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DIFFERENT SENSITIVITY OF Na^+,K^+-ATPase AND Mg^{2+}-ATPase TO ETHANOL AND ARACHIDONIC ACID IN RAT COLON SMOOTH MUSCLE UNDER PRETREATMENT OF CELLULAR MEMBRANES WITH Ds-Na

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The methodological procedure provides the detection of the relatively high Na^+,K^+-ATPase functional activity in the crude cellular membranes of rat colon smooth muscle (CSM) following standard detergent pretreatment (with Ds-Na vs digitonin). It includes the essential discrete steps: detergent membrane permeabilization under optimal detergent/protein ratio and active site protection by ATP (for Ds-Na) prior enzymatic reaction with substantial detergent dilution far below critical micelle concentration in the ATPase medium. The high level of the Na^+,K^+-ATPase activity, originally detected in CSM, did not differ for two detergents and was comparable with ouabain-resistant Mg^{2+},ATP-hydrolase activity. The features of ATPase protein-lipid complexes were evaluated by the enzyme sensitivity to the effect of ethanol and arachidonic acid with different membrane disordering effectiveness. The long-chain fatty acid is a more effective inhibitor as compared with aliphatic alcohol for both ATPases. Mg^{2+},ATP-hydrolase appeared to be much more resistant to inactivation than Na^+,K^+-ATPase. The data reflect the possible differences in lipid dependence of two enzymatic systems due to the peculiarities of the structural arrangement in membrane and importance of the hydrophobic microenvironment for mechanism of catalysis. Thus, the data represent the approach to the simple and reliable Na^+,K^+-ATPase activity determination in nonpurified CSM membranes, acceptable for different tissues and appropriate for quantitative comparison in pathophysiological studies and for testing the impact of diverse effectors on Na^+,K^+-ATPase.

K e y w o r ds: ATP-hydrolases, Na^+,K^+-ATPase, colonic smooth muscle, digitonin, Ds-Na, ethanol, arachidonic acid.

Na^+,K^+-ATPase is a key enzyme in the tissue-specific regulation of the active transport of sodium and potassium ions across plasma membrane and related electrochemical gradient-dependent processes in animal cells, including an indirect control of calcium homeostasis and electromechanical coupling at least in skeletal, cardiac and vascular smooth muscles [1, 2].

Being a crucial enzyme of the ion homeostasis readjustment, Na^+,K^+-ATPase is involved into mechanisms of miscellaneous diseases development accompanied by the changes of the enzymatic activity due to the direct defect or isozyme expression pattern remodeling [3, 4]. Na^+,K^+-ATPase participation in the cellular response as the plasma membrane target of cytotoxic impact is considered in oxidative stress and redox pathologies, ischemia-hypoxia, reprogramming of oxidative metabolism, impairment of transition metals detoxication mechanisms and appearance of a free iron or copper pool, etc [5-7]. The relationship exists between the decrease of the Na^+,K^+-ATPase activity and the severity of mucosal damage and the degree of inflammation in inflammatory large bowel diseases accompanied by impairment of the colonic smooth muscle (CSM) contractility [8]. However, the pathophysiological behavior of CSM Na^+,K^+-ATPase and its involvement in sarcolemmal disorders remain obscure.

Na^+,K^+-ATPase from different sources is used as an appropriate in vitro model for evaluation of the inhibitory potential and specificity of the membrane-acting biologically active compounds, applied

in medical, pharmacological and environmental researches [9,10]. To elucidate the functional involvement into the pathophysiological mechanisms the Na^+,K^+-ATPase expression is studied along with enzymatic detection in crude membrane preparation [4, 11].

Unfortunately, the reliable assay for Na^+,K^+-ATPase determination in crude CSM membranes has not been ever tested. In own researches the biochemical features of the rat CSM Na^+,K^+-ATPase were characterized for the first time under digitonin pretreatment in accordance with criteria of functionally adequate enzyme [12]. Mg^{2+}-ATPase belongs to the family of the non-P-type ATP-hydrolases with different way of ATP-hydrolyse in comparison with ion-transporting ATPases [12-14]. It is essential to evaluate the relative conformational resistance due to different structure-functional relations of these ATP-hydrolasing enzymes in membrane on the basis of different sensitivity to membranotropic agents.

