermic (b) and asthenozoospermic (c) men on ATP concentration ($M \pm m$; $n = 4-5$; $[MgCl_2] = 5$ mM)

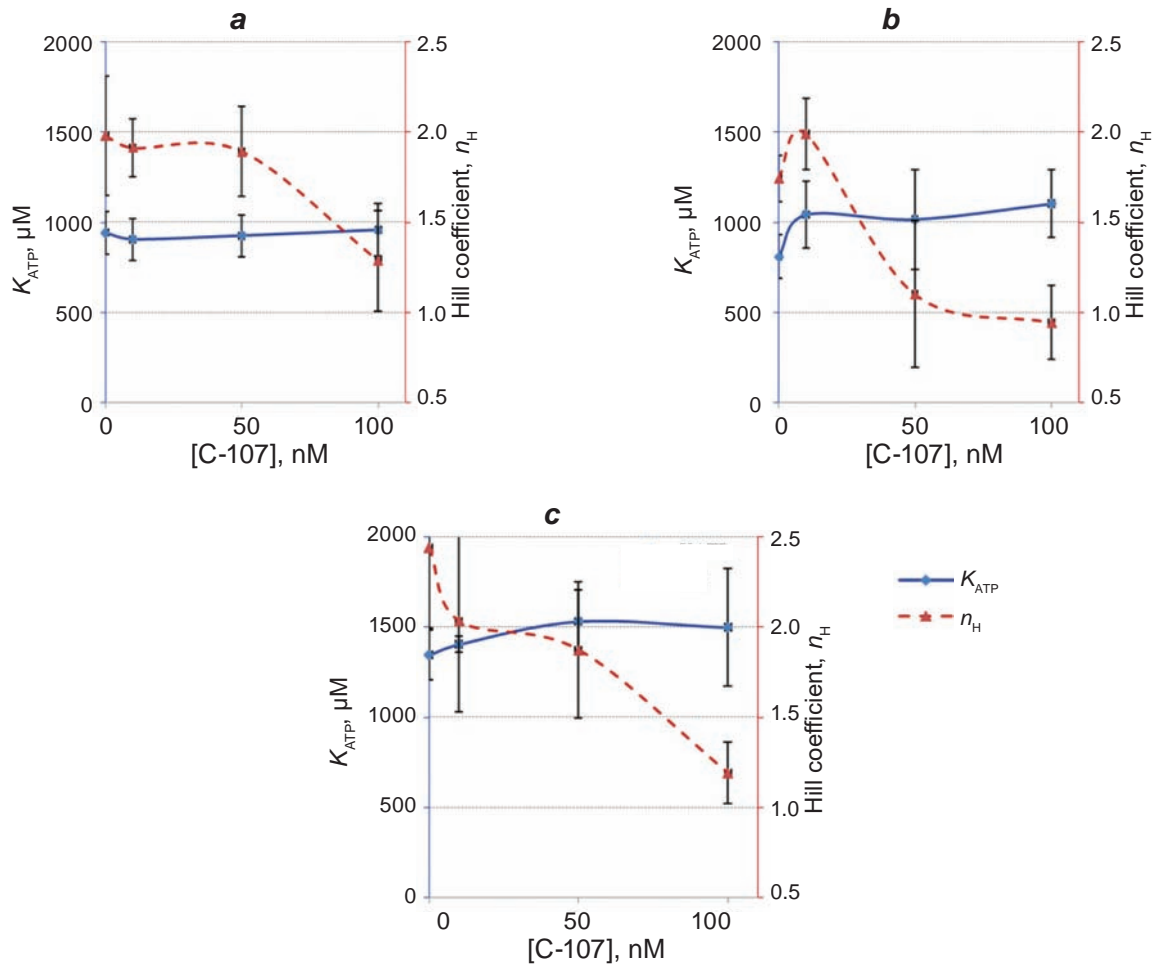


Fig. 3. Calix[4]arene C-107 effect on kinetic parameters (coefficient of (half-)activation by ATP K_{ATP} and Hill coefficient n_H) of Na^+, K^+ -ATPase in spermatozoa of normozoospermic (a), oligozoospermic (b) and asthenozoospermic (c) men ($M \pm m$; $n = 4-5$; $[\text{MgCl}_2] = 5 \text{ mM}$; control – $[\text{C-107}] = 0 \text{ nM}$)

calix[4]arene C-107 effect on enzymatic activity of Na^+, K^+ -ATPase of spermatozoa we studied its effects on the concentration dependence of ATPase activity on ATP and Mg^{2+} concentration.

