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Research Article

Adrenergic Receptors in the Mechanism of Regulation by Catecholamines of Mitochondrial and Cytoplasmic Enzymes of Cardiomyocytes and Hepatocytes

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Abstract

The involvement of adrenoreceptors in the regulation of the activity of mitochondrial and cytoplasmic enzymes in cardiomyocytes and hepatocytes by catecholamines and their metabolites was studied. Various adrenergic receptor (AR) agonists have been used that act on α-AR and β-AR, either on α-AR alone or on β-AR, as well as catecholamine quinoid metabolites. It has been established that the activating effect of the β-AR agonist isadrin on SDH in heart mitochondria is prevented by β-blockade. The activating effect of dopamine, epinephrine, and isadrine on cytochrome C - oxidase (CCO), and the inhibitory effect of dopamine, norepinephrine, epinephrine, and isadrine on Mg-activated ATPase are not mediated by adrenoreception.

Hormones-mediators of the sympathoadrenal system adrenaline, dopamine, norepinephrine, isadrine and catecholamine metabolites (adrenochrome and adrenoxyl), by changing the activity of the respiratory chain enzymes of the heart mitochondria, regulate the processes of tissue respiration, transferring mitochondria to a state of "loose" conjugation of respiration and phosphorylation.

In cardiomyocytes and in hepatocytes, adrenaline, as an agonist of β-adrenergic receptors, increases the activity of enzymes of metabolism of purine nucleotides AD and AMPD, and enzymes of antioxidant protection GPO and catalase, increases the level of MDA and DC. The blockade of β-adrenergic receptors with metoprolol removes the activating effect of adrenaline on GR, GPO and catalase, reduces the level of MDA and DC.

Keywords: Adrenoreceptors; Catecholamines; Adrenaline; Mitochondrial; Cytosol Enzymes of Cardiomyocyte; Hepatocytes

Introduction

The control of metabolism and physiological activity of all cells of the body is carried out with the help of a complexly organized specific polyfunctional hormonal ensemble [7,8]. In the neurohumoral regulation of physiological functions and metabolism, not only the presence of a native hormone-mediator molecule and metabolites of regulatory hormones is important, but also the functional state of receptors in target cells. Receptors are specific proteins that transmit any external signals to the cell. All effects of hormones are realized through receptors [4]. But these effects are reproduced through different types of receptors. For example, it has been shown that catecholamines have not only an activating, but also a weaker inhibitory effect - they reduce O_2 consumption through a_2-adrenergic receptors [5].

The aim of this study is to study the degree of participation of adrenoreceptors (AR) in the regulation of the activity of mitochon-

drial enzymes (succinate dehydrogenase (SDH), cytochrome c oxidase (CCO). ATPase and AMP deaminase (AMPD) and cytoplasmic enzymes of purine metabolism - adenosine deaminase (AD), AMP-deaminase (AMPD), 5-nucleotidase (5`H) and antioxidant defense enzymes - glutathione peroxidase (GPO), glutathione reductase (GR) and catalase.

For this, adrenoreceptor agonists acting on α-AR and β-AR or only on α-AR and β-AR, as well as quinoid metabolites of catecholamines, were used.

Method

Study design: experimental. The studies were carried out on 65 outbred white rats aged 3-3.5 months, weighing 160-180g. scientific purposes (Strasbourg, 18 March 1986). The experimental animals were kept in the same conditions of keeping the vivarium of the Center of the Research Laboratory of the Non-Commercial Joint Stock Company "Semey Medical University", in accordance with the main therapeutic and nutritional regimen. The experiment was approved by the Ethics Committee of the State Medical University of Semey in accordance with the Directive of the European Parliament on the protection of animals used for scientific purposes (Protocol No. 2, December 21, 2016). After decapitation, the blood and hearts of animals were taken. The hearts of the animals were washed with saline and homogenized with a Teflon pestle in a medium containing 0.25 M sucrose. Tissue homogenates were filtered through 2 layers of gauze and centrifuged for 10 min (0 ° - 2 ° C) at 800g to remove cell debris and nuclear fraction. The supernatant after centrifugation was used for research. Mitochondria from heart homogenates were isolated by differential centrifugation at 8500g for 15 minutes.

