

## THE INFLUENCE OF HEAVY METAL IONS, SPERMINE AND SODIUM NITROPRUSSIDE ON ATP-HYDROLASES OF CELL MEMBRANES OF RAT COLON SMOOTH MUSCLE

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*The specific features of functional lability of the rat colon smooth muscle (CSM) ATP-hydrolases were studied. Na<sup>+</sup>,K<sup>+</sup>-ATPase activity is effectively inhibited by divalent ions of both transition ( $\geq 0,1 \mu\text{M}$ ) and nontransition ( $\geq 1 \mu\text{M}$ ) heavy metals in succession by efficiency:  $\text{Cu}^{2+} > \text{Fe}^{2+} \geq \text{Cd}^{2+}$  ( $10 \mu\text{M}$ ). Polyamine spermine (0,5-1,0 mM) is a weak Na<sup>+</sup>,K<sup>+</sup>-ATPase inhibitor at saturation concentrations of ions and substrate. Sodium nitroprusside (1 mM) as nitric oxide-generating compound exhibits weak Na<sup>+</sup>,K<sup>+</sup>-ATPase inhibition only after prolonged preincubation with membranes. Mg<sup>2+</sup>-ATP-hydrolase activity in all cases is much more resistant to studied agents. Considering the example of the CSM Na<sup>+</sup>,K<sup>+</sup>-ATPase it is assumed that enzyme has specific biochemical features that contribute to its role as a potential target and redox-sensor, mediating the pathological mechanisms of heavy metal intoxication and cell oxidative damage.*

*Key words: ATP-hydrolases, Na<sup>+</sup>,K<sup>+</sup>-ATPase, colonic smooth muscle, heavy metals, spermine, sodium nitroprusside.*

**N**a<sup>+</sup>,K<sup>+</sup>-ATPase (EC 3.6.1.37) is a fundamental enzyme of the ion homeostasis regulation by providing energy-dependent electrogenic contradirectional transport of Na<sup>+</sup> and K<sup>+</sup> across plasma membrane of animal cells to maintain electrochemical gradient of monovalent ions, membrane potential, electrical excitability and associated processes of transport of ions and metabolites [1]. Na<sup>+</sup>,K<sup>+</sup>-ATPase via Na<sup>+</sup>/Ca<sup>2+</sup> exchanger is also involved in the control of calcium homeostasis and electromechanical coupling in smooth muscle cells [2, 3]. Being the main consumer of the ATP energy, synthesis of which requires 20-80% of the oxygen consumed by mammalian cell, Na<sup>+</sup>,K<sup>+</sup>-ATPase is believed to be involved into pathophysiological responses under oxidative stress, ischemia-hypoxia, mitochondrial dysfunction, reprogramming of the oxidative metabolism pathways, etc [4-6]. Labile enzymatic SH-groups are known to be the targets of oxidative modification by products of the interaction of the superoxide (O<sub>2</sub><sup>-</sup>), produced by plasma membrane NADPH oxidase or mitochondrial electron transport chain complexes, and nitric oxide (NO) with peroxynitrite (ONOO<sup>-</sup>) formation, O<sub>2</sub><sup>-</sup> dismutation to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and/or further generation of hydroxyl radical (OH<sup>•</sup>) in Haber-Weiss reaction, catalyzed by transition metals [7,8].

Chronic iron overload disorders (hemochromatosis, chronic drug or dietary intoxication) can be an aggravating factor of the pathogenesis, associated with accumulation of extremely toxic iron pool not linked to transferrin or ferritin in the plasma or inside the cell, respectively. It may cause the redox imbalance in tissues and entail cellular damage both at plasma membrane level and intracellularly. Toxic action of other heavy metals depends on mutual saturation and interactions [8-12]. Protein-free iron is also present in miscellaneous diseases primarily developed without iron overloaded conditions [10]. Na<sup>+</sup>,K<sup>+</sup>-ATPase along with respiratory chain enzymes may be the main targets of the toxic action of non-transferrin-bound iron, for example, in cardiomyocytes [9].

On the other hand, the dysperistalsis in inflammatory and ischemic large bowel diseases, colitis, tumor growth, that are accompanied by increase of cellular damaging factors such as reactive oxygen or nitrogen species, disorder of the epithelial barrier function and, finally, sarcolemmal defect, ultimately can be a result of impairment of the electromechanical coupling in smooth muscle. However, the functional features of the colonic smooth muscle (CSM) Na<sup>+</sup>,K<sup>+</sup>-ATPase are actually unexplored. It is important to ascertain the biochemical properties of

this enzyme in comparison with ATP-hydrolases not belonging to P-type family to evaluate the expected sarcolemmal targets participating in impairment of the smooth muscle motility of this intestine region.