Further research requires elucidation of the enzymatic dependence on membrane surrounding and its importance for maintenance of the functional conformation. In this regard the widely used procedure with mild Ds-Na pretreatment is applied for CSM membranes, which, as is known, unmasks latent Na^+,K^+-ATPase activity preserving the intrinsic membrane protein-lipid enzymatic complex (annular lipids) [15, 16]. It will allow standardizing enzymatic assay in smooth muscle membranes with commonly used approach for Na^+,K^+-ATPase purification in highly active membrane-bound form used for brain, kidney, salt glands, myocardium, etc. It is important to adopt the initial step of the procedure for determination of the functional Na^+,K^+-ATPase activity in crude membrane preparations from CSM in accordance with our previous researches [17]. This will enable to evaluate the dependence of the enzyme structural-functional complexes on intrinsic lipid integrity and impairment effect of the mebrane-acting agents on the membrane microenvironment of the ATP-hydrolases.

Thus, the aim of this study is to detect the functionally active membrane-bound Na^+,K^+-ATPase activity in the rat CSM crude membrane preparations by Ds-Na pretreatment and to determine the relative structure-function resistance in membrane of the ATPases (Na^+,K^+-ATPase vs Mg^{2+}-ATPase), affected by the lipophilic modifiers of the enzyme lipid microenvironment, such as aliphatic compounds with different hydrophobicity: ethanol and arachidonic acid (AA).

Materials and Methods

ATPase activity and protein determination were conducted in postmitochondrial membrane fraction isolated from rat CSM in accordance with the previously described methodical conditions [12]. ATPase activities (Na^+,K^+-ATPase and Mg^{2+}-ATPase) were detected after disrupture of the membrane vesicles by detergent digitonin or Ds-Na in accordance with 1 mM ouabain selectivity.

The pretreatment conditions were the following: 0.2% digitonin (detergent/protein ratio = 1/1 at 23 °C, 15 min) in the medium, contained: 30 mM tris-HCl buffer (pH 7.54), 0.16 M sucrose, 2 mg/ml protein, 2 mg/ml digitonin. Aliquots 5-10 µl immediately were added into ATPase reaction mixture (0.5 ml) at 37 °C with dilution 50-100 times [18, 19].

0.04% Ds-Na (detergent/protein ratio = 0.2 at 23 °C, 30 min) in the medium, contained: 30 mM tris-HCl buffer (pH 7.54), 0.16 M sucrose, additionally 1 mM EGTA and 3 mM ATP·Na_2 (for catalytic cite protection), 2 mg/ml protein, 0.4 mg/ml Ds-Na. The treatment was terminated by dilution 1/10 by chilled 30 mM tris-HCl buffer (pH 7.54) with 1 mM EGTA and 3 mM ATP. Aliquots 50-60 µl were added into ATPase reaction mixture (0.5 ml) at 37 °C. Final detergent dilution was 80-100 times. This procedure is more convenient and is based on the methodological principles previously applied [17]. The values of the enzymatic activities are given in Table 1.

Modifiers: 0.2-1.0 M ethanol or 1-330 µM AA (all-*cis*-5,8,11,14-eicosatetraenoic acid) were added into ATPase medium. AA stock solution was prepared on DMSO. The corresponding quantity of DMSO added into incubation mixture did nor alter the enzymatic activities.

Statistical analysis of the results was performed using Microsoft Office Excell 2007 and OriginPro 9.0. The data are given as means ± SEM. The significance of statistical differences between two groups was evaluated using Student's *t*-test ($P < 0.05$).

Results and Discussion

In the previous researches for the first time we estimated functionally adequate Na^+,K^+-ATPase activity in postmitochondrial cellular membrane fraction of the rat CSM by digitonin pretreatment [12, 18, 19]. This study is an application of the Ds-Na

pretreatment procedure used for the first time for the CSM membranes. Ultimately, rather high Na^+,K^+-ATPase activity was determined in crude smooth muscle membranes (Table 1) and further investigation of the membrane properties of the ATP-hydrolases using modifiers of the enzyme microenvironment in the membrane was conducted.