As substrate concentration in incubation medium increases (under constant level of cofactor) the Na^+, K^+ -ATPase activity of sperm cells from normo- (Fig. 2, a) and pathozoospermic men increases (Fig. 2, b, c). Similar concentration dependences were also obtained for oligoasthenozoospermic samples (results are not presented in the paper). The highest enzyme activity was observed under mM ATP in the incubation medium. Presence of calix[4]arene C-107 in a concentration range from 10 to 100 nM in the incubation medium leads to inhibition of sperm Na^+, K^+ -ATPase activity in both normo- and pathozoospermic men. The maximum Na^+, K^+ -ATPase activity of sperm cells decreases as the calixaren concentration increases.

The coefficient of (half-)activation by ATP (K_{ATP}) and Hill coefficient (n_H) were calculated using Hill's plot for all studied groups (Fig. 3).

As can be seen from Fig. 3, a the calix[4]arene C-107 did not affect coefficient of (half-)activation by ATP K_{ATP} and Hill coefficient n_H for Na^+, K^+ -ATPase in spermatozoa of normozoospermic men. The values of Hill coefficient n_H were higher than 1 with and without calix[4]arene C-107 in the incubation medium which indicates positive cooperation between enzyme and ATP. Since calix[4]arene C-107 inhibited sperm Na^+, K^+ -ATPase activity with respect to control [15] and did not change coefficient of (half-)activation by ATP and enzyme co-operation to ATP (values of K_{ATP} and n_H were unchanged) we assume that enzyme activity can be decreased due to the decrease in the maximum reaction rate (V_{\max}). Therefore, next, we calculated V_{\max} catalyzed by Na^+, K^+ -ATPase Hanes-Woolf plot. As can be

seen from Fig. 4 calix[4]arene C-107 effect on maximum reaction rate V_{\max} in a dose-dependent manner. Similar dependences of V_{\max} of Na^+, K^+ -ATPase were obtained for sperm cells from oligozoospermic and asthenozoospermic men.

Our results are in perfect agreement with studies carried out on uterus myocyte plasma membrane Na^+, K^+ -ATPase [18]. It was shown that calix[4]arene C-107 inhibited enzyme without affection of kinetic parameters (K_{ATP} , n_{H}) of reaction velocity dependence on substrate concentration.

For oligo- and asthenozoospermic samples the Hill coefficient n_{H} was almost twice lower in the presence of 100 nM calix[4]arene C-107 in the incubation medium compared to control. These values of Hill coefficient ($n_{\text{H}} > 1$) indicate the positive cooperation of Na^+, K^+ -ATPase to ATP in pathospermic samples obtained from infertile men. Changes in values of n_{H} might indicate subunit changes of enzyme structure under increasing concentration of calix[4]arene C-107 in the incubation medium. The maximum reaction rate (V_{\max}) of Na^+, K^+ -ATPase significantly decreases as calix[4]arene C-107 increases.

It is known that besides ATP, magnesium is required for the functioning of Na^+, K^+ -ATPase. The Mg^{2+} ions, which act as a cofactor, form a chelating complex $\text{Mg}\cdot\text{ATP}$, which is a substrate of enzymatic reaction. Also, Mg^{2+} ions bind to the regulatory center of Na^+, K^+ -ATPase [19]. The Na^+, K^+ -ATPase activity of sperm cells increases as MgCl_2 concentration increases in the range from 0.1 to 10 mM under constant concentrations of ATP (3 mM) in the incubation medium (Fig. 5, a, control). The dependences of enzyme activity on MgCl_2 concentration were similar to dependence without calix[4]arene C-107, however, the plateau level of Na^+, K^+ -ATPase activity decreases as the calix[4]arene concentration increases. Similar concentration dependences were obtained for oligozoospermic (Fig. 5, b), asthenozoospermic (Fig. 5, c) and oligoasthenozoospermic samples (results are not presented in the paper). Presented concentration dependences correspond not to Mg^{2+} , but to MgCl_2 , since we used in experiments MgCl_2 . It should be noted that $[\text{Mg}^{2+}]$ the incubation medium nonlinearly depends on $[\text{MgCl}_2]$.

