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ФІЗІОЛОГІЯ ЛЮДИНИ І ТВАРИН

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ATPase ACTIVITY OF RAT HEPATOCYTES MEMBRANE UNDER THE INFLUENCE OF NICOTINIC ACID ADENINE DINUCLEOTIDE PHOSPHATE

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This paper studied the effect of nicotinic acid adenine dinucleotide phosphate (NAADP) on rat hepatocytes membranes ATPase activity. We found a statistically significant reduction of specific Na^+/K^+-ATPase activity of microsomal fraction by (63.56±11.59)%, (p=0.03; n=8) at presence of NAADP. Also, it was found that NAADP had reduced "basal" Mg^{2+}-ATPase activity of rat hepatocytes membranes by (66.08±7.04)%, (p=0.01, n=10). However, it was observed that the specific Ca^{2+}-ATPase activity of microsomal fraction under the actions of NAADP have been increased by (70.83±5.37)% (p=0.025; n=9). We calculated that NAADP increased SERCA activity by (164.30±39.65)% (p=0.043, n=5), but increasing of PMCA was not statistically authentic. It was speculated that NAADP-released calcium is actively transported by SERCA that require close localization of endolysosomal membranes and EPR in membranes of rat hepatocytes.

Keywords: NAADP, ATPase activity, hepatocytes, microsomal fraction.

It is known that nicotinic acid adenine dinucleotide phosphate (NAADP) is Ca^{2+}-mobilizing messenger, so it evokes Ca^{2+} signals that is involved in regulation of different cell functions [13]. Thus the nature of NAADP receptor and their localization inside the cell, as well as mechanism of NAADP-sensitive Ca^{2+}-releasing is the object of intensive studding [see reviews 7, 21, 23]. It was shown that NAADP mobilizes Ca^{2+} from the endolysosomal system [7, 21, 23]. Two-pore channels (TPCs) appear to be exclusively localized to the endolysosomal system. Recent studies into the mechanisms of action of NAADP have demonstrated that TPCs are components of the NAADP receptor [12]. Despite a large number of evidences to support TPCs as NAADP-sensitive Ca^{2+}-channels, recently was published the reports postulating that TPCs are in fact sodium-selective channels activated by PI(3,5)P(2) and are not activated by NAADP [28]. Besides, TPCs make up an ion channel family that couples the cell's metabolic state to endolysosomal function and are crucial for physical endurance during food restriction [8]. In additions to TPCs, it also was proposed others channels transient receptor potential-mucolipin-1 (TRP-ML1) [29] and transient potential receptor melastatin-2 (TRPM2) [26], which are localized in lysosomal membranes, and ryanodine receptors (RyRs) of endoplasmatic reticulum [17, 24].

We found that activation of RyRs in microsomal fraction had increased the activation of basal Mg^{2+}-ATPase and Na^+/K^+-ATPase [4]. We supposed that activation of RyRs may be caused by increasing of calcium content inside acidic store due to close localization between membranes of endoplasmatic reticulum and endolysosomal systems [4].

Thus the aim of this study was to found out the influence of NAADP on ATPases activity of liver microsomal fraction. It allows us to understand the localization of NAADP-sensitive Ca^{2+}-channels and their correlation to different ATPases.

Materials and methods

Isolation of a microsomal membrane fraction. All experiments were conducted on male and female of wild type rats (0,18–0,2 kg). All work with animals was in accordance with the "International Convention for working with animals". After ether anesthesia the rats were decapitated.

Isolated rat liver was perfused briefly with homogenizing medium (buffer solution) containing (mmol/L): sucrose – 250.0; EDTA – 1.0; Tris-HCl – 10.0 (pH 7.4 t=4°C). Chilled tissue was then crushed by passing through the press. Next, buffer solution was added to the minced liver. Minced tissue was added buffer solution (in a ratio of 1:8) and tissue was homogenized with a Potter-Elvehjam teflon-glass homogenizer at a speed of 300 rev / min.

Isolation of a microsomal membrane fraction has been widely used for different tissues [5, 15] and is a common procedure conducted in studies of membrane proteins, as described by S. Fleischer and M. Kervina [11]. Microsomes can be concentrated and separated from other cellular debris by differential centrifugation. Unbroken cells, nuclei, and mitochondria sediment out at 10.000 g, whereas soluble enzymes and fragmented ER, PM, remain in solution. After precipitation of nuclei and mitochondria, supernatant, containing mainly plasmatic and reticular microsomes, was divided into aliquots and used for experiments or stored at -20°C.