The data were obtained for principally different detergents with distinct specificity towards membrane components [16, 20, 21]. It is known that at concentrations above critical micelle concentration (CMC) detergents solubilize membrane including its protein and lipid components into micelles. The solubilizing effectiveness also depends on detergent nature and detergent/protein ratio. Detergent characteristics are given in Table 1. As seen, the pretreatment concentration for Ds-Na is much lower than CMC. In such conditions the detergent selectively extracts some peripheral proteins and bulk lipids preserving intrinsic lipid Na^+,K^+-ATPase microenvironment [15, 16]. The activity values of two ATPase enzymes do not differ for used detergents. Taking into account the characteristics of the used detergents and enzyme activity values, the data indicate a high functional stability of protein-lipid complexes of Na^+,K^+-ATPase with annular lipids, which sustain the folded protein conformation in membrane fragments necessary for manifestation of high enzymatic activity. The minimal required conditions are the keeping of the optimal detergent/protein ratio, protection of the enzyme active center by substrate ATP and chelator presence in the case of pretreatment with negatively charged Ds-Na. It should be emphasized that CSM Na^+,K^+-ATPase activity (up to 30 μmoles of P_i/hour per 1mg of protein) is appreciably higher that is known for other smooth muscles including myometrium [11, 22]. This indicates the necessity for compliance with mild pretreatment procedure at room temperature, proper detergent/protein ratio and considerable detergent dilution in ATPase medium far below CMC

Summarizing in general, it is important to emphasize that the basic principles of the methodological procedure are determined by standard characteristics and precaution of commonly used detergents in membranological studies [15, 16, 20, 21] requiring mild pretreatment procedure at room temperature before incubation of permeabilized membranes with effectors or in ATPase medium at 37 °C. Thus, the methodological importance of the separation of the procedures of the detergent action, modification and/or ATPase reaction to minimize the deleterious unfolding detergent effect in the course of Na^+,K^+-ATPase activity determination, including CSM preparations, is highlighted [12, 17-19]. It also diminishes the possible combined effect of detergents and membrane-active agents, enhancing enzyme unfolding and inactivation [17]. Such approach

Table 1. ATPase activities under different detergent pretreatment in rat CSM membranes (M ± m, n = 5-8)

Parameter	Digitonin	Ds-Na
Activities, μmoles of P_i/hour per 1 mg of protein: Na^+,K^+-ATPase	25.72 ± 2.99	27.67 ± 1.79
Mg^{2+},ATP-hydrolase	32.57 ± 2.48	31.34 ± 1.42
Detergent type	nonionic	anionic
Critical micelle concentration, mM[#]	< 0.5	8.2
Optimal pretreatment concentration (at 2 mg/ml protein), mM	1.63	1.39
Detergent/protein ratio, mg/mg	1	0.2
Detergent dilution in ATPase medium	1/50-1/100	1/80-1/100
Specificity[#]	preserves protein-protein interaction, impairs protein-lipid and lipid-lipid interactions, specifically binds to cholesterol	impairs protein-protein, protein-lipid and lipid-lipid interactions
Na^+,K^+-ATPase molecular form[#]	oligomer $(\alpha\beta)_2$	monomer αβ

Note: [#] according to [16, 20, 21].

provides a possibility to adjust moderate but effective detergent concentration relative to membrane protein content, optimal for vesicles permeabilization and exposure of the latent activity. It also enables to preserve enzyme in fragmented membranes in a form of protein-lipid complexes with, at least, annular lipids, which are essential to maintain folded protein conformation of the functionally active enzyme, not achieving uncontrolled or complete solubilization of the membrane components. It is of great importance especially for denaturing anionic detergent Ds-Na. In this case ATP addition protects active site and enzyme inactivation [15-17]. The following introduction of the aliquots into incubation mixture enables to decrease detergent concentration up to negligible and ineffective, significantly lower CMC.

Thus, the data represent the first example of the Na^+,K^+-ATPase activity determination in CSM membranes in optimal conditions with different detergents for membranological and pathophysiological usage and testing the impact of diverse effectors on Na^+,K^+-ATPase.

