УДК 616:57.017

ISCHEMIA EFFECT ON ATPase ACTIVITY OF HEART MITOCHONDRIA IN YOUNG AND OLD RATS

Dzuba V.N., Nikitchenko Yu.V., Shmonina T.A.
Research Institute of Biology of Kharkov National V.N. Karazin University

SUMMARY

ATPase activity alterations in rats of different age groups during the total myocardial ischemia were studied. It was determined that only mitochondrial oligomycin-sensitive ATPase was sensitive to effect of the total myocardial ischemia. The fact that addition of magnesium ions in the incubation medium eliminated ischemic effect in 3-months old rats and weakened it in 24-months old rats pointed to important role of magnesium ions in structure-functional reconstruction of mitochondria under myocardial ischemia. Young 3-months old rats were established to be more tolerant to myocardial ischemic damage than old animals.

KEY WORDS: rats hearts, total myocardial ishemia. ATPase activity, age peculiarities

INTRODUCTION

To understand the pathogenesis of myocardial ischemic injury it's necessary to realize which metabolic changes are the motives of irreversible destruction of cell structures and cardiomyocyte death. One of the main links of cellular metabolism affected by ischemia and hypoxia is energy exchange. However the role of mitochondria and level of high energy compounds in the mechanism of development of irreversible ischemic changes of cells isn't finally ascertained.

Ischemic damage of myocardial and other tissues was established to depend to a large extent on the structural and functional changes of mitochondria [1-3]. There are a lot of studies concerning the investigation of magnesium ions role in the regulation of functions of mitochondria as a whole and ATPase in particular [4, 5]. It's clear from these studies that there are certain currents of Mg^{2+} both inwards and outwards mitochondria depending of mitochondrial functional state. One of the consequences of ischemia is disturbance of cellular ionic homeostasis that in its own turn leads to the change of mitochondrial functional state. The study of ATPase activity in the different medium ($\pm Mg^{2+}$, $\pm$ EDTA) seemed to be expedient for the clearing up the role of Mg^{2+} during myocardial ischemia.

The age aspect of ischemic damage is rather interesting but not finally decided [6 - 8]. The clearing up of the age peculiarities of ischemic damage may have practical importance for the elaboration of practical advices concerning treatment of the ischemia-suffering patients of different age groups.

Thus the main objective of present study was investigation of mitochondrial ATPase activity alterations in rats of different age groups during the total myocardial ischemia.

MATERIALS AND METHODS

ANIMALS. Wistar male-rats 3- and 24-months old were used in this study. All animals had been provided with feed and water ad libitum. During 16 - 18 hours before the experiment they were fasted.

MODEL OF TOTAL MYOCARDIAL ISCHEMIA AND PREPARATION OF HEART HOMOGENATE. After decapitation of rats hearts were rapidly excised and put in the glass moist chamber and incubated at $t = 37^0C$. Duration of ischemia was 30 or 120 min. After ischemic period hearts were immersed for 3 min to ice-cold isolation medium containing 170 mM KCl, 10 mM EDTA, 10 mM tris-HCl and 0,1% fatty-free bovine serum albumin (pH 7,4). Control hearts were rapidly excised and immersed for 3 min in ice-cold isolation medium without ischemic incubation period. After cooling the hearts were homogenized in isolation medium (tissue:medium - 1:3) for 1 min in homogenizer with Teflon pestle rotating at 800 rotations per min. The homogenates were filtered and kept at 0^0C.

MEASUREMENT OF ATPase ACTIVITY. ATPase activity was measured potentiometrically [9]. Incubation medium contained 100 mM sucrose, 75 mM KCl, 3 mM tris-HCl (pH 7,5). The sequence of additions to incubation medium was the following: 20 mcl heart homogenate, 1 mM ATP, 160 mcM 2,4-DNP, 4 mcg oligomycin. ATPase activity determination was carried out in three modes:

1) no other additions beside mentioned above ones;
2) 1 mM Mg^{2+} was added in the incubation medium before addition of homogenate and other reagents;
3) 250 mM EDTA was added in the incuba-

tion medium before the addition of homogenate.

All measurements were performed at t= 37^0C.

PROTEIN CONTENT DETERMINATION. Protein content was determined according to the method of Lowry in Miller's modification [10].

STATISTICAL ANALYSIS. The results are expressed as the mean ± SD. Differences between groups were analyzed by Student's t test. P value < 0,05 was considered statistically significant [11].

