Հայաստանի Գիտությունների Ազգային Ակադեմիա
Национальная Академия Наук Армении
National Academy of Sciences of Armenia

Հայաստանի Կենսաբանական Հանդես
Биологический Журнал Армении
Biological Journal of Armenia

• Փորձարարական և տեսական հոդվածներ • Экспериментальные и теоретические статьи • • Experimental and theoretical articles •

Biolog. Journal of Armenia, 4 (72), 2020

CHANGES OF ATPASE ACTIVITY AND LIPID PEROXIDATION IN CONDITIONS OF LONG-TERM HYPOKINESIA

A.A. SHIRVANYAN, H.M. KARAPETYAN, A.H. TRCHOUNIAN

Department of Biochemistry, Microbiology and Biotechnology, Faculty of Biology, Yerevan State University
anahit.shirvanyan@ysumail.am, hkarapetyan@ysu.am, Trchounian@ysu.am

The aim of this work was to investigate the dynamics changes in mitochondrial ATPase's activity and in lipid peroxidation in conditions of 30-day hypokinesia. It was shown that maximum lipid peroxidation level was observed in the kidneys and brain in the 20th day of stress, exceeding the norm by 20.87 % and 75.2 %, respectively. The maximum activity of Mg^{2+}-dependent proton F_0F_1-ATPase of mitochondria was promoted in the liver of rabbit after 20-day of stressful effect by 48.8 % compared to unstressed animals. Thus, the analysis of the nature of changes in the lipid peroxidation processes and in the activity of mitochondrial ATPase can be the basis for identifying pathological processes caused by hypokinesia and for creating various means to prevent them.

Hypokinesia – malonic dialdehyde – lipid peroxidation – the F_0F_1-ATPase

30-օրյա հիպոկինեզիայի պայմաններում ուսումնասիրվել է միտոքոնդրիումային ԱԵՖազի ակտիվության և լիպիդների գերօքսիդացման գործընթացների փոփոխությունների օրինաչափությունների բնույթը: Ցույց է տրվել, որ լիպիդների գերօքսիդացման առավելագույն մակարդակ դիտվում է ճագարի երիկամներում և ուղեղում սթրեսի 20-օրյա ազդեցության դեպքում՝ նորման գերազանցելով համապատասխանաբար 20.87 % և 75.2 %-ով: Միտոքոնդրիումային Mg^{2+}-կախյալ պրոտոնային F_0F_1-ԱԵՖազի ակտիվության առավելագույն խթանում բնականոն պայման-ների համեմատությամբ դիտվում է սթրեսի ազդեցության 20-րդ օրը ճագարի լյարդում՝48.8 %-ով:

Լիպիդների գերօքսիդացման գործընթացների և միտոքոնդրիումային ԱԵՖազի ակտիվության փոփոխությունների բնույթի վերլուծությունը կարող է հիմք հանդիսանալ հիպոկինեզիայով պայմանավորված ախտաբանական գործընթացների բացահայտման և դրանց կանխարգելման ուղղությամբ տարաբնույթ միջոցների ստեղծման համար:

Սակավաշարժություն – մալոնային երկալդեհիդ – լիպիդների գերօքսիդացում – F_0F_1-ԱԵՖազ

Изучен характер изменений процессов перекисного окисления липидов и активности митохондриальной АТФазы в условиях 30-дневной гипокинезии. Показано, что максимальный уровень перекисного окисления липидов наблюдается в почках и в мозге при 20-дневном стрессе, превышая норму на 20,87 % и 75,2 % соответственно. Максимальная стимуляция активности Mg^{2+}-зависимой митохондриальной F_0F_1-АТФазы по сравнению с нормой наблюдается в печени кролика на 48,8 %. Таким образом, анализ характера изменений процессов перекисного окисления липидов и активности митохондриальной АТФазы может служит основой для выявления патологических процессов, обусловленных гипокинезией, и создания в дальнейшем различных предотвращающих средств.

Гипокинезия – малоновый диальдегид – перекисное окисление липидов – F_0F_1-АТФаза

For organisms normal functioning, motoric activity is required, which enhances the production of energy, supplying the organism with oxygen, has a beneficial effect on the normal course of the physiological functions. Deficient motor activity causes stress reactions. Stress is a biochemical, physiological and mental specific reaction of body to the effects of various factors that cause tension in functions and ensure the mobilization and homeostasis of the body for adaptation to the different conditions [11]. Currently, one of the urgent tasks of medical physiology is the study of tissues and organs responses to these conditions, including changes in protein's, carbohydrate's and lipid's metabolism.