Measurement of the ATPase activity. Microsomes is a valuable tool for investigating of ATPase activity, determined by orthophosphate content released after ATP hydrolysis. At the beginning of the experiment aliquots of membrane vesicles were allowed to thaw and transferred to a standard incubation medium which contained (mmol/L): NaCI – 50.0; KCI – 100.0; Tris-HCI – 20.0; $MgCI_2$ – 3.0; $CaCI_2$ – 0.01; ATP – 3.0; pH 7.4 at 37°C.

The reaction was started by adding 3mM ATP and incubating samples for 15 min at 37°C at moderate shaking in a water bath. Before the end of incubation 0.4 ml of medium was selected for the determination of protein content by the method of Lowry [18]. Reaction was stopped by adding 5 ml of 10% trichloroacetic acid to samples and incubating them for 10 min followed by 10 min centrifugation at 1600 g. Supernatant obtained was used to determine the content of inorganic phosphorus by the spectrophotometric method of Fiske-Subbarow.

The total ATPase activity of microsomal fraction was calculated by the difference of inorganic phosphorus in the media with different composition (with Baf1A or NAADP) expressed as micromoles of inorganic phosphorus equivalent to 1 mg of protein per 1 h. Specific Na^+/K^+-ATPase activity was calculated as difference of inorganic phosphorus content in medium with or without ouabain (1 mmol/L). For the determination of Ca^{2+}/Mg^{2+}-ATPase activity, we quantified the difference between the total Ca^{2+}/Mg^{2+}- and Na^+/K^+-ATPase activity. Thapsigargin was used to calculate SERCA contribution into the total Ca^{2+}/Mg^{2+}-ATPase activity. Specific basal Mg^{2+}-ATPase activity was determined in incubation medium that contained 1 mmol/L EGTA and lacked ouabain. In all experiments, as a control for the absence of enzymatic ATP hydrolysis was incubation medium with no added microsomes to it.

Data analysis. For comparison of two groups was used Mann-Whitney test. Significance level of a test was considered under $p<0.05$ [3].

Results

The specific enzymatic activity of Na^+/K^+-ATPase of liver microsomal fractions under the presence of NAADP. The microsomal fraction is in fact a mixture of Ca^{2+} stores deriving from endolysosomes, endoplasmatic reticulum (ER) and plasmatic membranes (PM) contains IP3Rs, RyRs an NAADP receptors. This type of fusion of the different intracellular Ca^{2+} stores is an artifact of the preparation process itself [19]. It is important to note that The Na^+/K^+-ATPase is an electrogenic pump that is abundant in intracellular acidic vesicles where they profoundly affect pH_L [21]. In the organellar membrane, they reinforce a lumen-positive membrane potential and therefore will be inhibitory to H^+-pumping. It has been woven into the fabric of pH_L regulation across the endosomal spectrum [10]. Presumably, functional Na^+/K^+-ATPases are retained within the plasma membrane/endosomal axis as a result of endosome recycling [21]. Organelles rich in both H^+ and Ca^{2+} are called "acidic Ca^{2+} stores". These are lysosomes and lysosome-related

organelles, endosomes and secretory vesicles, present in certain secretory cell, and types Golgi complex [22]. Endosomes are formed during invagination of the plasma membrane and thus will incorporate extracelluar Ca^{2+} which is present at 1 mM. Ca^{2+} content of endosomal compartments is ~40 mkM. Lysosomes are also acidic organelles (pH ~4.8) that house a variety of hydrolytic enzymes. The lysosomal luminal Ca^{2+} concentration was determined about ~500 mkM. Thus, lysosomes and probably endosomes too are significant stores of Ca^{2+} [22]. The acid stores do not possess thapsigargin-sensitive Ca^{2+}-pumps, but have a Ca^{2+} uptake mechanism that depends on a bafilomycin-sensitive vacuolar H+ pump [14]. It is therefore likely that Ca^{2+} as well as Na^{+} accumulation into the acid store occurs through a Ca^{2+}/H^{+}-exchange mechanism and Na^{+}/H^{+}-exchangers, which utilize the H^{+} gradient to load the endolysosomes with Na^{+} and Ca^{2+} [16]. The acid Ca^{2+} store can release Ca^{2+} in response to NAADP.