RESULTS AND DISCUSSION

The data about alterations of total ATPase activity of heart homogenate in control and after total ischemia of different duration are presented in Table 1. As shown in Table 1 30 min of total ischemia led to the significant increase of total ATPase activity in both age groups and in all three sets of experiments. It may be conditioned by the rise of H^+ conductance of inner mitochondrial membrane during ischemia. Increase of ischemia duration from 30 to 120 min didn't lead to the additional rise of total ATPase activity. It may point to the fact that proton conductance of heart inner mitochondrial membrane was already maximal to 30 min of ischemia and the increase of ischemia duration didn't essentially affect this parameter.

In our study we didn't observed age peculiarities of ischemic effect on the total ATPase activity, it may indicate that proton conductance of inner mitochondrial membrane of young and old rats are of the same values.

Being the cofactor of ATPase magnesium ions are known to be necessary for normal functioning of this enzyme [4, 5]. Therefore in the presence of Mg^{2+} ATPase is functioning more active (Table 1). After addition of EDTA the enzyme activity decreases because of Mg^{2+} deficiency as a result of EDTA-Mg^{2+} - complex formation.

Table 1

The total ATPase activity of rat heart homogenates (nmol P_i /min·mg protein; n = 6 - 9)

Modes of experiment	Duration of ischemia, min		
	0 (control)	30	120
3 months			
-	100.5 ± 10.1	176.3 ± 11.6*	178.3 ± 13.3*
Mg^{2+}	251.6 ± 13.9	323.2 ± 24.5*	340.8 ± 12.2*
EDTA	45.0 ± 1.6	105.1 ± 11.6*	93.3 ± 16.6*
24 months			
-	90.2 ± 6.7	165.3 ± 10.2*	150.5 ± 10.2*
Mg^{2+}	259.2 ± 14.0	331.3 ± 17.7*	324.6 ± 29.4*
EDTA	41.6 ± 2.7	114.9 ± 14.4*	110.2 ± 12.6*

* - P < 0.05 in comparison with control

The addition of uncoupler 2,4-DNP in the incubation medium is known to lead to sharp increase of proton membrane conductance and ATPase activation as a result (Table 2).

It follows from the results presented (Table 2) that in the absence of Mg^{2+} DNP-stimulated ATPase activity in response to ischemia had obvious age peculiarities. There were no changes of this enzyme activity in young (3 months) animals after 30 min of ischemia. In old (24 months) rats the activity showed a tendency to decrease. More prolonged ischemia (120 min) caused statistically significant decrease of DNP-stimulated ATPase activity as compared with control (non-ischemic hearts), this decrease being more expressed in old rats. In old animals the decrease of the enzyme activity was statistically significant even in comparison with the level after 30 min of ischemia.

Table 2

DNP-stimulated ATPase activity of rat heart homogenates (nmol P_i /min·mg protein; n = 5 - 10)

Modes of experiment	Duration of ischemia, min		
	0 (control)	30	120
3 months			
-	259.6 ± 19.7	266.7 ±21.5	191.4 ± 22.4*
Mg^{2+}	285.3 ± 15.0	285.2 ± 19.5	275.1 ± 15.6
EDTA	214.2 ± 18.8	174.4 ± 13.8	141.3 ± 21.6*
24 months			
-	291.7 ± 15.5	244.0 ± 17.1**	174.6

			± 11.5*,**
Mg^{2+}	302.7 ± 16.0	294.1 ± 14.9	252.3 ± 18.1**
EDTA	244.3 ± 11.7	182.6 ± 6.7*	122.7 ± 11.7*,***

* - P < 0,05 in comparison with control;
** - 0,05 < P < 0,1 in comparison with control;
*** - P < 0,05 in comparison with 30 min ischemia

In the presence of Mg^{2+} DNP-stimulated ATPase activity wasn't altered after 30 and 120 min of ischemia in young rats and had only a tendency to decrease after 120 min of ischemia in old rats (Table 2).

