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ATP AS THE MARKER OF POWER EXCHANGE CONDITION AT THE EXPERIMENTAL ISCHEMIA OF THE MYOCARDIUM DUE TO METABOLIC DRUGS INTRODUCTION

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Abstract. For the purpose of definition of an adequate marker of a power exchange condition at the experimental ischemia of the myocardium due introduction of metabolic drugs, the experimental research on 60 rats-males of Wistar line has been spent. At experimental myocardial ischemia it has been found out energy-saving effects of trimetazidine, cytoflavin, meldonium, phosphocreatine, which were reached by different ways and in different degree. The indicator of ATP concentration in blood or erythrocytes closely correlated with the level of ATP in myocardial homogenates and with activity of mitochondrial heart enzymes – succinate dehydrogenase, citrate synthase, pyruvate dehydrogenase. Thus, this indicator can be recommended to study to assess the effectiveness of cardiocytoprotectors as the most appropriate marker of their energy-saving effect.

Keywords: ATP, myocardial metabolism, cardiocytoprotection, energy-saving effect, metabolic drugs, trimetazidine, meldonium, cytoflavin, phosphocreatine.

Introduction

The high indicators of prevalence and death rate from coronary heart disease (CHD), despite the accepted standards of pharmacotherapy, cause necessity of modernisation of the accepted schemes of treatment [1, 2]. One of the promising directions of improving the pharmacotherapy of CHD considered cardiocytoprotection [3, 4, 5, 6]. Metabolic-line drugs are widely used by doctors of the countries in post-Soviet space as ones that enhance the patients' life quality, whereas in Europe they are administered not more than in 1% of cases due to the lack of evidence of their impact on the patients' life expectancy [7]. One of the obstacles of overcoming the doubts of doctors in the effectiveness of the destination cardiocytoprotectors is absence of adequate methods of myocardium metabolism estimation in view of inaccessibility and ethical impossibility of performance of researches on human heart tissue.

The purpose of the present research was definition of an adequate marker of a myocardium power exchange condition at an experimental ischemia of a myocardium due the introduction of metabolic drugs.

Materials and methods of the research

For achievement of the purpose of work the experimental research which object were 60 rats-males of Wistar line aged by 10 months, which contained in standard conditions of vivarium, has been executed. The following groups of animals were used: 1) intact rats (n=10); 2) rats with an experimental ischemia of a myocardium (n=10); 3) rats with an experimental ischemia of a myocardium entered trimetazidine (n=10); 4) rats with an experimental ischemia of a myocardium entered meldonium (n=10); 5) rats with an experimental ischemia of a myocardium entered cytoflavin (n=10); 6) rats with an experimental ischemia of a myocardium entered phosphocreatine (n=10). Age of animals was chosen from a position of conformity of 10-month's rats – to middle age of human being. The choice of metabolic drugs spent from positions of their influence on various links of the power exchange of cardiomyocytes.

Modelling of an ischemia of a myocardium has been made on the method described by Gaman D.V. (2011) [8]: daily within 7 days subcutaneously the rats were entered 0,1 ml of 0,1 % adrenaline solution. A dose of medical drugs, entered with the therapeutic purpose, was counted under formula of Rybolovlev J.R. (1979), it has been made 1) for trimetazidine (a pure solid of firm «Sigma Aldrich») – 0,5mg/100g rats in 2 ml of 0,9% NaCl solution intragastric 2 times a day, that is equivalent to a recommended dose of trimetazidine for a human (35 mg 2 times a day per os); 2) for meldonium (Mildronate of "Grindex" firms,

Latvia) – 0,03ml/100g rats in 1,5 ml of 0,9 % NaCl solution intravenously 1 time a day, that is equivalent to the recommended dose for a human (5 ml intravenously 1 time a day; 3) for cytoflavin – 0,07ml/100g rats in 1,5 ml of 0,9 % NaCl solution intravenously 1 time a day, that is equivalent to the recommended dose for a human (10 ml intravenously in 200 ml of 0,9 % NaCl solution 1 time a day); 4) for phosphocreatine – 13mg/100g rats in 2 ml of 0,9 % NaCl solution intravenously 1 time a day, that is equivalent to the recommended dose for a human (2g/days intravenously of a medicine "Neotone").

The animals were deduced from experiment in 10 days after introduction of medicines by decapitation. The heart was perfused with a cooled solution of 0.9% NaCl. Preparation of myocardial homogenates and isolation of mitochondria was produced by the method described of M.V. Egorova, S.A. Afanasyev [9]. The erythrocytes were isolated from heparinized blood by centrifugation. The washed erythrocytes were used for determination of 2,3-diphosphoglycerat (2,3-DPG) and free nucleotides (ATP and ADP) (N.P. Meshkova, S.E. Severin, 1979). The content of ATP and ADP was measured in the serum of blood (I.S. Mranova, 1975). The activity of succinate dehydrogenase (SDH), citrate synthase (CS) and pyruvate dehydrogenase (PDH) was investigated in the myocardial mitochondria. The level of ATP was determined in myocardial homogenate (M.I. Prochorova, 1982).