We suppose that NAADP-induced Ca^{2+}-releasing could also change activity of Na^{+}/K^{+}-ATPases. We used NAADP in subthreshold concentrations 7 mkM. This concentration is suitable to experiment with microsomes fraction [14]. But in experiments in isolated cells the nanomolar concentrations of NAADP have routinely been found to be effective [24]. It was shown that NAADP at 0.01–1.0 μM significantly increased the openings of this reconstituted lysosomal channel from TRP-ML1$^{+/+}$ cells. However, NAADP at 100 μM could not produce more increase in channel activity compared with that at 1 μM [29]. We planned to get maximal activation of all population of NAADP-sensitive channels, especially TRP-ML1 and TRP-ML2.

It was established (Fig. 1), that specific Na+/K+-ATPase activity of rat liver microsomes was equal (1.85±0.59) mmol P/mg of protein for 1 hour, which made (21.07±5.95)% of common ATPase activity under NAADP presence. As it was established previously [4] in control specific enzymatic activity of Na^{+}/K^{+}-ATPase of liver microsomes made (3.67±0.73) mmol P/mg of protein for 1 hour, which also formed about (21.92±5.95)% of common ATPase activity.

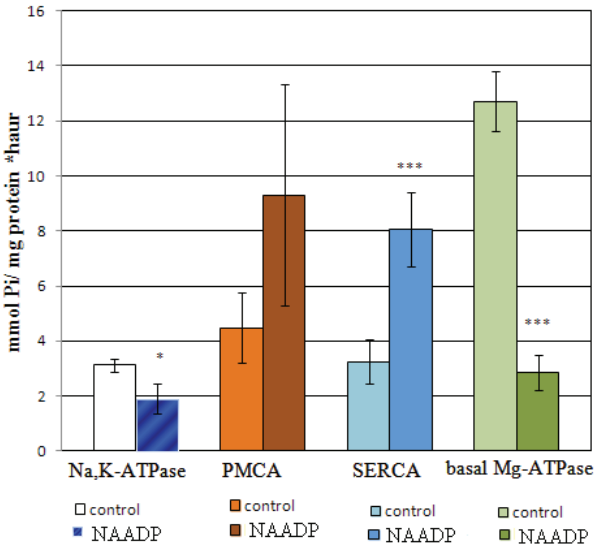

Fig. 1. Influence of NAADP on ATPase activity of liver microsomal fraction: * – p=0.03; n=8; *** – p=0.01, n=10.

So we found that NAADP had reduced the specific Na^{+}, K^{+}-ATPase activity of rat liver microsomal fraction by (63.56±11.59)%, (p=0.03; n=8). A possible explanation for this reduction of specific Na^{+}/K^{+}-ATPase activity by NAADP may be NAADP caused Na^{2+} current, which was

shown recently [23]. Besides, we found previously that activation of RyRs have been increased activity Na^+/K^+-pump [4]. Thus we speculated that RyRs and NAADP-sensitive Ca^{2+}-channels are localized in different Ca^{2+}-store, because of different effects of these channels activation on specific Na^+/K^+-ATPase activity. It is indirect evidence to support that NAADP-sensitive Ca^{2+}-channels are resident in membranes of endolysosomal acidic store while RyRs are normally localized in EPR.

The specific enzymatic activity of Ca^{2+}-ATPase of liver microsomal fractions under the presence of NAADP. We found that common activity of Ca^{2+}-pumps was equal (3.28±0.65) mmol P/mg of protein for 1 hour, which have been made (37.72±6.15)% of common ATPase activity with NAADP presence. In control specific enzymatic activity of rat liver microsomes Ca^{2+}-pumps made (1.13±0.24) mmol P/mg of protein for 1 hour, which formed only (6.03±1.76)% of common ATPase activity [4]. So we observed that NAADP increased activity of Ca^{2+}-pumps by (70.83±5.37)% (p=0.025; n=9) (Fig. 1). Probably NAADP-induced Ca^{2+}-releasing make a local sites of high calcium concentration, which activated Ca^{2+}-ATPases to transporte Ca^{2+}. Common Ca^{2+}-ATPase activity consists of activity Ca^{2+}-pump of EPR (SERCA) and Ca^{2+}-pump of PM (PMCA). We calculated that NAADP increased SERCA activity by (164.30±39.65)% (p=0.043, n=5), but increasing of PMCA was not statistically authentic. It may be explained by a close apposition between NAADP-sensitive Ca^{2+}-channels and SERCA, thus in microsomal fraction these proteins are also co localized. It is possible when membranes of endolysosomal systems and EPR have close contact. These membrane contact sites between acidic organelles and the ER were established [9, 25, 27]. For example, in mammalian cells the membrane contact sites between the ER and late endosomes have been described [25]. In this case the junction is formed by interaction between Rab7 and RILP (Rab-interacting lysosomal protein) on the late endosome and VAP [VAMP (vesicle-associated membrane protein)- associated ER protein] (on the ER membrane) through ORP1L (oxysterol-binding protein-related protein 1, long form) [25]. These would be akin to junctions between the PM and SPR formed by junctophilin in the dyadic and triadic junctions of muscle cells which are critical for excitation–contraction coupling [27]. So we speculated that these contact sites between acidic organelles and the ER exists in rat liver cells, which is unbroken in microsomal fraction.

