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SELECTIVE SODIUM PUMP INHIBITOR CALIX[4]ARENE C-1130 INCREASES CONCENTRATION OF Ca IONS I N SMOOTH MUSCLE CELLS

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Na^+,K^+-ATPase is an electrogenic Mg^{2+}, Na^+, K^+-ATP-dependent transport system of the plasma membrane (PM) that actively transports monovalent Na and K ions and thereby maintains their electrochemical gradients, which are essential for normal cell functioning [1]. Moreover, Na^+, K^+-ATPase is important for the regulation of intracellular Ca concentration due to the physical connection between Na^+,K^+-ATPase, Na^+/Ca^{2+}-exchanger and Ca stores in the sarcoplasmic reticulum [2]. The function of the sodium pump is susceptible to alterations in various pathological conditions, including diabetes and ischemia. Therefore, it is promising to search for a compound that would allow changing the activity of the PM sodium pump. In our previous experiments, calix[4]arene C-1130 showed the selective inhibitory effect on the activity of Na^+,K^+-ATPase ($I_{0.5}$ = 38 ± 6 nM) relative to other ATP hydrolases of the PM, so calixarenes are promising agents to alter the activity of sodium pump. However, the properties and mechanism of the inhibitory effect of this calix[4]arene on Na^+,K^+-ATPase activity have not been determined.

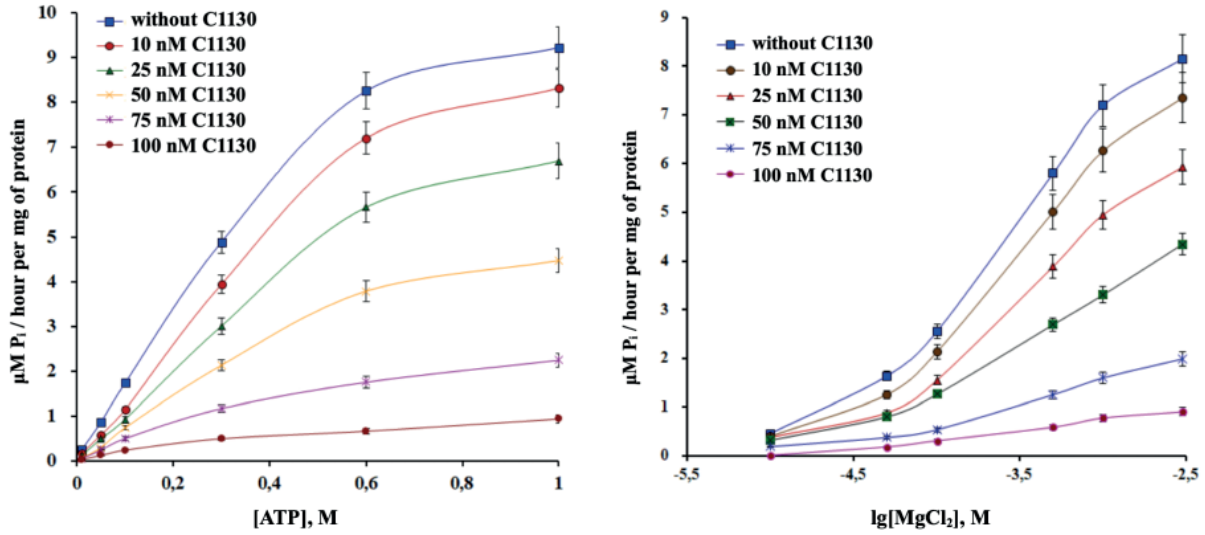
Aim. This work aimed to determine the biochemical regularities of calix[4]arene C-1130 effect on the activity of Na^+, K^+-ATPase and Ca^{2+} level in PM of smooth muscle cells (SMCs).

Methods. Calix[4]arene C-1130 (5,17-di(phosphono-2-pyridylmethyl)amino-11,23-di-tert-butyl-26,28-dihydroxy-25,27-dioctyloxycalix[4]arene) was synthesized and characterized by NMR and infrared spectroscopy by V.I. Kalchenko and his colleagues (Institute of Organic Chemistry of the National Academy of Sciences of Ukraine). Biochemical studies were carried out in the Department of Muscle Biochemistry of Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine. The experiments were performed on a suspension of myometrial cells and the PM fraction treated with a 0.1% digitonin solution. The protein concentration in the membrane fraction was determined by Bradford method using Kumasi-G250 reagent. Determination of the intracellular Ca^{2+} concentration was done by confocal microscopy. The hydrodynamic diameter of myocytes was determined by dynamic light scattering.

Results and Discussion. We have studied the dependence of the specific activity of Na^+,K^+-ATPase of PM on the concentration of Mg ions and ATP in the incubation medium at different concentrations of calix[4]arene C-1130 (from 10 to 100 nM).

The study evaluated the impact of calix[4]arene C-1130 concentration on the Na^+,K^+-ATPase affinity for ATP and Mg ions and its influence on the cooperative effect and maximum velocity of ATP hydrolysis (Figure). Surprisingly, the affinity of Na^+, K^+-ATPase for ATP remained largely unaffected by the presence of calix[4]arene C-1130, suggesting no competition between the binding centers of ATP and C-1130. Likewise, there was no discernible effect on the affinity constant or cooperative effect of Mg ions. However, a notable reduction in the initial maximum velocity of enzymatic ATP hydrolysis was observed. In combination with unchanged affinity constants, it indicates a non-competitive mechanism of inhibition of Na^+, K^+-ATPase by C-1130.

Through confocal microscopy analysis, we have demonstrated that calix[4]arene C-1130 elevated Ca^{2+} concentration within myometrial myocytes. According to the change in fluorescence of the Ca^{2+}-sensitive fluo-4 probe, a temporary increase in the Ca^{2+} concentration in SMCs under the influence



Effect of calix[4]arene C-1130 on the dependence of Na^+, K^+-ATPase activity in PM of myometrium cells on the ATP concentration and on the Mg ion concentration ($n = 5$)

of calix[4]arene C-1130 occured. Within 2–3 min, the Ca^{2+} concentration gradually decreased, indicating the involvement of compensatory mechanisms.

Additionally, photon-correlation spectroscopy revealed that C-1130 reduced the effective hydrodynamic diameter of smooth muscle cells by 55% compared to the control, indicative of myocyte contraction.

Conclusions. Thus, we have shown that calix[4]arene C-1130 did not affect the activation coefficient for Mg ions, the apparent Michaelis constant K_m for ATP. However, in both these cases, C-1130 reduced the maximum initial velocity V_{max} of the ATP hydrolysis reaction. Thus, calix[4] arene C-1130 acted as a complete non-competitive inhibitor of Na^+,K^+-ATPase of PM.

The experimental data obtained using calix[4]arene C-1130 might be significant for elucidating the role of the PM sodium pump in controlling the intracellular concentration of Ca ions in uterine myocytes. It may serve as a basis for the effective inhibitors of Na^+,K^+-ATPase development based on calix[4]arene C-1130 as well.

Key words: calix[4]arenes, Na^+,K^+-ATPase, plasma membrane, smooth muscle cells, myometrium, enzymatic hydrolysis of ATP.

Authors' contribution. VVM (conducted a study of the dependence of the specific activity of Na^+,K^+-ATPase of PM on the concentration of Mg ions and ATP in the incubation medium at different concentrations of calix[4]arene C-1130, wrote and designed the publication), OVM (conducted a study of changes in the effective hydrodynamic diameter of SMCs under the influence of calix[4] arene C-1130), TOV (conducted a study of changes in intracellular Ca^{2+} concentration in myometrial myocytes, summarized and analyzed the results).

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