Calix[4]arene C-107 effect on the coefficient of (half-)activation by MgCl_2 of Na^+, K^+ -ATPase in spermatozoa of normo- and pathozoospermic men was different. Specifically, in normozoospermic samples of spermatozoa, the coefficient of (half-)activation by MgCl_2 K_{MgCl_2} decreases at high concentrations

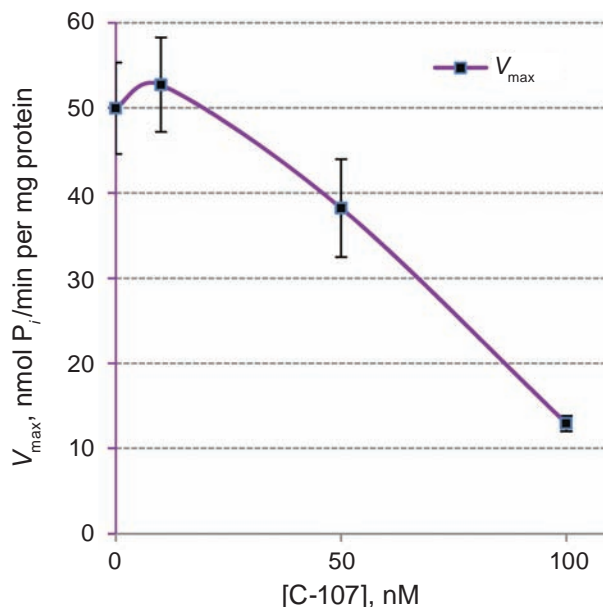


Fig. 4. Calix[4]arene C-107 effect on maximum reaction rate V_{\max} of Na^+, K^+ -ATPase by ATP in spermatozoa of normozoospermic men ($M \pm m$; $n = 5$; $[\text{MgCl}_2] = 5 \text{ mM}$; control – $[\text{C-107}] = 0 \text{ nM}$)

of calix[4]arene C-107 ($\geq 50 \text{ nM}$) in the incubation medium (Fig. 6). Contrary to this in oligozoospermic samples of spermatozoa, K_{MgCl_2} increases only at high concentration (100 nM) of calix[4]arene C-107. For sperm Na^+, K^+ -ATPase from asthenozoospermic men, an increase in calix[4]arene C-107 concentration leads to an increase in K_{MgCl_2} in the entire range of investigated concentrations of calix[4]arene. Previously it was shown complex two-phase nature of K_{MgCl_2} dependence on the concentration on calix[4]arene C-107 for uterus myocyte plasma membrane Na^+, K^+ -ATPase [18]. The Hill cooperativity coefficient n_{H} of activation by MgCl_2 decreases in the presence of calix[4]arene C-107. This may indicate changes in the subunit composition of the enzyme as a result of calix[4]arene C-107 effect on it.

The obtained results indicate that highly effective inhibitory effect of calix[4]arene C-107 on Na^+, K^+ -ATPase in normozoospermic samples has non-competitive character and is associated with a decrease in the maximum reaction rate (Fig. 7). In oligo- and asthenozoospermic men inhibitory effect of calix[4]arene C-107 causes an increase in K_{MgCl_2} for MgCl_2 .