Farther, the effect of ethanol on CSM ATPases was compared for two detergents used for membrane pretreatment. As it was shown earlier using fluorescent probe 1-anilinonaphthalene-8-sulfonate (ANS) of the membrane surface localization (in phospholipids polar heads and glycerin residues region), short chain aliphatic alcohol ethanol caused structural modification of the membrane surface area [23]. Na^+,K^+-ATPase activity is sensitive to ethanol in greater extent compared with Mg^{2+}-ATPase (Fig. 1, Table 2). Actually this indicates different dependence of the ATP-hydrolases to membrane surface modification. Regardless membrane permeabilization with digitonin or Ds-Na the dependence of the Na^+,K^+-ATPase activity inhibition by ethanol and I_{50} values does not differ significantly for two detergents. This indicates the functional similarity of protein-lipid complexes of the enzyme produced by both detergents. Mg^{2+}-ATPase is less susceptible to membrane alterations caused by ethanol.

The obtained data for CSM ATPases almost completely coincide with our previous researches for rat cerebral cortex enzymes with the use of Ds-Na for membrane permeabilization: the I_{50} values for Na^+,K^+-ATPase ethanol inhibition are the same both for smooth muscle and brain (Table 2) [17]. It is possible to make a conclusion about the existence of the general differences of significance of the membrane surface microenvironment for ATP hydrolysis

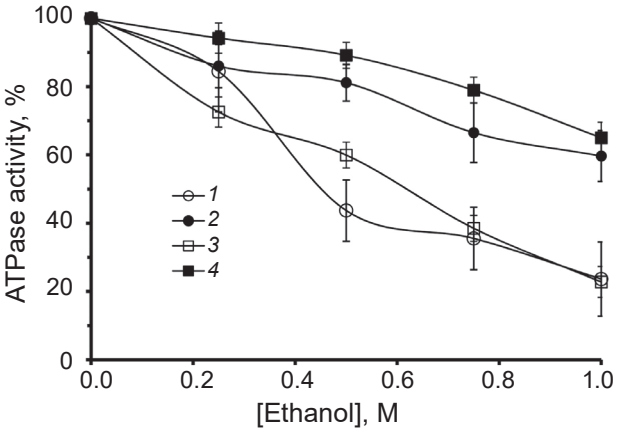

Fig. 1. The effect of ethanol on rat CSM Na^+,K^+-ATPase (1, 3) and Mg^{2+}-ATPase (2, 4) activity in permeabilized by digitonin (1,2) and Ds-Na (3,4) membranes (M ± m, n = 4-5). 100% – corresponding activity without effector

mechanism by the two enzyme systems in various tissues.

In further experiments the effect of the hydrophobic modifier AA on CSM ATP-hydrolases was studied in membrane fragments that were made leaky by Ds-Na pretreatment. The long-chain polyunsaturated AA is the structural component of the membrane phospholipids. It is released in membrane by phospholipase A_2, modifies membrane matrix packaging and together with its metabolites inhibits Na^+,K^+-ATPase, in pulmonary artery or colonic mucosa in particular [24-27]. AA and its metabolites also are well known physiological regulators and pathophysiological factors, inflammation mediators, that participate also in neurotransmission, signal transduction, regulation of vascular tonus and smooth muscle contractility.

It is shown (Fig. 2, Table 2), that in CSM membranes AA inhibits Na^+,K^+-ATPase in micromolar range with I_{50} ~ 30 μM, while Mg^{2+}-ATPase is more resistant with I_{50} value eight times higher, namely ~ 240 μM. These results for CSM Na^+,K^+-ATPase fully coincide with parameters for enzyme from other tissues [24, 27]. The data indicate the differences of the ATPases dependence on native hydrophobic membrane matrix, deep modification of the enzyme microenvironment with long aliphatic chains, annular lipid packaging, thus representing the major specific features of structural arrangement in membrane of the two ATP-hydrolytic enzyme systems.