The fastest response to various stressful conditions is the activation of lipid peroxidation, the main substrates of which are lipoproteins and polyunsaturated fatty acids that are part of the cell membrane. In result of this the modification and inactivation of membrane enzymes as well as the increasing of membrane's ion-penetration and disturbance of the lipid bilayer stability can happen [5].

During stress the body, at first, strives to provide the tissues, especially nervous, with energy, so that is why researchers are interested in studying the body energy-ensure substrates and systems. In aerobic conditions, the main metabolic pathway of ATP synthesis is the oxidative phosphorylation, the final reaction of which catalyzes by the F_0F_1-ATP synthase (3.6.1.4). It is one of the most common cell membrane enzymes, widely detected in the biological world, including microbe cell membranes, the inner membrane of the mitochondria in animals and in the thylakoids of chloroplasts in plants. Enzyme catalyzes the synthesis of the ATP from adenosine diphosphate (ADP) and inorganic phosphorus using the electrochemical potential of membrane. When the electrochemical potential is insufficient, the enzyme may act in the opposite direction: to hydrolize the ATP and transfer the proton through the membrane by the released energy [2].

Different diseases may bring structural and functional changes of the F_0F_1-ATP synthase. [4,8,10,12,13,15,16]. In the available literature studied, we did not meet discussions about the effect of prolonged hypokinesia on rabbits. Prolonged hypokinesia causes a range of violations affecting almost all body systems. In the medical literature resulting changes are defined as "hypokinetic disease" [14].

Therefore of this work was to investigate the dynamics changes of mitochondrial ATPase activity in organs and lipid peroxidation under conditions of 30-day hypokinesia.

Materials and methods. The experimental protocol was made according to the norms established by the National Committee of Bioethics (Armenia), as well as the recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (Strasbourg, 2 December 2005). The experimental animals had the same gender (male) and weight (2.5 kg) *Oryctolagus cuniculus domesticus* rabbits, which were in the same conditions of nutrition and care during experiments. For limitation of mobility they have been kept in the experimental boxes during 22 h daily, for 30 days. After 10, 20 and 30 days of stress influence the animals were decapitated and the malonic dialdehyde (lipid peroxidation product) content and ATPase activity were determined in the liver, kidneys, heart and brain.

Malonic dialdehyde amount was determined by the spectrophotometric method [9].

ATPase activity was determined by increasing of inorganic phosphorus in the incubation medium by the method of Taussky and Shorr using a spectrophotometer Genesis 10s UV-VIS, as described [1]. Mitochondrias were isolated by the method [3]. Isolated mitochondria's were treated with the inhibitor 0.2 mM *N,N'*-dicyclohexylcarbodiimide for 10 min, which almost completely inhibited ATPase activity, thus confirming the presence of the F_0F_1-ATP synthase [1].

Protein content determination was made by the method of Lowry [1].

The obtained data were subjected to statistical analysis with the computer program "BIOSTAT". Trust was determined by the ''t'' criterion of Student.

Results and Discussion. One of the risk factors for development of pathological processes in the body is hypokinesia, which suppresses the adaptive capabilities of organism and changes it‘s responses to various extreme factors. The adaptation of organism largely depends from energy metabolism and synthesis of macroergic compounds.

In stress conditions as energetic substrates fatty acids and lipids can also be used, including the polyunsaturated fatty acids contained in the membranes, so that is why in stress conditions it is particularly important to study the lipid peroxidation. Taking this into account, the aim of this work was to study the nature of changes in the amount of the final product of lipid peroxidation-molonedialdehyde (MDA) during hypokinesia. Obtained data are presented in fig. 1.

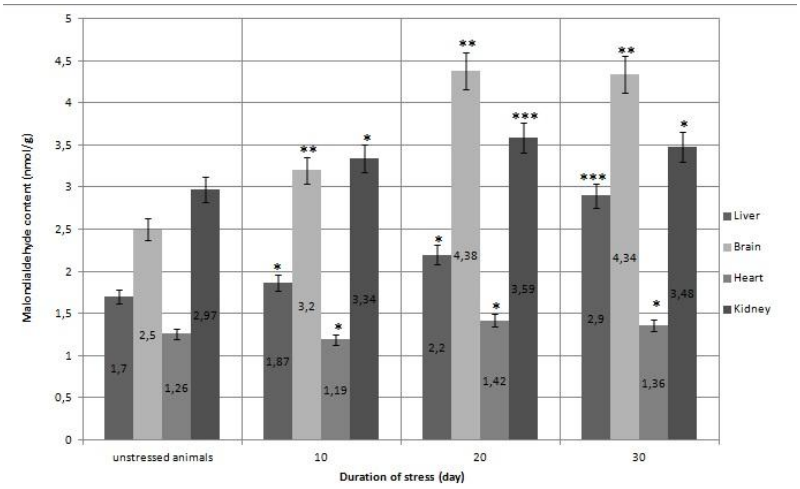

Fig.1. Malonic dialdehyde content (nmol/g) in the various organs of rabbits in conditions of prolonged hypokinesia (n=3, *p<0,05, **p<0,01, ***p<0,001 -is for difference between the data of the serie and those of unstressed animals)