The activity of succinate dehydrogenase (SDH) [EC 1.3.99.1] of mitochondria was determined in an incubation medium containing 0.05 M phosphate buffer solution pH = 7.4, 10 μM 0.05 M $MgCl_2$ solution, KCL 2.5 μM 1.0 m solution, 50 μM succinate and 0.5 ml 1% solution 2.3 .5. triphenyltetrazolium chloride. Activity of cytochrome c-oxidase (CCO) [EC 1.9.3.1] by the monometric method in a Warburg apparatus in a medium containing 1.4 ml of 0.05 M borate buffer, 0.2 ml of 0.2% dimethylparaphenylenediamine solution. Activity was expressed in μat oxygen per mg protein. The activity of mitochondrial DNP of activated ATPase (EC 3.6.1.3) was determined by the increase in inorganic phosphate in a medium containing 30 μm Tris buffer pH = 7.5, 2 μM $MgCl_2$, 50 μM α-dinitrophenol, 0.1 ml of 0.2% solution ATP. The activity of adenosine deaminase (AD) [EC 3.5.4.4], AMP deaminase (AMPD) [EC 3.5.4.6] was determined by the method of S.O. Tapbergenov [9] and expressed in nmol of ammonia per mg of protein per minute in tissues or nmol/ml in blood. The activity of 5'-nucleotidase (5'H) [EC 3.1.3.5] was judged by the rate of AMP hydrolysis to adenosine and phosphoric acid and was expressed as μmol H3PO4 per mg of protein per minute. The amount of protein was determined by the conventional Lowry method. The activities of glutathione reductase (GR) [EC 1.8.1.7] and glutathione peroxidase (GPO) [EC 1.11.1.9] were determined by the method of S.N. Vlasova., *et al*. [1], the activity of catalase [EC 1.11.1.6] according to the method of M.A. Korolyuk and co-author [3]. The determination of the amount of MDA was carried out according to the method of Uchiyama M., Mihara M. [13], diene conjugates (DC) according to the method of V.B. Gavrilov and coauthors [2]. The amount of protein was determined by the conventional Lowry method.

The research results were processed using Student's t-test. The work presents arithmetic mean data ± error of means (X ± m). Differences were considered significant at $p < 0.05$.

Results of Studies

Studies have found (Table 1) that dopamine administered to animals at a dose of 1.5 mg/100g 15 minutes before the study activates succinate dehydrogenase in the mitochondria of the heart. Norepinephrine (an agonist of α-adrenergic receptors), administered to animals at a dose of 0.5 mg/100g and adrenaline (an agonist of α- and β-adrenergic receptors), at a dose of 0.015 mg/100g, also cause activation of SDH in the heart. It can be assumed that the effects of the main representatives of catecholamines on SDH are to some extent associated with adrenergic reception. Using izadrin as a specific agonist of β-adrenergic receptors (0.1 mg/100g), an activation of SDH in the mitochondria of the heart similar to the effect of dopamine, norepinephrine and adrenaline was established. The activating effect of izadrin on SDH of cardiac mitochondria is prevented by β-blockade of propranolol administered at a dose of 0.1 mg/100g for 2 hours (Table 2).

Adrenoxyl (adrenochrome monosemicarbosone) at a dose of 0.2 mg/100g and adrenochrome administered 30 minutes before

the study, like dopamine, norepinephrine and adrenaline, causes SDH activation in the heart (Table 1). Earlier, we found that adrenoxyl has an activating effect on myocardial adenylcyclase and cAMP level, independent of β-adrenoreception [10].

Dopamine increases the activity of CCO in the mitochondria of the heart (Table 1). Since dopamine can be converted to norepinephrine under the influence of dopamine beta-hydroxylase, a similar effect of norepinephrine could be assumed. However, norepinephrine does not increase mitochondrial activity in the heart. At the same time, adrenaline as an agonist of alpha- and beta-adrenergic receptors and the stabilized product of the quinoid oxidation of adrenaline adrenoxyl and adrenochrome activate CCO in the heart (Table 1). Studies have shown that the activating effect of the β-adrenergic receptor agonist izadrin on the CCO is not prevented by β-adrenoblockade propranolol administered at a dose of 0.1 mg/100g for 2 hours (Table 2).