It seems expedient to compare the effectiveness of membranotropic action of the aliphatic

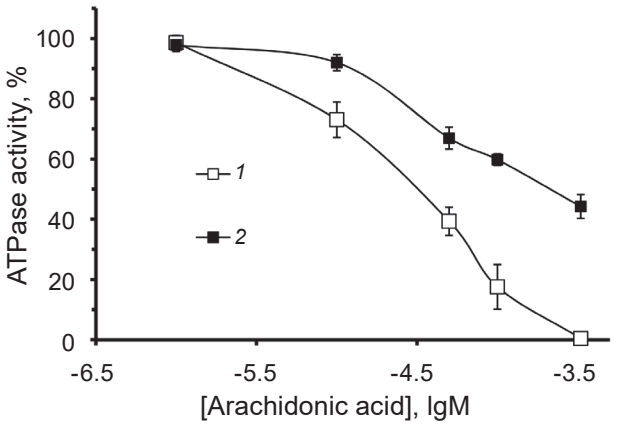

Fig. 2. The effect of arachidonic acid on rat CSM Na^+,K^+-ATPase (1) and Mg^{2+}-ATPase (2) activity (M ± m, n = 4-6) in permeabilized by Ds-Na membranes. 100% – corresponding activity without effector

compounds despite the nonhomologous series on ATPases from various tissue. The extent of the inhibition sharply enhanced with the chain length according to the increased hydrophobicity in the range: alcohols < long chain unsaturated fatty acids (ethanol < butanol < AA). I_{50} decreased from high millimolar range to micromolar values for two ATPases (Table 2). But Mg^{2+}-ATPase in all cases was much more resistant than Na^+,K^+-ATPase. For ethanol the inhibition parameters for Na^+,K^+-ATPase appeared to be the same for CSM and brain cortex membranes pretreated with Ds-Na [17]. I_{50} values for ethanol inhibition of the CSM Na^+,K^+-ATPase for two detergents do not differ significantly. Taking into account our previous researches that ethanol is a moderate modifier of the structural state of the surface membrane area [23], these data indicate the structural-functional similarity of the Na^+,K^+-ATPase protein-lipid complexes formed by both detergents, but in tendency somewhat more stable in the case of Ds-Na pretreatment.

Much higher resistance of the Mg^{2+}-ATPase in comparison with Na^+,K^+-ATPase to inactivation by hydrophobic aliphatic membrane-acting agents reveals the different importance of the lipid environment for their functional activity and peculiarities of the mechanism of the enzymatic ATP hydrolysis.

Thus, the optimal conditions were chosen for Na^+,K^+-ATPase activity determination in rat CSM crude membrane preparation. The universal methodical approach, proven in other tissues [15-17], is adapted in our case. It provides simple and reliable detection of the CSM Na^+,K^+-ATPase functional activity in a relatively nonpurified membranes – the highest of the known for smooth muscle enzyme, including myometrial plasma membranes [11, 22], with comparable Mg^{2+}-ATPase acivity. The technique is appropriate for membranological assays, quantitative comparison in pathophysiological studies and for testing the impact of diverse effectors on Na^+,K^+-ATPase. The key feature is that the enzyme stays embedded in intrinsic membrane environment due to the mode of the detergent exposure [15, 16, 20]. The presence of the protein-lipid complexes is essential for determination of the enzyme dependence on the membrane microenvironment under the influence of the lipophilic modifiers. The higher Na^+,K^+-ATPase susceptibility in comparison with Mg^{2+}-ATPase to the membrane modification by ethanol and arachidonic acid was revealed in this study, thus proving the essential importance of the hydrophobic environment for the maintenance of the properly folded functional enzymatic conformation.

In the previous own researches the following biochemical features of the CSM ATP-hydrolases were revealed for the first time in accordance with general insights of structure-functional enzymatic

*Table 2. Inhibition parameters for ATP-hydrolases (I_{50}) by membrane-active modifiers (M ± m, n = 4-5). * Significant differenses vs corresponding Mg^{2+}-ATPase activity*

I_{50}	Na^+,K^+-ATPase	Mg^{2+}-ATPase
Ethanol, mM, CSM, digitonin	467.92 ± 64.88*	> 1000
Ethanol, mM, CSM, Ds-Na	606.96 ± 40.38*	> 1000
Ethanol, mM, brain cortex, Ds-Na [17]	690.00 ± 10.00*	> 2000
Butanol, mM, brain cortex, Ds-Na#	79.52 ± 1.35*	234.26 ± 6.83
Arachidonic acid, μM, CSM, Ds-Na	30.55 ± 4.37*	237.87 ± 43.35