Studies have shown that lipid peroxidation in the liver is gradually activated with an increase of stress duration: in the 10^{th}, 20^{th} and 30^{th} days of hypokinesia it exceeds the norm by 10 %, 29.4 % and 70.58 % respectively. Significant changes in lipid peroxidation in the heart were not observed, only after 20-day stressful effect it increased by 12.7 % compared with the norm. The maximum lipid peroxidation level was observed in the kidneys and brain in the 20^{th} day of stress, exceeding the norm by 20.87 % and 75.2 %, respectively. Further increase of stressful effect led to decrease of malondialdehyde content, which probably confirms the emergence of adaptation mechanisms, which was also shown in literature [7]. Hypokinesia causes oxidative stress - the inbalance between processes of lipid peroxidation and antioxidant system in the body, and thus becomes the cause of the formation of various structural and functional disorders in different organs [15], which is associated with the need of prevention and correction of stress reactions [11].

For implementation a wide range of such endergonic reactions, a large amount of ATP is required. This requirement may increase in stressful conditions. Therefore we studied the effect of 30-day hypokinesia in mitochondrial ATP-synthase activity in the liver, heart, kidney, and brain of rabbit. The results are presented in tab. 1.

Table 1. The activity of ATPase (μmol P_{in} /s in g protein) of mitochondria in the various organs of rabbit in condition of long-term hypokinesia

The studied organ	The activity of ATPase			
	Unstressed animals	10-day stress	20-day stress	30-day stress
Liver	6.24± 0.31	8.28± 0.41 $p^*<0.01$	9.29± 0.46 $p<0.05$	5.35± 0.26 $p>0.5$
Brain	1.94± 0.09	2.32± 0.11 $p<0.01$	2.7± 0.13 $p<0.05$	2.83± 0.14 $p<0.05$
Heart	5.46±0.27	6.52±0.32 $p<0.05$	7.75±0.38 $p<0.01$	6.34± 0.31 $p<0.05$
Kidney	4.38±0.21	5.36±0.26 $p<0.05$	7.7±0.38 $p<0.01$	2.14± 0.1 $p<0.01$

*p is for difference between the data of the serie and those of unstressed animals (n=3)

Data analysis showed that in long-term hypokinesia the directed changes in the activity of membrane - bound enzymes in the rabbit organs are different. Thus, after 10 and 20 days of stress in the liver, the Mg^{2+}-dependent F_0F_1-ATPase activity increases by 32.7 % and 48.8 %, respectively, and in the 30^{th} day it decreases by 14.2 % compared with unstressed animals. ATPase activity in the brain gradually increases with increasing duration of stress so that in the 10^{th}, 20^{th} and 30^{th} days it exceeds the norm by 19.6 %, 39.2 % and 45.8 %, respectively. Changes in ATPase activity in the heart and kidneys are similar to changes in the liver. The maximum ATPase activity was observed in the kidneys and heart after 20-day stress effect, respectively, exceeding the normal level by 75.8 % and 41.9 %.

In the studied organs, the activation of ATPase in the 10^{th} and 20^{th} days of stressful effects can probably be explained, on the one hand, by the features of the respiratory chain in different organs and, on the other hand, by not uniformly using of free fatty acids as energy substrates. In the studied organs a similar change in ATPase activity in 20 days of stress was associated with the activation of enzymes phospholipase, lipase and lipid peroxidation due to the limitation of mobility, what in turn changes the lipid circles of membrane enzymes, especially changes in the activity of ATPase. During prolonged hypokinesia observed enzyme activity's reduction in the liver can be explained by a decrease in the protein content in mitochondria [13]. Mounting evidence has revealed that mitochondria become damaged and dysfunctional during prolonged hypokinesia of muscles, and this mitochondrial dysfunction is a causal event in the initiation of muscle-inactivity induced atrophy [6].

However, the specific mechanisms employed by dysfunctional mitochondria that enact disease have yet to be fully elucidated.

During stress, lipid peroxidation processes in rabbit kidney, liver and brain are gradually activated. In long-term hypokinesia, in the mitochondriums of different organs the dynamic changes in the F_0F_1-ATPase activity have stepwise character and are evidence of mitochondrial functional disorders, and hence changes in cells energy exchange.