The specific enzymatic "basal" Mg^{2+}-ATPase activity of liver microsomal fractions under the presence of NAADP. Mg^{2+}-dependent-ATPase activity was identified in PM of different cells type [2]. Besides this ATPase activity may be related to proton pump [6], so the "basal" Mg^{2+}-ATPase activity is also correlated to acidic store function. Recently was shown that Mg^{2+}_{cyt} specifically inhibits the outward current (cations flowing from the cytosol into the lysosomes) with an apparent affinity within the physiological Mg^{2+} concentration [16]. Hence, TPC2 functions as Mg^{2+}_{cyt} sensor, with Mg^{2+} cyt determining the lysosomal membrane potential [16]. Changes in Mg^{2+}cyt that are observed with receptor stimulation and with changes in cytoplasmic ATP and cell energetics are thus transmitted to the lysosome, through rapid acute changes in Mg^{2+}_{cyt} [16]. As we found before [4] in control specific enzymatic activity of "basal" Mg^{2+}-ATPase of liver microsomes made (12.76±2.13) mmol P/mg of protein for 1 hour, which formed (79.90±8.02)% of common ATPase activity. We observed that the specific enzymatic activity of Mg^{2+}-ATPase made (3.35±0.67) mmol P/mg of protein for 1 hour under NAADP presence in incubation medium of rat liver microsomes, which formed only (44.20±7.05)% of common ATPase activity under NAADP presence. It was less than control by (66.08±7.04)%, (p=0.01, n=10) (Fig. 1). These results are opposite to obtained early effect of ryanodine [4] and inositol-1,4,5-trisphosphate [1] on activity of Mg^{2+}-pump. It is also indirect evidence to support that NAADP-sensitive Ca^{2+}-channels are corresponding to other Ca^{2+} store, not to EPR. The alkalinization causing with NAADP in egg homogenate [20] may be the reason, which makes direct effect on H^+-pump and indirect

changing the activity of Mg^{2+}-ATPase. Other explanation may be negative feedback between NAADP-caused Ca^{2+}-releasing and activity of Mg^{2+}-ATPase due to Mg^{2+}_{cyt}.

So we established that NAADP caused inhibition of Na^+/K^+-ATPase activity and "basal" Mg^{2+}-ATPase activity of rat liver membranes while activity of the SERCA was increased.