Due to the amphiphilicity of calix[4]arenes molecule (hydrophilic acid groups and lipophilic macrocycle skeleton), calix[4]arenes might be embedded in lipid biomembranes, forming channels for

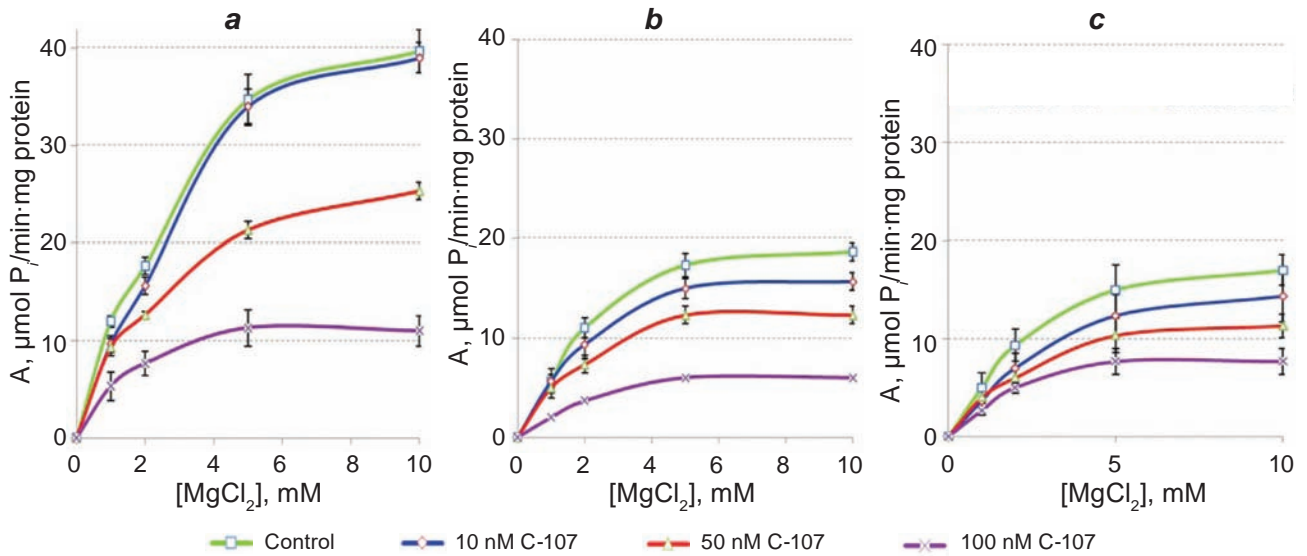


Fig. 5. Calix[4]arene C-107 effect on the dependence of Na^+, K^+ -ATPase activity of spermatozoa of normozoospermic (a), oligozoospermic (b) and asthenozoospermic (c) men on MgCl_2 concentration ($M \pm m$; $n = 4-5$; $[\text{ATP}] = 3 \text{ mM}$)