Series	SDG	CCO	Mg-ATP-ase	AMP deaminase
Control	24,84 ± 0,59	16,24 ± 0,72	59,60 ± 3,96	86,59 ± 2,28
Dopamine (1.5 mg / 100g)	48,32 ± 4,42*	19,83 ± 1,41*	27,19 ± 1,51*	78,40 ± 2,72*
Norepinephrine (0.5 mg / 100 g)	30,34 ± 2,01 *	16,59 ± 1,80	38,83 ± 4,17 *	104,98 ± 1,31*
Adrenaline (0.15mg / 100g)	29,40 ± 2,17 *	27,62 ± 1,36 *	40,51 ± 2,38 *	-
Izadrin (0.1mg / 100g)	34,04 ± 2,04*	19,92 ± 0,37 *	25,88 ± 2,12 *	39,65 ± 7,88 *
Adrenoxil (0.2 mg / 100g)	35,07 ± 3,01 *	20,16 ± 1,26 *	45,45 ± 3,00 *	71,90 ± 3,96 *
Adrenochrome (0.2 mg / 100g)	43,06 ± 3.00*	28,76 ± 4.64*	47,79 ± 2.81 *	86,10 ± 12,66
Note: * - $p < 0.05$ in comparison with control				

Table 1: Influence of catecholamines on the activity of heart mitochondrial enzymes.

Dopamine, norepinephrine and adrenaline reduce the activity of ATP-ase in the mitochondria of the heart (Table 1). Administration of the beta-adrenergic receptor agonist izadrin (0.1 mg/100g) to animals 15 minutes before the study causes a decrease in the activity of ATPase of heart mitochondria similar to dopamine. Administration of adrenochrome and adrenoxyl (0.2 mg/100g) to animals 30 minutes before the study also leads to a decrease in the activity of ATPase in the mitochondria of the heart, but to a lesser extent than dopamine. Studies have shown that the effect of the β-adrenergic receptor agonist isadrin on Mg-activated ATP-ase is not prevented by β-adrenergic blockade (Table 2).

Dopamine and isadrin reduce the activity of AMP deaminase in the mitochondria of the heart (Table 1). Norepinephrine, unlike dopamine, increases the activity of AMP deaminase in the mitochondria of the heart. The introduction of the stabilized adrena-

line quinoid oxidation product adrenoxil 30 minutes before the study leads to a decrease in the activity of AMP deaminase in the mitochondria of the heart. Analysis of these data suggests that the beta-adrenoreceptor apparatus is not used for catecholamine control over the activity of AMP-deaminase of cardiac mitochondria, since the action of izadrin on AMP-deaminase is not prevented by β-adrenoblockade (Table 2).

Earlier, we found that when adrenaline was administered to animals at a dose of 0.1 mg 100g 60 minutes before the study, there was some activation of respiration and a decrease in the P/O ratio in the mitochondria of the heart [8]. This effect of adrenaline on oxidative phosphorylation is probably associated with the peculiarities of the effect of the metabolites of adrenaline quinoid oxidation on the enzymes of the mitochondrial respiratory chain. This is confirmed by the data that adrenochrome and adrenoxyl reduce the activity of ATPase and increase the activity of SDH and CCO (Table 1) in the mitochondria of the heart. Analyzing our data on the effect of catecholamines and their metabolites on the activity of enzymes in the heart mitochondria, we can assume that the metabolism of hormones-mediators of the sympathoadrenal system is a regulatory factor of mitochondrial energy transformation processes in the cell.