Analyzing the patterns of lipid peroxidation and the change in activity of the mitochondrial ATPase, we can conclude that enzyme activity's changes are probably due to the reduction of amount of the fatty acids present in the membrane due to lipid peroxidation, which can lead to structural changes in mitochondria. Elucidation of the nature of changes in enzyme activity influenced by various factors can become the basis for the creation of new therapeutic agents for the treatment of diseases caused by reactive oxygen species.

REFERENCES

1. *Blbulyan, S.*, *Trchounian A.* Impact of membrane-associated hydrogenases on the F_OF_1-ATPase in *Escherichia coli* during glycerol and mixed carbon fermentation: ATPase activity and its inhibition by *N,N′*-dicyclohexylcarbodiimide in the mutants lacking hydrogenases. *Archives of Biochemistry and Biophysics*, 2015, 579, 67-72.
2. *Bonora M., Wieckowski M., Chinopoulos C., Kepp O., Kroemer G., Galluzzi L. and Pinton P.* Molecular mechanisms of cell death: central implication of ATP synthase in mitochondrial permeability transition. *Oncogene*, 34, 1475-1486, 2014.
3. *Frezza, C., Cipolat, S., & Scorrano, L.* Organelle isolation: functional mitochondria from mouse liver, muscle and cultured filroblasts. Nature protocols, *2*, 2, 287, 2007.
4. *Gauba E., Guo L. and Du H.* Cyclophilin D promotes brain mitochondrial F_OF_1-ATP synthase dysfunction in aging mice. *Journal of Alzheimer's Disease*, *16*, 1-12, 2016.
5. *Gelpi R.J., Boveris, A., & Poderoso, J.J. Biochemistry of Oxidative Stress*. Springer International Pu., Argentina, p. 9-45, 2017.
6. *Hyatt H., Deminice, R., Yoshihara, T.,& Powers, S.K.* Mitochondrial dysfunction induces muscle atrophy during prolonged inactivity: A review of the causes and effects. *Archives of Biochemistry and Biophysics, 662*, 49-60, 2019.
7. *Juravlyova O. A., Markin A.A., Kuzichkin D.S., Loginov V.I., Zabolotskaya, I.V., & Vostrikova L.V.* Dynamics of oxidation stress markers during long-term antiorthostatic hypokinesia: A retrospective study. *Human Physiology, 42*, 1, p.79-83, 2016.
8. *Kim Y.,Konno H., Sugano Y., Hisabori T.* Redox regulation of rotation of the cyanobacterial F_1-ATPase containing thiol regulation switch. *Journal of. Biological Chemistry*, *286*, 9071-9078, 2011.
9. *Ljubuncic P., Z. Tanne, and A. Bomzon.* Evidence of a systemic phenomenon for oxidative stress in cholestatic liver disease. *Gut., 47*, 5, 710-716, 2000.
10. *Rasmo D., Micelli L., Santeramo A., Signorile A., Lattanzi P., Papa S.* cAMP regulates the functional activity, coupling efficiency and structural organization of mammalian F_OF_1-ATP synthase. Biochimica et Biophysica Acta, 1857, 350-358, 2016.
11. *Sies H., Berndt C. and Jones P.* Oxidative stress. *Annual Review of Biochemistry*, 86, 715-748, 2017.
12. *Tangvarasittichai S.* Oxidative stress, insulin resistance, dyslipidemia and type 2 diabetes mellitus. *World Journal of Diabetes 6*, 3, 456-480, 2015.
13. *Terni B., Boada J., Portero-Otin M., Pamplona R., Ferrer I.* Mitochondrial ATP-synthase in the entorhinal cortex is a target of oxidative stress at stages I/II of Alzheimer's disease pathology. *Brain Pathology, 20*, 222-233, 2010.
14. *Ulugbekova, G., Ubaydullaev, R., Zulfizar, U., Rokhatoy, K., & Diloram, R.* Secretion of enzymes by the salivary glands and homeostasis of enzymes in various ambient temperature. *Journal of Critical Reviews, 13*, 7, 494-497, 2020.
15. *Vasilaki A., Millan D.* Lipid Peroxidation. Encyclopedia of Cancer, L-3373, p. 1968-1991, 2017.
16. *Wang Sh., Foster D., Rucker J., O'Rourke B., Kass D., Van Eyk J.* Redox regulation of mitochondrial ATP synthase; Implications for cardiac resynchronization therapy. *Circulation Research, 109*, 750-757, 2011.

Received on 04.11.2020