REFERENCES

1. *Бичкова С., Чорна Т.* Вплив $ІФ_3$ на АТФ-азну активність мембранних везикул гепатоцитів щурів після перфузування печінки інсуліновим розчином // Біологічні Студії / Studia Biologica. 2012. Т. 6. №1. С. 47–54.
2. *Костерін С. О., Векліч Т. О., Прилуцький Ю. І., Бориско П. О.* Кінетичне тлумачення pH-залежності ферментативної активності «базальної» Mg^{2+}-АТФ-ази сарколеми гладенького м'яза // Укр. біохім. журнал. 2005. Т. 77. № 6. С 37–45.
3. *Лапач С. Н., Губенко А. В., Бабич П. Н.* Статистические методы в методико-биологических исследованиях с использованием Exel. К.: Морион, 2000. 320с.
4. *Чорна Т., Бичкова С.* Вплив ріанодину на АТФ-азну активність мембран гепатоцитів щурів після перфузування печінки інсуліновим розчином // Вісн. Львів. ун -ту. Сер. біол. 2011. Вип. 55. С. 170–178.
5. *Báez-Ruiz A., Cázares-Gómez K., Vázquez-Martínez O.* et al. Diurnal and nutritional adjustments of intracellular Ca^{2+} release channels and Ca^{2+} ATPases associated with restricted feeding schedules in the rat liver // J. Circadian. Rhythms. 2013. Vol. 11. N 8. P. 1–17.
6. *Bhatnagar V., Ramalah A.* Characterization of Mg^{2+}-ATPase activity in isolated B16 murinemelanoma melanosomes // Mol. Cell Biochem. 1998. Vol. 189. P. 99–106.
7. *Bychkova S.* Ca^{2+} releasing process and nicotinic acid adenine dinucleotide phosphate // Біологічні Студії / Studia Biologica. 2013. Т. 7. № 2. С. 195–206.
8. *Chunlei C., Zhou Y., Navarro B.* et al. mTOR Regulates Lysosomal ATP-Sensitive Two-Pore Na^+ Channels to Adapt to Metabolic State // Cell. 2013. Vol. 152. N 4. P.778–790.
9. *Eden E. R., White I. J., Tsapara A., Futter C. E.* Membrane contacts between endosomes and ER provide sites for PTP1B-epidermal growth factor receptor interaction // Nat. Cell. Biol. 2010. Vol. 12. N 3. P. 267–272.
10. *Feldmann T., Glukmann V., Medvenev E.* Role of endosomal Na^+-K^+-ATPase and cardiac steroids in the regulation of endocytosis // Am. J. Physiol. Cell Physiol. 2007. Vol. 293. P. 885–896.
11. *Fleischer S, Kervina M.* Subcellular fractionation of rat liver // Methods Enzymol. 1974. Vol. 31. P. 6–41.
12. *Galione A., Morgan A. J., Arredouani A.* et al. NAADP as an intracellular messenger regulating lysosomal calcium-release channels // Biochem. Soc. Trans. 2010. Vol. 38. P. 1424–1431.
13. *Galione A., Parrington J., Funnell T.* Physiological roles of NAADP-mediated Ca^{2+} signaling // Sci. China. Life. Sci. 2011. Vol. 54. N 8. P. 725–732.
14. *Huss M., Wieczorek H.* Inhibitors of V-ATPases: old and new players // J. Exp. Biol. 2009. Vol. 212. (Pt 3). P. 341–346.
15. *Janicki P. K., Wise P. E., Belous A. E., Pinson C. W.* Interspecies differences in hepatic Ca^{2+}-ATPase activity and the effect of cold preservation on porcine liver Ca^{2+}-ATPase function // Liver Transpl. 2001. Vol. 7. P. 132–139.
16. *Jha A., Brailoiu E., Muallem S.* How does NAADP release lysosomal Ca^{2+}? // Channels. 2014. Vol. 8. N 3. P. 174-175.
17. *Kinnear N. P., Wyatt C. N., Clark J. H.* et al. Lysosomes co-localize with ryanodine receptor subtype 3 to form a trigger zone for calcium signalling by NAADP in rat pulmonary arterial

smooth muscle // Cell. Calcium. 2008. Vol. 44. N 2. P. 190–201.
18. *Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J.* Protein measurement with the folin phenol reagent // J. Biol. Chem. 1951. Vol. 193. P. 265–275.
19. *Mandi M., Toth B., Timar G.* et al. Ca^{2+} release triggered by NAADP in hepatocyte microsomes // Biochem. J. 2006. Vol. 395. N 2. P. 233–238.
20. *Morgan A. J., Davis L. C., Wagner S. K.* et al. Bidirectional Ca^{2+} signaling occurs between the endoplasmic reticulum and acidic organelles // J. Cell. Biol. 2013. Vol. 18. N 200(6). P. 789–805.
21. *Morgan A. J., Platt F. M., Lloyd-Evans E.* et al. Molecular mechanisms of endolysosomal Ca2+ signalling in health and disease // Biochem. J. 2011. Vol. 439. P. 349–374.
22. *Patel S., Docampo R.* Acidic calcium stores open for business: expanding the potential for intracellular Ca^{2+} signaling // Trends. Cell. Biol. 2010. Vol. 20. N 5. P. 277–286.
23. *Patel S., Marchant J. S., Brailoiu E.* Two-pore channels: Regulation by NAADP and customized roles in triggering calcium signals // Cell Calcium. 2010. Vol. 47. N 6. P. 480–90.
24. *Petersen O. H., Gerasimenko O. V., Tepikin A. V.* et al. Aberrant C^{a2+} signalling through acidic calcium stores in pancreatic acinar cells // Cell Calcium. 2011. Vol. 50. Iss. 2. P. 193–199.
25. *Rocha N., Kuijl C., van der Kant R.* et al. Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7–RILP–p150 Glued and late endosome processing // J. Cell Biol. 2009. Vol. 185. P. 1209–1225.
26. *Sumoza-Toledo A., Penner R.* TRPM2: a multifunctional ion channel for calcium signaling // J. Physiol. 2011. Vol. 589. N (Pt 7). P. 1515–1525.
27. *Takeshima H., Komazaki S., Nishi M.* et al. Junctophilins: a novel family of junctional membrane complex proteins // Mol. Cell. 2000. Vol. 6. P. 11–22.
28. *Wang X., Zhang X., Dong X. P.* et al. TPC proteins are phosphoinositide- activated sodium-selective ion channels in endosomes and lysosomes // Cell. 2012. Vol. 151. N 2. P. 372–383.
29. *Zhang F., Xu M., Han W. Q., Li P. L.* Reconstitution of lysosomal NAADP-TRP-ML1 signaling pathway and its function in TRP-ML1(-/-) cells // Am. J. Physiol. Cell. Physiol. 2011. Vol. 301. N 2. P: C421–30.