Probably Mg2+ efflux from mitochondrial matrix takes place during ischemia [5]. And it leads to lowering of H^+ ATPase activity since the true substrate for this enzyme is Mg^{2+} - ATP complex (Table 2). Adding Mg^{2+} to the medium we supply Mg^{2+} level and in that way normalize the enzyme functioning in 3 months old rats and lower the rate of decrease of DNP-stimulated ATPase activity in 24 months old animals. Inhibition of the enzyme activity in old rats after 120 min of ischemia is most likely connected with the irreversible ischemic damage of membranes. In the medium with EDTA DNP-stimulated ATPase activity was reduced. In this case age peculiarities were clearly expressed. The enzyme activity was reduced in 1,2 and 1,5 times in comparison with control group after 30 and 120 min of ischemia in young rats, respectively, and in 1,3 and 2,0 times in old rats, respectively. The decrease of this activity in old rats was already statistically significant after 30 min of ischemia (Table 2). So far the study was carried out on the system of homogenate where other ATPases, not only H^+ - ATPase, function it's important to elucidate if the observed alterations were connected with mitochondrial ATPase. With the purpose of elucidating this question oligomycin, the specific inhibitor of mitochondrial ATPase, was added in the incubation medium. In such a case mitochondrial ATPase was inhibited and only oligomycin-resistant activity was remained. It follows from results presented (Table 3) there were no age peculiarities of this activity. The value of this activity was lesser than total DNP-stimulated activity value (Table 2). This fact gives evidence that extramito-chondrial oligomycin-resistant ATPase is a very little part of total ATPase activity. We didn't observe ischemic effect on the level of oligomycin-resistent ATPase activity in both sets of experiments (-Mg^{2+} and +Mg^{2+}, Table 3). It indicated that extramitochondrial DNP-stimu-lated oligomycin-resistant ATPase practically wasn't sensitive to ischemia.

Table 3

Oligomycin-resistant ATPase activity of rat heart homogenates (nmol P_i /min·mg protein; n = 5 - 10)

Modes of experiment	Duration of ischemia, min		
	0 (control)	30	120
3 months			
-	56.2 ± 4.7	64.2 ± 2.7	63.2 ± 6.3
Mg^{2+}	123.9 ± 8.0	129.7 ± 10.4	127.1 ± 5.5
24 months			
-	63.9 ± 1.7	63.9 ± 3.6	57.8 ± 4.8
Mg^{2+}	131.3 ± 14.2	138.5 ± 13.3	132.1 ± 8.2

The difference of total DNP-stimulated ATPase activity and oligomycin-resistant ATPase one is DNP-stimulated oligomycin-sensitive ATPase activity (Table 4). This parameter reflects the state of especially mitochondrial ATPase. In course of comparison of absolute values of activity of oligomycin-resistant ATPase (Table 3) and mitochondrial DNP-stimulated oligomycin-sensitive ATPase (Table 4) one can see that activity of the last enzyme in the medium without Mg^{2+} was 3.6 - 3.7 - fold higher than oligomycin-resistant one in control group. This ATPase is clearly shown from the presented data (Table 4) to be sensitive to ischemia. These facts testify to just mitochondrial ATPase makes the main contribution to DNP-stimulated ATPase activity of cells and just it is affected by ischemia in the first step.

Table 4

Oligomycin-sensitive DNP-stimulated ATPase activity of rat heart homogenates (nmol P_i /min·mg protein; n = 6 - 9)

Modes of experiment	Duration of ischemia, min		
	0 (control)	30	120
3 months			
-	210.5 ± 23.6	216.3 ± 15.7	143.1 ± 19.4*,**

Mg^{2+}	161.4 ± 9.0	155.6 ± 11.7	148.0 ± 14.0
EDTA	166.4 ± 19.8	132.9 ± 11.0	104.1 ± 16.9*
24 months			
-	230.3 ± 14.6	180.2 ± 16.5*	120.3 ± 8.8*,**
Mg^{2+}	183.0 ± 9.9	145.7 ± 8.0*	120.2 ± 12.9*,***
EDTA	198.3 ± 10.8	135.1 ± 9.9*	92.8 ± 9.9*,**

* - P < 0,05 in comparison with control;
** - P < 0,05 in comparison with 30 min ischemia;
*** - 0,05 < P < 0,1 in comparison with 30 min ischemia

From the data presented (Table 4) the clear age peculiarities of the enzyme functioning (medium without Mg^{2+}) during ischemia are shown. 30 min of ischemia did no effect in young rats while this ischemic period resulted in significant decrease of oligomycin-sensitive DNP-stimulated ATPase activity in old animals. 120 min of ischemia led to significant drop of the enzyme activity in both age groups in comparison with control and even with the activity levels after 30 min of ischemia. Addition of Mg^{2+} in the assay medium normalized situation in 3-months old rats whereas in 24-months old animals Mg^{2+} only slightly ameliorated situation as compared with the results obtained in the medium without Mg^{2+}.