The data were treated by variation statistics with arithmetic mean values and their errors, correlation analysis, assessment of significant differences in the student t-test using the software "Microsoft Excel 2000" and "SPSS for Windows 11.0".

Results and their discussion

At modelling of the ischemia of a myocardium of 10 months rats a significant increase in 2,3-DPG and a decrease in ATP concentration in erythrocytes was found, suggesting the development of tissue hypoxia and energy deficiency (Table 1). At the mitochondria a significant decrease in the activity of studied enzymes – SDG, CS and PDG was found, which indicates a decrease in the intensity of oxidative phosphorylation and oxidative decarboxylation of pyruvate. The consequence of this fact a significant decrease in the concentration of ATP in myocardial homogenates was observed.

At the animal experiments the effectiveness of all 4 metabolic drugs due to chronic myocardial ischemia have been studied. We found significant differences in the mechanisms of action of each of them.

We considered the main indicator of energy saving effect of metabolic drugs the ATP levels in myocardial homogenates, which reflects the amount of ATP in cardiomyocytes. Introduction of trimetazidine, cytoflavin and phosphocreatine increased ATP concentration in cardiomyocytes, but without reaching the level of intact rats. Introduction of meldonium led to the recovery of the amount of ATP in the heart homogenates to the level of the intact rats, without excessive accumulation of nucleotides, which in our opinion, indicates the absence of doping properties of the drug.

ATP levels in serum and red blood cells due to introduction of metabolic correctors have similar dynamics, as reflected in Table 1, and indirectly confirmed by correlation analysis. Positive correlations were found between the concentration of ATP in myocardial homogenates and blood serum ($r = 0,66$, $p < 0,0001$), in red blood cells ($r = 0,66$, $p < 0,0001$). ATP levels in myocardial homogenate is also positively correlated with the activity of mitochondrial enzymes – succinate dehydrogenase ($r = 0,67$, $p < 0,0001$), citrate synthase ($r = 0,65$, $p < 0,0001$) and pyruvate dehydrogenase ($r = 0,75$, $p < 0,0001$). There is an inverse relationship between ATP concentration in the myocardium and 2,3DPG level in red blood cells ($r = -0,33$, $p < 0,01$). Thus, the more active oxidation-reduction reactions take place in the mitochondria, the higher levels of ATP detect in myocardial homogenates , blood serum and erythrocytes with the lower level of tissue hypoxia. So that, in terms of ATP concentrations in serum and / or erythrocytes it can be indirectly judged the content of ATP in the myocardium and the intensity of the oxidation-reduction reactions taking place in the mitochondria of the cardiomyocytes.

We studied the effect of metabolic correctors on the activity of mitochondrial enzymes in comparative aspect. Thus, the succinate dehydrogenase activity in experimental myocardial ischemia was significantly reduced. Introduction of cytoflavin and meldonium led to partial activation of SDH, but not to the level of the intact rats, administration of trimetazidine and phosphocreatine was accompanied by decreased activity of this Krebs cycle enzyme (Table 1).

Activity of citrate synthase in experimental myocardial ischemia was significantly reduced. Introduction of trimetazidine, meldonium and cytoflavin led to a slight increase in CS activity in mitochondria, while the introduction of phosphocreatine was accompanied by inactivation citrate synthase in the mitochondria of rats.

Pyruvate dehydrogenase activity in mitochondria of cardiomyocytes in experimental myocardial ischemia was significantly reduced. Introduction of cytoflavin to the animals was contributed to increase the activity of PDH to the level of the intact rats, administration of phosphocreatine contributed to a slight increase in the activity of PDH, while trimetazidine had no significant effect on the activity of this enzyme.

Table 1

Indicators of myocardial metabolism in 10 months rats in normal, at experimental myocardial ischemia and on the background of metabolic correctors (M ± m)