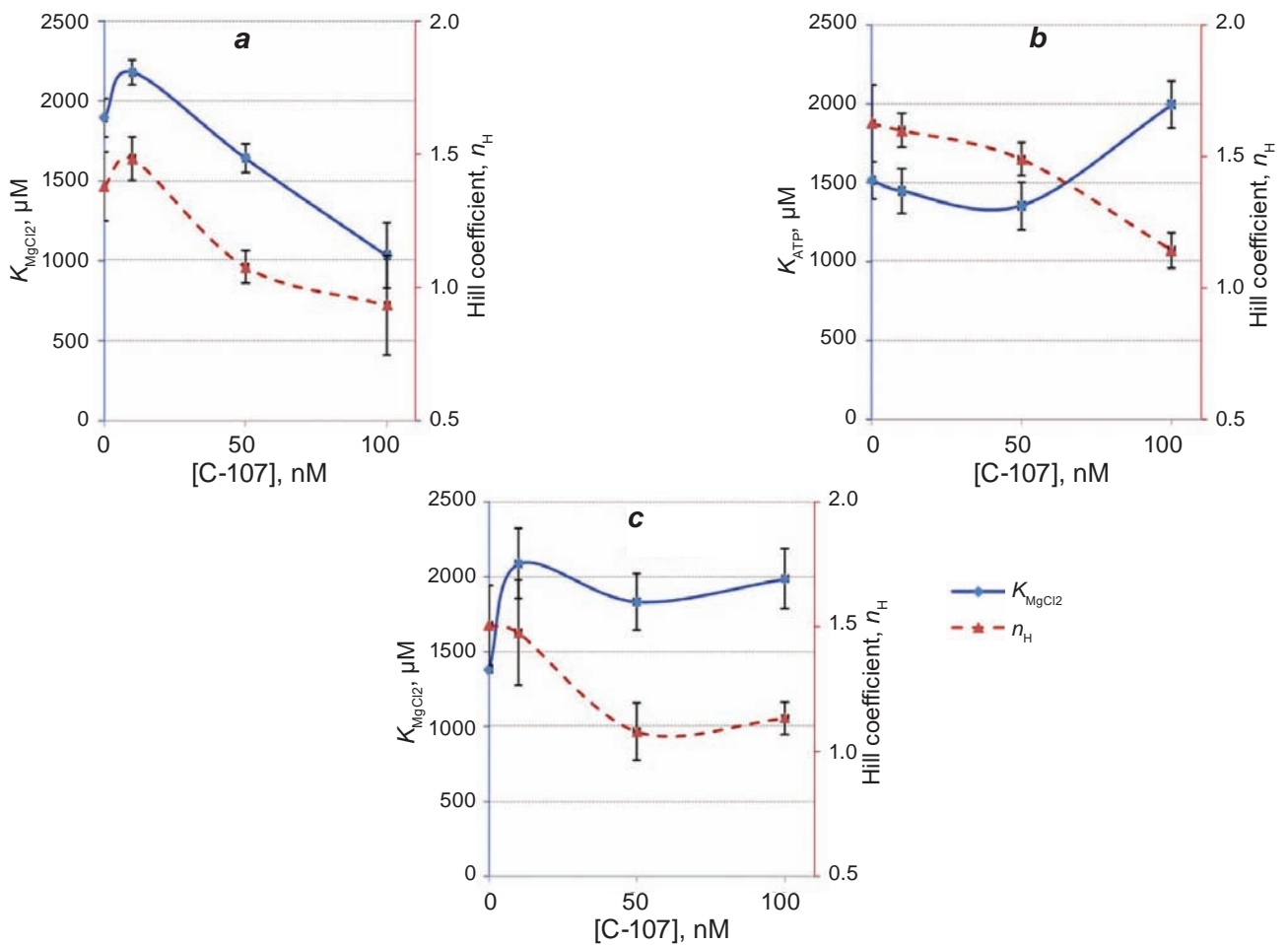


Fig. 6. Calix[4]arene C-107 effect on kinetic parameters (coefficient of (half-)activation by MgCl_2 and Hill coefficient n_H) of Na^+, K^+ -ATPase in spermatozoa of normozoospermic (a), oligozoospermic (b) and asthenozoospermic (c) men ($M \pm m$; $n = 4-5$; $[\text{ATP}] = 3 \text{ mM}$; control – $[\text{C-107}] = 0 \text{ nM}$)

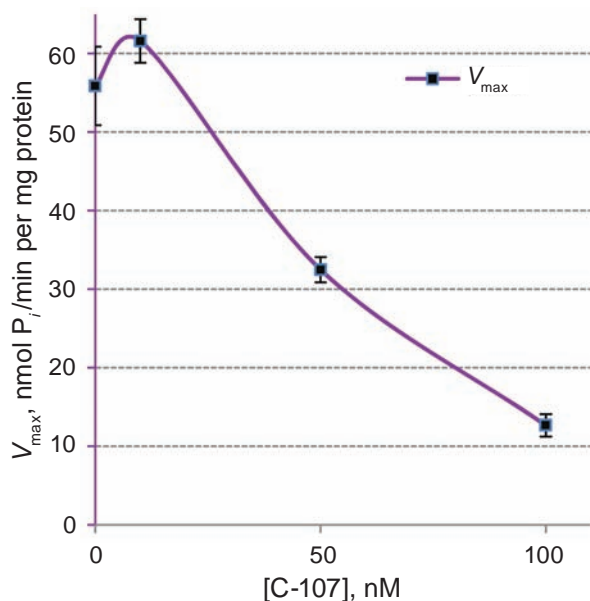


Fig. 7. Calix[4]arene C-107 effect on maximum reaction rate V_{max} of Na^+,K^+ -ATPase by $MgCl_2$ in spermatozoa of normozoospermic men ($M \pm m$; $n = 5$; $[ATP] = 3 \text{ mM}$; control – $[C-107] = 0 \text{ nM}$)