Note: # brain cortex microsomes were obtained according [17]

properties. Rat CSM Na^+,K^+-ATPase is mainly represented by rodent species-specific ouabain resistant α1-isofrm with I_{50} ~ 70 μM, prevailing in membrane fraction [18]. By the way, the determination anywhere of the low apparent ouabain affinity (high I_{50}, μM) of the native, non-mutant, not rodent enzyme indicates the mismatch of conditions for stationary glycoside binding. Na^+,K^+-ATPase of rat CSM in comparison with Mg^{2+},ATP-hydrolase is characterized by a greater functional importance of SH-groups corresponding to the higher sensitivity to inhibition by divalent heavy metal ions. Sodium nitroprusside as nitric oxide donor and polyamine spermine (mM) are the weak inhibitors also of Na^+,K^+-ATPase but not of Mg^{2+}-ATPase [12, 19]. Contrary, in membrane preparations Na^+,K^+-ATPase and Mg^{2+}-ATPase both are insensitive to submillimolar H_2O_2 concentrations (in the presence of EGTA), but unlike Mg^{2+}-ATPase, Na^+,K^+-ATPase is highly sensitive to hydroxyl radical, generated in the presence of transition metals. Thus, the above data may reflect the differences between two types of ATP-hydrolases in enzyme structural arrangement in membrane, susceptibility to oxidation and catalytic mechanism.

Taken together [12, 19], the data reflect the differences of the structural arrangement in the membrane for two ATP-hydrolases, important for the functional manifestation. The CSM Na^+,K^+-ATPase biochemical properties revealed in the own researches correspond to the specific features of the enzyme from different sources [12, 18, 19]. Thus, the data also represent the approach of the simple and reliable determination of the activity of Na^+,K^+-ATPase in nonpurified CSM membranes, present in membrane-bound form with native microenvironment, necessary for functional congruence. It is acceptable for different tissues and appropriate for membranological assays, quantitative comparison in pathophysiological studies and for testing the impact of diverse effectors on Na^+,K^+-ATPase [28]. Primarily, the high conformational mobility of the Na^+,K^+-ATPase, performing the cyclic intramolecular conformational transitions in the process of the catalytic turnover, is supported by native lipid environment, at least by annular lipids, susceptible to be disordered by detergents and/or lipophilic modifiers [16, 17, 28]. Such properties are important for optimization of the functioning of the enzyme under normal conditions. However, they determine the sensitivity of the enzyme to the action of pathological factors, when diverse homeostatic mechanisms in the cell go out of control. It may be of biological relevance in cellular response, for example, under the effect of phospholipase A_2, endogenic AA acid and its metabolites or other hydrophobic compounds. The data reflect the possible functional differences in lipid dependence and importance of the integrity of the protein and intrinsic microenvironment for two enzymatic system (Na^+,K^+-ATPase and Mg^{2+}-ATPase) due to the peculiarities of the structural arrangement in membrane and importance of the hydrophobic surrounding for mechanism of catalysis.

ВІДМІННОСТІ В ЧУТЛИВОСТІ Na^+,K^+-АТРази ТА Mg^{2+}-АТРази ГЛАДЕНЬКИХ М'ЯЗІВ ОБОДОВОЇ КИШКИ ЩУРА ДО ЕТАНОЛУ ТА АРАХІДОНОВОЇ КИСЛОТИ ЗА ПОПЕРЕДНЬОЇ ОБРОБКИ КЛІТИННИХ МЕМБРАН Ds-Na