It was shown earlier that Na<sup>+</sup>,K<sup>+</sup>-ATPase in the membrane preparations of rat CSM is represented by prevailing catalytic subunit  $\alpha 1$ -isoform bearing the rodent specific resistance to cardiac glycosides, i. e. being inhibited by millimolar but not micromolar ouabain concentrations [13]. As follows from our previous data CSM Na<sup>+</sup>,K<sup>+</sup>-ATPase is characterized by low sensitivity to hydrogen peroxide, but in the presence of micromolar Fe<sup>2+</sup> concentrations is inhibited in physiological (nanomolar) range of H<sub>2</sub>O<sub>2</sub> evidently due to hydroxyl radical production [14]. Considering that Na<sup>+</sup>K<sup>+</sup>-ATPase activity is used as analytical tool for determination of the potential toxicity of a number of compounds of different nature [11], the appeared enhancement of the CSM Na<sup>+</sup>,K<sup>+</sup>-ATPase sensitivity to H<sub>2</sub>O<sub>2</sub> in the absence of EGTA may serve as sensitive and reliable test for the presence of the transition metals contaminants in the medium [14]. Contrary to Na<sup>+</sup>,K<sup>+</sup>-ATPase, the greater resistance to oxidation of Mg<sup>2+</sup>-ATP-hydrolase corresponds to differences in functional importance of SH-groups in these enzyme types [14]. It is also important to evaluate CSM Na<sup>+</sup>,K<sup>+</sup>-ATPase inhibitory pattern for other compounds with different effector groups and action mechanisms.

The aim of this study is to comparatively examine the sensitivity of the ATP-hydrolases (namely Na<sup>+</sup>,K<sup>+</sup>-ATPase and Mg<sup>2+</sup>-ATPase) of rat CSM cellular membranes to divalent transition and non-transition metal ions and to evaluate the inhibitory potency of polyamine spermine and nitric oxide-generating compound sodium nitroprusside for these enzymatic systems.

### Materials and Methods

The isolation of the nonmitochondrial membrane fraction from rat CSM, protein and ATPase activity determination after unmasking procedure by digitonin pretreatment of the membranes were carried out in accordance with the previously described methodical conditions [14]. Male Wistar rats were fed with the standard diet of the vivarium and deprived of food the day before experiment. Animals were anesthetized by diethyl ether inhalation and decapitated. The experiments were done in accordance with guidelines for keeping and work with laboratory animals laid down in the European Conven-

tion for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986). To estimate the effect of divalent heavy metals (0.1-10.0  $\mu$ M FeSO<sub>4</sub>, CuSO<sub>4</sub> or CdCl<sub>2</sub>) the digitonin-permeabilized membranes were preincubated for 30 min at 37 °C in the ATP- and EGTA-free medium. ATPase reaction was started by simultaneous addition of 3 mM ATP and 1 mM EGTA mixture (final concentrations) [14]. To evaluate the spermine effect (0.1-1.0 mM) this polyamine was added directly into the ATPase reaction mixture, but sodium nitroprusside action (1 mM) was determined after membrane preincubation with this agent at 37 °C for different time intervals in the ATPase reaction medium in the presence of 1 mM EGTA with subsequent addition of 3 mM ATP to start the ATPase reaction. Concrete concentration and preincubation time range are given in figure captions. Corresponding enzymatic activity in membranes treated under the same conditions but without effector was taken as 100%. Summarizing briefly, basic principles of methodological procedure are defined by standard characteristics and precautions of the detergent use in membranological studies. The mild detergent pretreatment of membranes at room temperature precedes the incubation with effector and/or the enzymatic reaction (at 37 °C). It provides the possibility to set a moderate detergent concentration in accordance with the protein concentration of the membranes (1/1 ratio), necessary for permeabilization of the vesicles, but not for the full membrane solubilization. Following addition of the aliquots into the incubation medium at 37 °C leads to a drastic decrease in the detergent concentration to ineffective, much lower than critical micelle concentration, preventing the distortion of the experimental results due to deleterious or the combined effect of detergent and membrane-acting compound. Possible interactions of effector with ligands and targets are also taken into account in such experimental conditions. Agents in used concentrations did not affect the color intensity by Chen (phosphomolybdate formation in the presence of ascorbate) [15], did not change the intensity of the spontaneous ATP hydrolysis and did not cause turbidity when using solubilizing concentrations of Ds-Na to stop the reaction.

Statistical analysis of the results was performed using Microsoft Office Excell 2007. The data are given as means  $\pm$  SEM, *n* – number of used preparations. The significance of statistical differences between two groups was evaluated using Student's *t*-test (*P* < 0.05).