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АТФ-АЗНА АКТИВНІСТЬ МЕМБРАН ГЕПАТОЦИТІВ ЩУРІВ ЗА ДІЇ НІКОТИНА ЦИДАДЕНІНДИНУКЛЕОТИДФОСФАТУ

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У статті вивчали вплив нікотинацидаденіндинуклеотидфосфату (НААДФ) на АТФ-азну активність мембран гепатоцитів щурів. Нами виявлено статистично достовірне зменшення питомої Na^+, K^+-АТФ-азної активності мікросомальної фракції на (63,56±11,59)% (p=0,03; n=8) за дії НААДФ. Також встановлено, що НААДФ знижує базальну Mg^{2+}-АТФ-азну активність мембран гепатоцитів щурів на (66,08±7,04)% (p=0,01, n=10) щодо контролю. Однак показано, що питома Ca^{2+}-АТФ-азна активність мікросомальної фракції за дії НААДФ зростає на (70,83±5,37)% (p=0.025; n=9). При

цьому виявилось, що активність Ca^{2+}-помпи ЕПР (SERCA) за дії НААДФ зросла на (164,30±39,65)% (p=0,043, n=5), тоді як активування Ca^{2+}-помпи ПМ (PMCA) не виявилось статистично достовірним. Висловлено припущення, що вивільнений у процесі дії НААДФ кальцій активно транспортується SERCA, а це передбачає тісну локалізацію мембран ендо-лізосомального депо та ЕПР у гепатоцитах щурів.

Ключові слова: НААДФ, АТФ-азна активність, гепатоцити, мікросомальна фракція.

АТФ-АЗНАЯ АКТИВНОСТЬ МЕМБРАН ГЕПАТОЦИТОВ КРЫС ПРИ ДЕЙСТВИИ НИКОТИНАЦИДАДЕНИНДИНУКЛЕОТИДФОСФАТА

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В статье изучали влияние никотинацидадениндинуклеотидфосфата (НААДФ) на АТФ-азную активность мембран гепатоцитов крыс. Нами показано статистически достоверное уменьшение удельной Na^+, K^+-АТФ-азной активности микросомальной фракции на (63,56±11,59)% (p=0,03; n=8) под действием НААДФ. Также установлено, что НААДФ снижает базальную Mg^{2+}-АТФ-азную активность мембран гепатоцитов крыс на (66,08±7,04)% (p=0,01, n=10) относительно контроля. Однако показано, что удельная Ca^{2+}-АТФ-азная активность микросомальной фракции под действием НААДФ увеличивается на (70,83±5,37)% (p=0,025; n=9). Установлено, что активность Ca^{2+}-насоса ЭПР (SERCA) под действием НААДФ возрастала на (164,30±39,65)% (p=0,043, n=5), в то время как активация Ca^{2+}-насоса ПМ (PMCA) не была статистически значимой. Высказано предположение, что освободившийся в процессе действия НААДФ кальций активно транспортируется SERCA, а это предусматривает тесную локализацию мембран эндо-лизосомальной системы и ЭПР в гепатоцитах крыс.

Ключевые слова: НААДФ, АТФ-азная активность, гепатоциты, микросомальная фракция.