Indicators		Intact rats of 10 months, n=10	Rats with myocardial ischemia, n=10	Rats with myocardial ischemia + cytoflavin, n=10	Rats with myocardial ischemia + trimetazidine, n=10	Rats with myocardial ischemia + meldonium, n=10	Rats with myocardial ischemia + phosphocreatine, n=10
Erythrocytes	ATP, mkmol/l	664,54±14,49 $^{\Delta\Delta}$##	594,44±5,75**	582,47±5,24**	587,05±6,40**	638,88±14,96$^{\Delta}$	589,28±5,43**
	ADP, mkmol/l	315,11±8,78	330,53±16,05	316,05±3,01	323,57±5,40	319,27±7,51	328,86±2,93
	2,3DPG, mkmol/l	4,82±0,29 $^{\Delta\Delta}$##	7,21±0,32**	6,84±0,15**	6,12±0,19 $^{\Delta}$	5,70±0,18* $^{\Delta\Delta}$	6,69±0,21**
Serum of blood	ATP, mkmol/l	200,08±3,47 $^{\Delta\Delta}$##	162,81±4,57**#	177,70±2,15**$^{\Delta}$	171,81±1,61**	192,32±5,36 $^{\Delta\Delta}$	192,02±4,76$^{\Delta\Delta}$
	ADP, mkmol/l	75,92±1,58##	79,31±1,13##	91,21±0,79** $^{\Delta\Delta}$	85,00±3,22*	77,33±2,29	90,91±1,56**$^{\Delta\Delta}$
Mitochondria	SDH, nmol/min·mg	17,82±1,10 $^{\Delta\Delta}$##	11,83±0,47**#	13,45±0,47**$^{\Delta}$	10,77±0,68**$^{\Delta\Delta}$	13,68±0,65*	10,12±0,29**$^{\Delta}$
	CS, nmol/min·mg	3,94±0,23 $^{\Delta\Delta}$#	2,38±0,21**#	3,21±0,20*$^{\Delta}$	3,08±0,27**	3,12±0,10*$^{\Delta}$	2,00±0,08**
	PDH, mkmolNAD/min·mg	31,04±0,89 $^{\Delta\Delta}$	21,68±0,90**##	31,34±0,73 $^{\Delta\Delta}$	22,32±0,57**	27,30±0,45** $^{\Delta\Delta}$	24,45±0,34** $^{\Delta}$
	ATP in myocardial homogenate, mkmol/l	3,08±0,24 $^{\Delta\Delta}$#	1,18±0,08**##	2,09±0,16* $^{\Delta\Delta}$	2,00±0,05**$^{\Delta\Delta}$	3,16±0,09 $^{\Delta\Delta}$	1,96±0,08**$^{\Delta\Delta}$

Note. ATP – adenosine triphosphate, ADP – adenosine diphosphate, 2,3DPG – 2,3-diphosphoglycerat, SDH – succinate dehydrogenase, CS – citrate synthase, PDH – pyruvate dehydrogenase. The significance of differences: *p<0,05; ** p<0,01 as compared to the intact rat; $^{\Delta}$ p<0,05; $^{\Delta\Delta}$ p<0,01 as compared to the group of "myocardial ischemia"; # p<0,05; ## p<0,01 as compared to the group of "myocardial ischemia + cytoflavin"; $^{\$}$p<0,05; $^{\$\$}$p<0,01 as compared to the group of "myocardial ischemia + meldonium";$^{!}$ p<0,05; $^{!!}$ p<0,01 as compared to the group of "myocardial ischemia + phosphocreatine".

We found a strong direct correlations between all studied mitochondrial enzymes: SDH and CS (r = 0,75, p <0,0001), SDH and PDH (r = 0,70, p <0,0001), the CS and SDH (r = 0,74, p <0,0001), and between them and the ATP levels in the myocardium, erythrocytes and blood serum, what has been said above.

Our findings, firstly, confirm the presence of energy-saving effect in all we have studied metabolic correctors – trimetazidine, cytoflavin, phosphocreatine and meldonium, secondly, they show that the achievement of energy economization within cardiomyocytes occurs in different ways and to varying degrees because of different mechanisms of action. The studied results are consistent with published data [10, 11, 12]

Really, trimetazidine contributes switch in energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting acetyl-CoA acyltransferase, a key enzyme in fatty acid oxidation in mitochondria [13, 14]; cytoflavin contains four components (succinic acid, riboxinum, nicotinamide and riboflavin), each of which contributes to the stimulation of Krebs cycle inside the mitochondria [15, 16]; phosphocreatine is an exogenous source of substrate phosphorylation reacti

+ons inside cardiomyocytes [17]; meldonium blocks synthesis of carnitine, transporter of fatty acids from the mitochondria into the cytosol, thereby switches the energy exchange from lipid to carbohydrate types, meldonium also stimulates the synthesis of nitric oxide [17, 18].

The most important result of our research we consider found out the strong correlations between the concentration of ATP into the miocardial homogenate and the level of this nucleotide in serum and red blood cells, which are also linked with the activity of cardiomyocytes' mitochondrial enzymes. This fact is the basis to offer for use in clinical practice index of ATP concentration in blood serum or red blood cells as the most adequate and accessible marker of energy metabolism state in cardiomyocytes, and in the case of the definition of this indicator during the treatment of patients with cardiocytoprotectors it can be judged the energy-saving effect of the latest .

Conclusions

1. ATP concentration in serum or red blood cells is the most appropriate measure of the energy metabolism in the heart muscle due to the close correlation with the level of ATP in myocardial homogenates, and the activity of mitochondrial enzymes of the heart – succinate dehydrogenase, citrate synthase, pyruvate dehydrogenase.

2. In the experimental myocardial ischemia it has been detected energy-saving effects of trimetazidine, cytoflavin, phosphocreatine and meldonium, which were achieved in different ways and to varying degrees.

3. Introduction of meldonium to the animals with experimental myocardial ischemia resulted in recovery amount of ATP in heart homogenate to the level of intact rats without excessive accumulation of this nucleotide, indicating a lack of doping properties of the drug.

4. To assess the effectiveness of cardiocytoprotectors in complex treatment of patients with cardiac pathology, the study of ATP concentration in erythrocytes and serum can be recommended during the treatment as the most appropriate marker of their energy-saving effect.

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