## Results and Discussion

It was shown that divalent heavy metal ions, regardless of whether they are transition metals in the reduced ( $\text{Fe}^{2+}$ ) or oxidized form ( $\text{Cu}^{2+}$ ), or nontransition metals ( $\text{Cd}^{2+}$ ) and despite their ability to induce peroxidation processes, efficiently inhibited  $\text{Na}^+, \text{K}^+$ -ATPase activity (Fig. 1, A). The enzyme sensitivity to transition metals appeared from submicromolar concentrations and was practically equal for divalent ions of iron and copper at 0.1 and 1.0  $\mu\text{M}$ , respectively.  $\text{Cd}^{2+}$  inhibited only in the micromolar range, but to a lesser extent than transition metals. At 10  $\mu\text{M}$   $\text{Cd}^{2+}$  the  $\text{Na}^+, \text{K}^+$ -ATPase activity decreased by 44%. Copper ions are most potent at this concentration inhibiting enzymatic activity by 90%. Hence, the ions are arranged in the order by inhibitory potency:  $\text{Cu}^{2+} > \text{Fe}^{2+} \geq \text{Cd}^{2+}$ .

It is generally accepted that inhibition by heavy metals is determined by a selective affinity to  $\text{SH}$ -,  $\text{NH}_2$ - and  $\text{COOH}$ -groups, but most of toxic effects are caused by interaction with sulfhydryl groups [11]. While the effect of the transition metals is obviously associated with both their interaction with functionally important groups of the enzyme along with lipid peroxidation processes, the cadmium influence is only due to the interaction with functional groups [12, 14, 16].

CSM  $\text{Mg}^{2+}$ -ATPase is significantly more resistant to the inhibition by heavy metals and in the case of cadmium ions is almost insensitive at studied concentrations (Fig. 1, B). Primarily it corresponds to lower importance of the sulfhydryl groups for the function of this enzymatic system in comparison with  $\text{Na}^+, \text{K}^+$ -ATPase in CSM [14].

The numerous high-affinity specific binding sites distinct for  $\text{Fe}^{2+}$  and  $\text{Cu}^{2+}$  were detected on  $\text{Na}^+, \text{K}^+$ -ATPase subunits [17-19]. Adding hydrogen peroxide and ascorbate (i.e. conditions of intense generation of the hydroxyl radical) results in metal-catalyzed site-specific polypeptide cleavage into the distinct multiple fragments. Such highly reproducible cleavage of peptide bonds selectively catalyzed by submicromolar concentrations of iron or copper is sensitive to conformational state and used to study spatial organization of the  $\text{Na}^+, \text{K}^+$ -ATPase molecule and intramolecular conformational transitions [17-19]. Our previous finding clearly showed [14] that kidney (exclusively  $\alpha 1$ -isozyme) and CSM  $\text{Na}^+, \text{K}^+$ -ATPase inactivation in nanomolar range of  $\text{H}_2\text{O}_2$  occurred only in the presence of iron ions and increased at  $\text{Fe}^{2+}$  micromolar range compared to inhi-

bition resistance to hydrogen peroxide alone up to submillimolar concentrations (in EGTA presence). Revealed  $\text{Na}^+, \text{K}^+$ -ATPase hypersensitivity to  $\text{H}_2\text{O}_2$  may serve as a reliable and sensitive test for the contaminations of transition heavy metals in the reaction mixture [14]. In the presence of ATP the high-affinity  $\text{ATP-Mg}^{2+}$  complex is formed and cleavage sites are localized within active site of enzyme [20]. To prevent such phenomenon the ATPase reaction was run under EGTA chelation after enzyme preincubation with divalent ions.

Thus, it is likely that binding of heavy metals to  $\text{Na}^+, \text{K}^+$ -ATPase molecule modifies specific functional groups and separately from lipid peroxidation leads to conformational changes that affects functionally determined  $\text{Na}^+, \text{K}^+$ -ATPase conformational mobility during the catalytic cycle. This may account for the effect of  $\text{Cd}^{2+}$ . It is well-known that this heavy metal is extremely toxic for organism, it influences on membrane and cellular levels [12]. According to the obtained data its effect is specific for  $\text{Na}^+, \text{K}^+$ -ATPase as compared with  $\text{Mg}^{2+}$ -ATP-hydrolase in CSM (Fig. 1). It should be emphasized that the mechanism of  $\text{Na}^+, \text{K}^+$ -activated,  $\text{Mg}^{2+}$ -dependent ATP hydrolysis fundamentally depends on the enzyme native conformation, essential for cyclic conformational turnover in the membrane and cation occlusion/deocclusion [1]. So, CSM  $\text{Mg}^{2+}$ -ATP-hydrolase is resistant to destabilizing effect of transition and nontransition heavy metals and can be the criterion of the membrane enzymatic resistance in comparative analysis. The data correspond to the results obtained for brain enzymes [11, 21], specifying the common structural and functional differences, existing between the examined ATP-hydrolase families.