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Уніфікований методичний підхід дозволяє визначати досить високий рівень функціональної активності Na^+,K^+-АТРази в неочищеній фракції клітинних мембран гладенького м'яза ободової кишки (ГМОК) щура з використанням стандартної попередньої обробки детергентом (Ds-Na порівняно з дигітоніном). Необхідний обов'язковий поділ етапів: пермеабілізації мембран детергентом за оптимального співвідношення детергент/протеїн в умовах захищення активного центру ензиму АТР (для Ds-Na) попередньо до ензиматичної реакції за значного розведення детергенту набагато нижче критичної концентрації міцелоутворення в середовищі АТРази. Високий рівень Na^+,K^+-АТРазної активності, яка вперше визначена в ГМОК, не відрізнявся для двох детергентів і був порівняним з активністю уабаїн-резистентної Mg^{2+},АТР-гідролази. Специфічні особливості протеїново-ліпідних комплексів АТРаз оцінювали за чутливістю до мембранотропного впливу етанолу і арахідонової кислоти з різною дезорганізуючою мікрооточення ензимів ефективністю. Довголанцюгова жирна кислота є ефективнішим інгібітором обох АТРаз, ніж аліфатичний спирт. У свою чергу

Mg^{2+},АТР-гідролаза характеризується значно більшою стійкістю до інактивації, ніж Na^+,K^+-АТРаза в обох випадках. Передбачається можливе існування відмінностей двох ензимних систем у ліпідній залежності як результат особливостей їх структурної організації в мембрані і важливості гідрофобного оточення в механізмі каталізу. Таким чином, застосований простий і надійний підхід визначення активності Na^+,K^+-АТРази в неочищеній мембранній фракції клітин ГМОК, уніфікований для різних тканин, який може використовуватися в мембранологічних і порівняльних патофізіологічних дослідженнях, а також для тестування впливу різних ефекторів на Na^+,K^+-АТРазу.

Ключові слова: АТР-гідролази, Na^+,K^+-АТРаза, гладенький м'яз ободової кишки, дигітонін, Ds-Na, етанол, арахідонова кислота.

РАЗЛИЧИЕ В ЧУВСТВИТЕЛЬНОСТИ Na^+,K^+-АТРазы И Mg^{2+}-АТРазы ГЛАДКИХ МЫШЦ ОБОДОЧНОЙ КИШКИ КРЫСЫ К ЭТАНОЛУ И АРАХИДОНОВОЙ КИСЛОТЕ В УСЛОВИЯХ ПРЕДОБРАБОТКИ КЛЕТОЧНЫХ МЕМБРАН Ds-Na

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Унифицированный методический подход позволяет определять достаточно высокий уровень функциональной активности Na^+,K^+-АТРазы в неочищенной фракции клеточных мембран гладкой мышцы ободочной кишки (ГМОК) крысы с использованием стандартной предобработки детергентами (Ds-Na в сравнении с дигитонином). Необходимо обязательное разделение этапов: пермеабилизации мембран детергентом при оптимальном соотношении детергент/протеин в условиях защиты активного центра энзима АТР (для Ds-Na), предшествующей энзиматической реакции при значительном разведении детергента намного ниже критической концентрации мицеллообразования в среде АТРазы. Высокий уровень Na^+,K^+-АТРазной активности, впервые определенной в ГМОК, одинаков для двух детергентов и сопоставим с активностью уабаинрезистентной Mg^{2+},АТР-гидролазы. Специфические особенности протеиново–липидных комплексов АТРаз оценивали по чувствительности к мембранотропному воздействию этанола и арахидоновой кислоты с разной эффективностью дезорганизации микроокружения энзимов. Длинноцепочечная жирная кислота является более эффективным ингибитором обеих АТРаз, чем алифатический спирт. В свою очередь Mg^{2+},АТР-гидролаза характеризуется значительно большей устойчивостью к инактивации, чем Na^+,K^+-АТРаза в любом случае. Предполагается возможное существование различий двух энзимных систем в липидной зависимости как результат особенностей их структурной организации в мембране и важности гидрофобного окружения в механизме катализа. Таким образом, применен простой и надежный подход определения активности Na^+,K^+-АТРазы в неочищенной мембранной фракции клеток ГМОК, унифицированный для разных тканей, который может использоваться в мембранологических и сравнительных патофизиологических исследованиях, а также для тестирования воздействия различных эффекторов на Na^+,K^+-АТРазу.

Ключевые слова: АТР-гидролазы, Na^+,K^+-АТРаза, гладкая мышца ободочной кишки, дигитонин, Ds-Na, этанол, арахидоновая кислота.

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