Thus in result of our study we came to the following conclusions:

1) from all studied enzymes only mitochondrial oligomycin-sensitive ATPase was sensitive to effect of total myocardial ischemia;
2) young 3-months old rats were more tolerant to ischemic damage than old ones;
3) addition of Mg^{2+} in the incubation medium eliminated ischemic effect in 3-months old rats and weakened it in 24-months old rats. It points to important role of magnesium ions in structure- functional reconstruction of mitochondria under myocardial ischemia.

REFERENCES

1. Биленко М.В. Ишемические и реперфузионные повреждения органов. - М.:Медицина, 1989. -368 с.
2. Griffiths E.J., Halestrap A.P. // Biochem. J. - 1995. - Vol. 307. - P. 93 - 98.
3. Almeida A., Allen K.L., Bates T.E., et.al. // J. Neurochem. - 1995. - Vol. 65, № 4. - P. 1698 - 1703.
4. Fleury B., Di P.A., Godinot C. et al. // Biochimie. - 1980. - Vol. 62, № 10. - P. 733 - 737.
5. Лемешко В.В., Маланда П. // Укр. биохим. журн. - 1986. - Т. 61, № 1. - С. 64 - 71.
6. Tani M., Suganuma Y., Hasegawa H., et al. // J. Mol. Cell.Cardiol. - 1997. - Vol. 29, № 11. - P. 3081 - 3089.
7. McCully J.D., Levitsky S. // Ann. N. Y. Acad. Sci. - 1996. - Vol. 793. - P. 305 - 318.
8. Tani M., Suganuma Y., Hasegawa H. et al. // Circulation. -1997. - Vol. 95, № 11. - P. 2559 - 2566.
9. Болдырев А.А. Транспортные иденозинтрифосфатазы. - М.: Изд-во Моск. ун-та , 1977. - 126 с.
10. Miller G.D. // Anal. Chem. - 1959. - Vol. 31, № 5. - P. 964 -966.
11. Терентьев П.В., Ростова Н.С. Практикум по биометрии. - Л.: Изд-во ЛГУ, 1977. - 151 с.

ВПЛИВ ІШЕМІЇ НА АТФазну АКТИВНІСТЬ МІТОХОНДРІЙ СЕРЦЯ МОЛОДИХ І СТАРИХ ЩУРІВ

Дзюба В.Н., Нікітченко Ю.В., Шмоніна Т.А.
Науково-дослідний інститут біології Харківського національного університету ім. В.Н. Каразіна

РЕЗЮМЕ

Вивчено зміни АТФазної активності у щурів різного віку при тотальній ішемії міокарда. Встановлено, що тільки мітохондріальна олігоміцин-чутлива АТФаза виявила чутливість до тотальної ішемії міокарда. Додавання іонів магнію до середовища інкубації знімає вплив ішемії у 3-місячних щурів та послаблює його у 24-місячних щурів, що свідчить про важливу роль іонів магнію в структурно-функціональній перебудові мітохондрій при ішемії міокарда. Встановлено, що молоді, 3-місячні, щури більш стійкі до ішемічного пошкодження міокарда, ніж старі тварини.

КЛЮЧОВІ СЛОВА: серця щурів, тотальна ішемія міокарда, АТФазна активність, вікові особливості

ВЛИЯНИЕ ИШЕМИИ НА АТФазную АКТИВНОСТЬ МИТОХОНДРИЙ СЕРДЦА МОЛОДЫХ И СТАРЫХ КРЫС

Дзюба В.Н., Никитченко Ю.В., Шмонина Т.А.
Научно-исследовательский институт биологии Харьковского национального университета
им. В.Н. Каразина

РЕЗЮМЕ

Изучены изменения АТФазной активности у крыс разного возраста при тотальной ишемии миокарда. Установлено, что только митохондриальная олигомицин-чувствительная АТФаза проявляла чувствительность к тотальной ишемии миокарда. Добавление ионов магния в среду инкубации снимает влияние ишемии у 3-месячных крыс и ослабляет его у 24-месячных крыс, что свидетельствует о важной роли ионов магния в структурно-функциональной перестройке митохондрий при ишемии миокарда. Установлено, что молодые, 3-месячные, крысы более устойчивы к ишемическому повреждению миокарда, чем старые животные.

КЛЮЧЕВЫЕ СЛОВА: сердца крыс, тотальная ишемия миокарда, АТФазная активность, возрастные особенности