The diverse inhibitors were used for further study. Evaluating the spermine effect, it was shown that this polyamine at concentrations of 0.5-1.0 mM in the ATPase incubation medium in the presence of saturating concentrations of essential ions and ligands ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$  and ATP) is a weak inhibitor of SMS  $\text{Na}^+, \text{K}^+$ -ATPase (Fig. 2).  $\text{Mg}^{2+}$ -ATP-hydrolase appeared to be stable to this polyamine. The obtained data for CSM enzymes are in accordance with other investigations in different tissues. Polyamines are considered as  $\text{Na}^+, \text{K}^+$ -ATPase inhibitors that exhibit complex interactions with ions and the substrate depending on different conformational states during enzyme catalytic turnover [22, 23]. Lack of the effect on  $\text{Mg}^{2+}$ -dependent hydrolysis of ATP compared

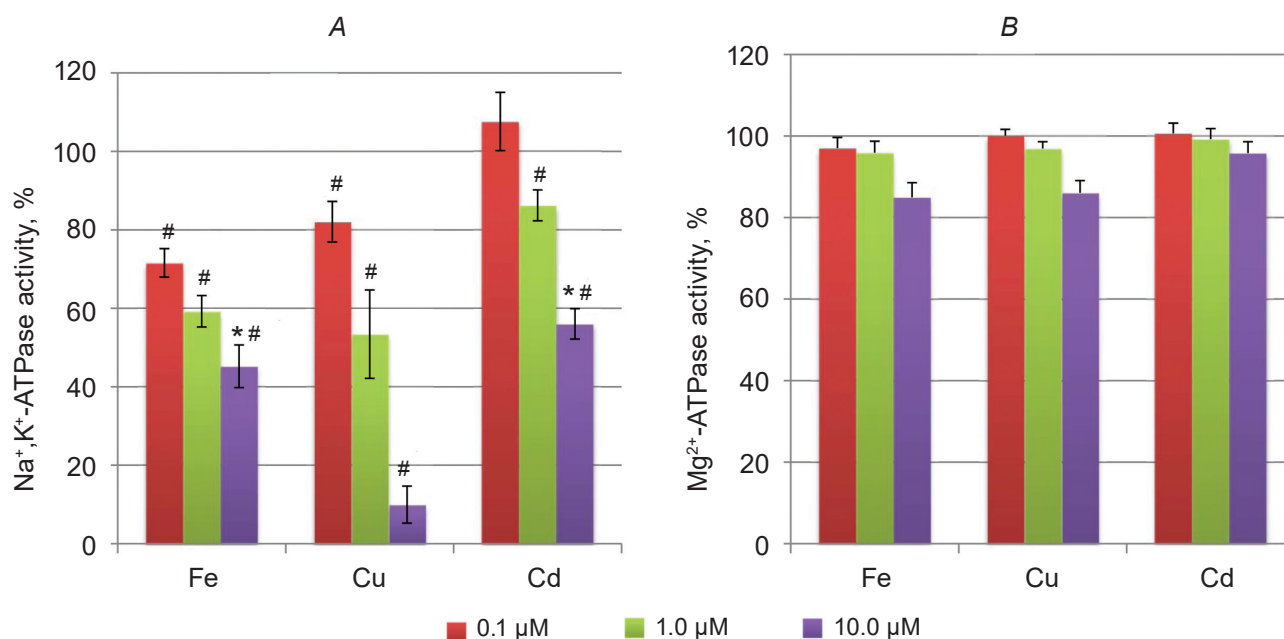


Fig. 1. The influence of the divalent heavy metal ions on the activity of  $\text{Na}^+, \text{K}^+$ -ATPase (A) and  $\text{Mg}^{2+}$ -ATPase (B) of rat CSM ( $M \pm m$ ,  $n = 4-6$ ). 100% - corresponding activity without effector. The concentrations of heavy metals are given in the legend. \* Significant differences vs value for  $\text{Cu}^{2+}$ , # vs corresponding  $\text{Mg}^{2+}$ -ATPase activity

with  $\text{Na}^+, \text{K}^+$ -ATPase may reflect the specifics of the mechanism of ATP hydrolysis by two enzyme systems. These data confirm the specific spermine inhibitory action just on  $\text{Na}^+, \text{K}^+$ -ATPase at saturation ion concentrations and may indicate the modulation of the reaction cycle of ( $\text{Na}^+, \text{K}^+$ )-dependent ATPase hydrolytic activity.