the transport of cations [20, 21]. Therefore calix[4]arenes can potentially affect enzymatic or transport activity of membrane-bound proteins. It is assumed that obtained experimental data may be important to clarify membrane mechanisms of ions exchange and homeostasis in spermatozoa in normal and pathological states since they explain the inhibitory effect of calix[4]arene C-107. In addition, our data can be useful for the development of potential pharmacological agents for modulating sperm motility, specifically to improve the sperm motility of asthenospermia patients or to create new spermicidal compounds. In particular, the effect of a number of calix[4]arenes is currently being studied not only on the ATPase system, but also on the sperm motility. We assume that calix[4]arene acting on membrane-bound proteins can lead to a change in sperm motility, which is extremely relevant in particular for urology and andrology.

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Conflict of interest. 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.

КІНЕТИЧНІ ВЛАСТИВОСТІ Na^+,K^+ -АТРАЗИ СПЕРМАТОЗОЇДІВ ПЛІДНИХ І НЕПЛІДНИХ ЧОЛОВІКІВ ЗА ДІЇ КАЛІКС[4]АРЕНУ C-107

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Досліджували ефекти калікс[4]арену C-107 (5,17-ди(фосфоно-2-піридилметил)аміно-11,23-ди-трет-бутил-26,28-дигідрокси-25,27-дипропоксикалікс[4]арен) на кінетичні характеристики Na^+,K^+ -АТРАЗИ сперматозоїдів фертильних (нормозооспермія) та інфертильних (оліго- та астенозооспермія) чоловіків. Показано, що інгібування Na^+,K^+ -АТРАЗНОЇ активності калікс[4]ареном C-107 у сперматозоїдах нормозооспермічних чоловіків відбувається внаслідок зниження максимальної швидкості АТР-гідролазної реакції і не пов'язане зі зміною коефіцієнта (напів)активації АТР та коефіцієнта Хілла n_H . Проте в оліго- та астенозооспермічних зразках сперматозоїдів спостерігається зниження коефіцієнта кооперативності n_H інгібування АТР-азної реакції калікс[4]ареном C-107 майже в 2 рази. Встановлено, що калікс[4]арен C-107 впливає на коефіцієнт (напів)активації $MgCl_2$ Na^+,K^+ -АТРАЗИ в сперматозоїдах нормо- та патозооспермічних чоловіків по-різному. Зокре-

ма, у нормозооспермічних зразках сперматозоїдів K_{MgCl_2} знижується при високих концентраціях калікс[4]арену C-107 (≥ 50 нМ) в інкубаційному середовищі. Натомість, в олігозооспермічних чоловіків K_{MgCl_2} зростає лише при високих концентраціях калікс[4]арену C-107 (100 нМ). Для Na^+, K^+ -АТРази сперматозоїдів астенозооспермічних чоловіків збільшення концентрації калікс[4]арену C-107 приводить до зростання K_{MgCl_2} в усьому діапазоні досліджуваних концентрацій калікс[4]арену. При цьому величина коефіцієнта кооперативності Хілла n_H активуючої дії $MgCl_2$ знижується у присутності калікс[4]арену C-107.

Ключові слова: калікс[4]арен C-107, Na^+, K^+ -АТРаза, чоловіча безплідність, патоспермія.