Other results indicate, that potent NO-releasing compound sodium nitroprusside (SNP) at concentrations of 0.1-1.0 mM has no effect on  $\text{Na}^+, \text{K}^+$ -ATPase activity under standard preincubation conditions of the CSM membranes in ATPase reaction mixture without ATP (for 30 min) in the presence of 1 mM EGTA. Chelator addition makes the interference with divalent metal contaminations unlikely. The weak inhibition was revealed only after prolonged pretreatment with 1 mM SNP, enhancing enzyme inactivation by 25% (Fig. 3). Such inhibitory effect was also revealed for numerous NO-generating compounds in vitro indicating the direct action of NO on the enzyme through SH-groups oxidation [24]. The formation of the other reactive nitrogen species is uncertain. Despite the mechanism of action and effector groups, such conditions are obviously necessary for nonenzymatic decomposition of the compound. SNP does not affect the  $\text{Mg}^{2+}$ -ATPase activity. The data correspond to the time course of

the  $\text{Na}^+, \text{K}^+$ -ATPase inhibition in the cerebral cortex preparations [24], where SH-sensitive isoforms, which are much more susceptible to oxidation, are present [16].

Thus, the  $\text{Mg}^{2+}$ -ATP-hydrolase in all cases is stable to used agents with different mechanism of

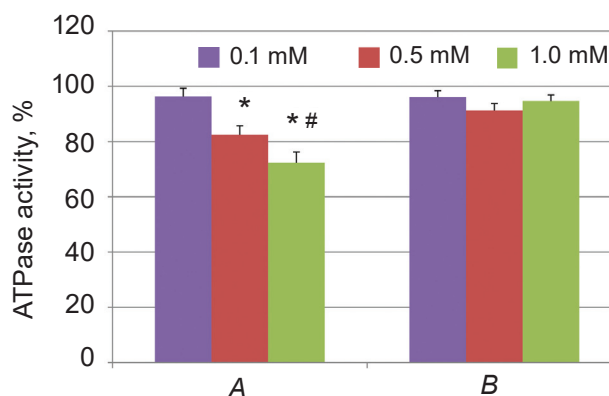


Fig. 2. The influence of the spermine on the activity of  $\text{Na}^+, \text{K}^+$ -ATPase (A) and  $\text{Mg}^{2+}$ -ATPase (B) of rat CSM ( $M \pm m$ ,  $n = 4-5$ ). \* Significant differences vs control value without spermine, # vs corresponding  $\text{Mg}^{2+}$ -ATPase activity. 100% - corresponding activity without effector. Spermine concentrations are given in the legend

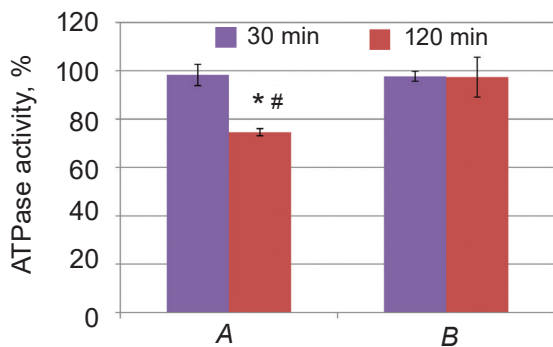


Fig. 3. The influence of the 1 mM sodium nitroprusside (SNP) on the activity of Na<sup>+</sup>,K<sup>+</sup>-ATPase (A) and Mg<sup>2+</sup>-ATPase (B) of rat CSM ( $M \pm m$ ,  $n = 3-4$ ). \* Significant differences vs corresponding control without SNP, # vs corresponding Mg<sup>2+</sup>-ATPase activity. 100% - corresponding activity without effector. Preincubation time are given in the legend

action and different effector groups. We demonstrated earlier that Mg<sup>2+</sup>-ATPase was also more resistant in comparison with Na<sup>+</sup>,K<sup>+</sup>-ATPase to membrane active compounds such as aliphatic alcohols (ethanol), inducing structural membrane disorders [25, 26]. Taken together, these findings are attributed to the specifics of the membrane structural organization of the active conformation of the mentioned ATPases and indicate their definite ATP-hydrolyzing mechanisms and particular inhibitor action for conformationally labile Na<sup>+</sup>,K<sup>+</sup>-ATPase.

In accordance with previous studies [14], taking into account the high sensitivity of the CSM Na<sup>+</sup>,K<sup>+</sup>-ATPase to inhibition by transition metals and significant increase in the efficiency of the hydrogen peroxide inhibition in the presence of ferrous ions, which is manifested from H<sub>2</sub>O<sub>2</sub> physiological nanomolar range (under conditions of the Haber-Weiss reaction and generation of the hydroxyl radical as the most potent membrane oxidant [8]), it is suggested that Na<sup>+</sup>,K<sup>+</sup>-ATPase can be a potential effective oxidative target under certain pathophysiological conditions, a marker of oxidative stress and oxidative plasma membrane structural disorders.

These versatile modulators of Na<sup>+</sup>,K<sup>+</sup>-ATPase from other sources with different mode of action enable to further characterize two CSM enzymatic systems that differ by way of ATP hydrolysis [14]. The similarity of the enzymatic properties in smooth muscle and Na<sup>+</sup>,K<sup>+</sup>-ATPase from other tissues revealed by the effect of studied inhibitors should be noted [21-24]. They are determined by the molecu-

lar structure and membrane topology, importance of conformational turnover during the catalytic cycle, SH-dependence and sensitivity to lipid environment. Despite also existing tissue-specific individual differences of Na<sup>+</sup>,K<sup>+</sup>-ATPase isozymes in sensitivity to oxidants, SH- and lipid dependence [16], the potential defect of the enzyme in target tissues foremost should be determined by the combined intensity of redox imbalance and antioxidant deficiency and can be expected in conditions of oxidative stress, ischemia, hemochromatosis, iron and copper overloading, heavy metals intoxication.

Evidently, the studies on Na<sup>+</sup>,K<sup>+</sup>-ATPase preparations *in vitro* do not exactly simulate the complex relationships that exist in the cells under pathophysiological conditions. However, despite the revealed high sensitivity of enzyme to heavy metal ions they indicate tendency of possible processes *in vivo* and can occur in the cell only in particular conditions of simultaneous hyperproduction of the reactive oxygen or nitrogen species, depletion of the antioxidant defense system and introduction of some sensitizing factors. In addition, it is important to distinguish between reversible regulatory processes under stress conditions to maintain or tune redox homeostasis and adjust redox-dependent signal transduction in the cell and irreversible processes leading to structural and functional disorders and, ultimately, to cell death.

Indeed, numerous studies found functional changes in Na<sup>+</sup>,K<sup>+</sup>-ATPase in the pathogenesis of ischemia and hypoxia in the kidneys and excitable tissues [27, 28]. It was also shown that during hypoxia, mitochondrial ROS contribute to the inhibition of Na<sup>+</sup>,K<sup>+</sup>-ATPase activity of alveolar epithelial cells by stimulating its endocytosis via PKC-mediated phosphorylation of the  $\alpha 1$ -subunit of the enzyme [5, 29].

In vascular smooth muscle Na<sup>+</sup>,K<sup>+</sup>-ATPase is an important determinant of their tone, mediating the regulation of [Ca<sup>2+</sup>]<sub>i</sub> [3]. The physiological mechanism of the reversible oxidative regulation of the Na<sup>+</sup>,K<sup>+</sup>-pump in the sarcolemma of the myocardium and vessels is mediated by glutathionylation under oxidative stress of  $\beta 1$ -subunit cysteine residues in the close vicinity to the site of the association with the Na<sup>+</sup>,K<sup>+</sup>-ATPase  $\alpha 1$ -subunit that reduces the turnover number of the  $\alpha 1\beta 1$ -heterodimer by inducing conformational changes and slowing the rate-limiting conformational transition E<sub>2</sub>→E<sub>1</sub> in a catalytic cycle of Na<sup>+</sup>,K<sup>+</sup>-ATPase pump [7, 30].

In colonic smooth muscles anticipated oxidative defect in the sarcolemma is possible under colon

pathologies, including colitis, inflammation and erosion of the mucosa with impaired barrier function of the epithelial layer and external smooth muscle exposure to heavy metals and oxidants, nutritional or pharmacological overloading of an organism with iron and copper, hemochromatosis, intoxication with heavy metals. It is obvious that direct defect in  $\text{Na}^+, \text{K}^+$ -ATPase may be followed by partial depolarization of the sarcolemma and impairment of the ion homeostasis, including  $[\text{Ca}^{2+}]_i$  via  $\text{Na}^+, \text{Ca}^{2+}$ -exchanger, osmoregulation, electromechanical coupling, large intestine motility.