References

- Atamas LI, Boyko VI, Drapaylo AB, Yesypenko AA, Kalchenko OI, Klyachina MA, Matveev YuI, Miroschnichenko SI, Rodik RV, Chrenok SA, Kalchenko VI. Supramolecular chemistry of calixarenes. *J Org Pharm Chem*. 2009; 7(2(26)): 28-36. (In Ukrainian).
- Tsybalyuk OV, Kosterin SO, Rodik RV, Kalchenko VI. Comparative study in the *in vitro* and *in vivo* experiments of influence of ouabain and calixarene C107 on Na^+, K^+ -ATPase activity of rat hepatocyte plasmatic membranes. *Ukr Biokhim Zhurn*. 2010; 82(4): 78-85. (In Ukrainian).
- Da Silva E, Lazar AN, Coleman AW. Biopharmaceutical applications of calixarenes. *J Drug Sci Tech*. 2004; 14(1): 3-20.
- Rodik RV, Boyko VI, Kalchenko VI. Calixarenes in Biotechnology and Bio-Medical Researches. *Front Med Chem*. 2016; 8: 206-301.
- Nimse SB, Kim T. Biological applications of functionalized calixarenes. *Chem Soc Rev*. 2013; 42(1): 366-386.
- Darbost U, Rager MN, Petit S, Jabin I, Reinaud O. Polarizing a hydrophobic cavity for the efficient binding of organic guests: the case of calix[6]tren, a highly efficient and versatile receptor for neutral or cationic species. *J Am Chem Soc*. 2005; 127(23): 8517-8525.
- Lamb DJ. Semen analysis in 21st century medicine: the need for sperm function testing. *Asian J Androl*. 2010; 12(1): 64-70.
- Omu AE. Sperm parameters: paradigmatic index of good health and longevity. *Med Princ Pract*. 2013; 22(Suppl 1): 30-42.
- Sanchez G, Nguyen AN, Timmerberg B, Tash JS, Blanco G. The Na, K -ATPase $\alpha 4$ isoform from humans has distinct enzymatic properties and is important for sperm motility. *Mol Hum Reprod*. 2006; 12(9): 565-576.
- Xie Z, Askari A. $Na(+)/K(+)$ -ATPase as a signal transducer. *Eur J Biochem*. 2002; 269(10): 2434-2439.
- Wagoner K, Sanchez G, Nguyen AN, Enders GC, Blanco G. Different expression and activity of the $\alpha 1$ and $\alpha 4$ isoforms of the Na, K -ATPase during rat male germ cell ontogeny. *Reproduction*. 2005; 130(5): 627-641.
- Woo AL, James PF, Lingrel JB. Sperm motility is dependent on a unique isoform of the Na, K -ATPase. *J Biol Chem*. 2000; 275(27): 20693-20699.
- Jimenez T, Sánchez G, Blanco G. Activity of the Na, K -ATPase $\alpha 4$ isoform is regulated during sperm capacitation to support sperm motility. *J Androl*. 2012; 33(5): 1047-1057.
- Koçak-toker N, Aktan G, Aykaç-toker G. The role of Na, K -ATPase in human sperm motility. *Int J Androl*. 2002; 25(3): 180-185.
- Veklich TO, Kocheshkova NS, Rodik RV, Boyko VI, Vorobets ZD, Kosterin SO. Comparative research of calixarens influence on Na^+, K^+ -ATPase activity in plasma membrane of contractile and mobile cells. *Ukr Biokhim Zhurn*. 2007; 79(3): 19-28. (In Ukrainian).
- Lychkovska N, Fafula R, Efremova U, Vorobets Z. A study of Na^+, K^+ -ATPase and arginase activity in peripheral blood lymphocytes in patients with rheumatic diseases. *Annales Universitatis Mariae Curie-Sklodowska, Sectio DDD: Pharmacia*. 2011; 1(20): 171-177.
- Meskalo OI, Fafula RV, Lychkovskyj EI, Vorobets ZD. Na^+, K^+ -ATPase and Ca^{2+} , Mg^{2+} -ATPase activity in spermatozoa of infertile men with different forms of pathospermia. *Studia Biologica*. 2017; 11(2): 5-12.
- Veklich TO, Shkrabak OA, Rodik RV, Kalchenko VI, Kosterin SO. The effect of calixarene C-107 on kinetic parameters of Na^+, K^+ -ATPase of uterus myocyte plasma membrane. *Ukr Biokhim Zhurn*. 2011; 83(2): 36-44. (In Ukrainian).

19. Boldyrev AA. Na/K-ATPase as an oligomeric ensemble. *Biochemistry (Mosc)*. 2001; 66(8): 821-831.
20. Houel E, Lazar A, Da Silva E, Coleman AW, Solovyov A, Cherenok S, Kalchenko VI. Interfacial interactions of cations with amphiphilic dihydroxyphosphonyl-calix-[4]-arene mesosystems. *Langmuir*. 2002; 18(4): 1374-1379.
21. Yoshino N, Satake A, Kobuke Y. An artificial ion channel formed by a macrocyclic resorcin. *Angew Chem Int Ed*. 2001; 40(2): 457-459.