Hypothetical conditions to ensure a defect at the plasma membrane level require the following: disturbances of the redox homeostasis in the cell, hyperproduction of the reactive oxygen species (superoxide anion radical and hydrogen peroxide) mainly of mitochondrial origin or by plasma membrane NADPH oxidase, depletion of antioxidant defense system, primarily the reduction in the activity of mitochondrial catalase, existence of free labile intracellular iron pool. Hydrogen peroxide is the most stable reactive oxygen compound; it is membrane permeable and acts over long distances in the cell, including plasma membrane [6]. The presence of a labile pool of transition metals (ions of iron or copper) accelerates toxic hydroxyl radical generation.

The question arises about the  $\text{Na}^+, \text{K}^+$ -ATPase involvement in the mechanisms of cell death and induced cytotoxicity in the context of deregulation of redox homeostasis in the cell. It is known that in some malignant neoplasms  $\text{Na}^+, \text{K}^+$ -ATPase inhibition by cardiac glycosides, accompanied with the increase in  $[\text{Na}^+]_i$ , concomitant  $[\text{Ca}^{2+}]_i$  increase and  $[\text{K}^+]_i$  depletion, leads to apoptosis [31]. In cultures of vascular smooth muscle, endothelial cells and astrocytes the cytotoxic action of ouabain (3  $\mu\text{M}$ ), developed in 24 hours period, is shown only for human cells, but this cardiac steroid did not affect cell viability of rat cells (at concentrations up to 5 mM). However, the same complete inhibition of  $\text{Na}^+, \text{K}^+$ -ATPase, accompanied by dramatic increase of  $[\text{Na}^+]_i/[\text{K}^+]_i$ , occurred. Certainly, it is a result of the species-dependent differences in the unique receptor properties of the ouabain-sensitive and rodent ouabain-resistant  $\text{Na}^+, \text{K}^+$ -ATPase catalytic subunit  $\alpha 1$ -isoform, rather than in features of the signaling mechanisms of cell death [32].

Recently, it was established that  $\text{Na}^+, \text{K}^+$ -ATPase plays a crucial role in the regulation of autosis development – new morphologically unique type of

autophagy-dependent cell death occurring under autophagy, triggered by certain peptides and stress conditions of starvation, ischemia-hypoxia of the brain in both *in vitro* and *in vivo*. Blockers of apoptosis and necrosis do not affect the autosis development, which, however, is inhibited by cardiac glycosides – pharmacological  $\text{Na}^+, \text{K}^+$ -ATPase antagonists, pharmacological and genetic autophagy blockers and genetic knockdown of the  $\text{Na}^+, \text{K}^+$ -ATPase  $\alpha 1$ -subunit [33].

On the model of smooth muscle pathology of pulmonary arterial hypertension in rats it was shown that the acceleration of oxidative phosphorylation by sodium dichloroacetate (SDA) – the inhibitor of pyruvate dehydrogenase kinase, which expression is increased in this pathology, was accompanied by increased generation of mitochondrial ROS, namely  $\text{H}_2\text{O}_2$ , capable to diffuse into the cytoplasm promoting intracellular effects, including opening and activation of sarcolemmal voltage-gated  $\text{K}^+$ -channels, associated vasodilation, hypertension reversal, ultimately inducing mitochondrial-dependent apoptosis [34]. Obviously, the differences in the expression of respiratory chain enzymes, Mn-superoxide dismutase and, actually, the efficiency of  $\text{H}_2\text{O}_2$  production in mitochondria, that affects smooth muscle cell membrane potential and cytosolic calcium, may determine the opposite response to hypoxia of pulmonary and renal arteries, causing their constriction or dilation, respectively [35]. A similar mechanism of SDA action, increasing mitochondrial metabolism and ROS production under mitochondrial dysfunction is specific for many malignant tumors, which energy metabolism, as known, is characterized by the Warburg effect against the background of the mitochondrial structure-functional disorders [6]. In our previous studies, the cytotoxic action of SDA was observed only in transplanted sarcoma 37, but not in Lewis lung carcinoma, as a possible result of the biological properties of tumors, characteristics and extent of energy metabolism reprogramming, structural and functional abnormalities of the mitochondrial membranes and prooxidant-antioxidant homeostasis impairment [6, 36, 37].

Our researches have found a continuation in further investigations. It was shown that exogenous ascorbate is able to induce necrosis and apoptosis in the cells of many tumors treated with SDA without loading with exogenous iron preparations, but due to involvement of the intracellular free labile iron pool. It specifically increased in tumors and catalyzed

Haber-Weiss reaction and generation of hydroxyl radicals [38]. The contribution of the  $\text{Na}^+, \text{K}^+$ -ATPase defect in induced cytotoxicity as a sensor or a target of the oxidative-mediated damage of the plasma membrane is not excluded under these conditions.

Thus, this study shows that CSM  $\text{Na}^+, \text{K}^+$ -ATPase is highly sensitive to heavy metals and is also inhibited by polyamine spermine as modulator of conformational turnover and SNP as nitric oxide donor. Taking into account the available scientific evidences, presented data and previous studies on  $\text{Na}^+, \text{K}^+$ -ATPase specific sensitivity to heavy metals and prooxidants and enzyme inhibitory lability in CSM [14] it is suggested that this enzyme system is characterized by biochemical features that allow it to be a potential target and redox sensor mediating pathophysiological mechanisms of intoxication by heavy metals and oxidative cell damage in different tissues.

### **ВПЛИВ ІОНІВ ВАЖКИХ МЕТАЛІВ, СПЕРМІНУ ТА НІТРОПРУСИДУ НАТРІЮ НА АТР-ГІДРОЛАЗИ КЛІТИННИХ МЕМБРАН ГЛАДЕНЬКИХ М'ЯЗІВ ОБОДОВОЇ КИШКИ ЩУРА**

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Досліджено особливості функціональної лабільності АТР-гідролаз клітинних мембран гладеньких м'язів ободової кишки (ГМОК). Показано, що  $\text{Na}^+, \text{K}^+$ -АТРаза ефективно інгібується двовалентними іонами як перехідних ( $\geq 0,1$  мкМ), так і неперехідних ( $\geq 1$  мкМ) важких металів у послідовності за ефективністю:  $\text{Cu}^{2+} > \text{Fe}^{2+} \geq \text{Cd}^{2+}$  (10 мкМ). Поліамін спермін (0,5–1,0 мМ) – слабкий інгібітор  $\text{Na}^+, \text{K}^+$ -АТРази ГМОК за насичуючих концентрацій іонів і субстрату. Донор оксиду азоту нітропруssid натрію (1 мМ) виявляв слабкий інгібувальний ефект на  $\text{Na}^+, \text{K}^+$ -АТРазу тільки за довготривалої попередньої інкубації з мембранами.  $\text{Mg}^{2+}$ -АТРаза у всіх випадках була значно стійкішою до дії досліджуваних агентів. На прикладі  $\text{Na}^+, \text{K}^+$ -АТРази ГМОК припускається, що ензим характеризується біохімічними особливостями,

які забезпечують можливість бути потенційною мішенню та редокс-сенсором, що опосередковує патофізіологічні механізми інтоксикації важкими металами та оксидативного ушкодження клітин.

**Ключові слова:** АТР-гідролази,  $\text{Na}^+, \text{K}^+$ -АТРаза, гладенькі м'язи ободової кишки, важкі метали, спермін, нітропруssid натрію.

### **ВЛИЯНИЕ ИОНОВ ТЯЖЕЛЫХ МЕТАЛЛОВ, СПЕРМИНА И НИТРОПРУССИДА НАТРИЯ НА АТР-ГИДРОЛАЗЫ КЛЕТОЧНЫХ МЕМБРАН ГЛАДКИХ МЫШЦ ОБОДОЧНОЙ КИШКИ КРЫСЫ**

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Исследованы особенности функциональной лабільности АТР-гідролаз клеточных мембран гладких мышц ободочной кишки (ГМОК). Показано, что  $\text{Na}^+, \text{K}^+$ -АТРаза ефективно інгібується двухвалентними іонами як перехідних ( $\geq 0,1$  мкМ), так и неперехідних ( $\geq 1$  мкМ) тяжелых металлов в последовательности по эффективности:  $\text{Cu}^{2+} > \text{Fe}^{2+} \geq \text{Cd}^{2+}$  (10 мкМ). Полиамин спермин (0,5–1,0 мМ) – слабкий інгібітор  $\text{Na}^+, \text{K}^+$ -АТРази ГМОК при насыщающих концентрациях ионов и субстрата. Донор оксид азота нітропруssid натрію (1 мМ) проявлял слабкий інгібіруючий ефект на  $\text{Na}^+, \text{K}^+$ -АТРазу только при долговременной инкубации с мембранами.  $\text{Mg}^{2+}$ -АТРаза во всех случаях была значительно более устойчива к действию исследованных агентов. На примере  $\text{Na}^+, \text{K}^+$ -АТРази ГМОК предполагается, что энзим характеризуется биохимическими особенностями, позволяющими выступать потенциальной мишенью и редокс-сенсором, опосредующим патофизиологические механизмы интоксикации тяжелыми металлами и оксидативного повреждения клеток.

**Ключевые слова:** АТР-гідролазы,  $\text{Na}^+, \text{K}^+$ -АТРаза, гладкие мышцы ободочной кишки, тяжелые металлы, спермин, нітропруssid натрію.

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