Cells	Series	SDG	CCO	Mg-ATP-ase	AMP deaminase
heart	Control	24,84 ± 0,59	16,24 ± 0,72	59,60 ± 3,96	86,59 ± 2,28
	Izadrin (0.1mg/100gr)	34,04 ± 2,04*	19,92 ± 0,37 *	25,88 ± 2,12 *	39,65 ± 7,88 *
	Izadrin and propranolol 0.1 mg/100g in 2 hours	22,72 ± 0,46 **	19,28 ± 1,26	19,68 ± 1,50 **	34,87 ± 3,57
liver	Control	21,32 ± 1,50	10,89 ± 0,80	14,78 ± 1,43	55,86 ± 1,24
	Izadrin (0.1mg/100gr)	17,00 ± 1,13*	8,10 ± 0,37 *	6,53 ± 0,56 *	24,64 ± 7,70 *
	Izadrin and propranolol 0.1 mg/100g in 2 hours	11,24 ± 0,57 **	7,86 ± 0,49	6,31 ± 1,39	28,24 ± 3,74

Table 2: Influence of β-adrenergic blocker propranolol and isadrin on the activity of enzymes of mitochondria of the heart and liver.

Note: * - $p < 0.05$ in comparison with the control

* * - $p < 0.05$ in comparison with izadrin.

In the liver (Table 3), in contrast to the heart, the studied adrenoceptor agonists cause a decrease in the activity of SDH, CSO, and ATPase. And only norepinephrine and adrenoxyl activate AMP deaminase.

In cardiomyocytes (Table 4), the administration of adrenaline to animals at a dose of 0.4 mg/100g 60 minutes before the study is accompanied by the activation of enzymes of metabolism of purine nucleotides AD, AMPD, a decrease in 5'H activity and an increase in the ratio of the activities of AD + AMPD/5 'H. An increase in the ratio of the activities of enzymes of metabolism of purine nucleotides AD + AMPD/5'H is directed towards an increase in the deamination of adenosine and AMP with the formation of inosine and IMP.

Series	SDG	CCO	Mg-ATP-ase	AMP deaminase
Control	21,32 ± 1,50	10,89 ± 0,80	14,78 ± 1,43	55,86 ± 1,24
Dopamine (1.5 mg/100g)	15,41 ± 2,45*	6,12 ± 0,90*	4,07 ± 1,72*	59,64 ± 8,48
Norepinephrine (0.5 mg/100g)	11,66 ± 2,15 *	5,79 ± 0,56 *	9,79 ± 1,29 *	99,59 ± 13,41*
Adrenaline (0.15mg/100g)	26,96 ± 1,42 *	14,30 ± 1,37 *	17,20 ± 1,33	-
Izadrin (0.1mg/100g)	17,09 ± 1,13*	8,10 ± 0,37 *	6,53 ± 0,86 *	24,64 ± 7,70 *
Adrenoxil (0.2 mg/100g)	15,00 ± 1,12 *	8,86 ± 0,64	8,75 ± 3,00 *	121,20 ± 9,74 *
Adrenochrome (0.2 mg / 100g)	13,58 ± 0.91*	10,48 ± 0.74*	11,84 ± 0.69 *	-

Table 3: Influence of catecholamines on the activity of liver mitochondrial enzymes.
Note: * - p <0.05 in comparison with control.

It is known that the catabolism of adenosine and AMR in the xanthioxidase reaction is accompanied by the appearance of toxic forms of oxygen [11,12], which leads to the activation of the antioxidant defense enzymes GPO and catalase, which we found in the heart upon administration of adrenaline. The introduction cardioselective beta-adrenoblocker metoprolol [6] of against the background of adrenaline leads to the activation of AD, AMPD and 5'H, to a decrease in the amount of MDA and DC and, correspondingly, to a decrease in the activity of GR, GPO and catalase (Table 4).

Index	Control n = 20	Adrenalin n = 15	Adrenaline + Metoprolol n = 15
AD μmol/mg per min	0,19 ± 0,01	0,26 ± 0,02*	0,45 ± 0,01**
AMPD μmol/mg per min	0,09 ± 0,01	0,13 ± 0,01*	0,21 ± 0,01**
5'H μmol/mg per min	0,02 ± 0,001	0,01 ± 0,001*	0,02 ± 0,001**
AD+AMPD/5'H	14,0 ± 0,15	39,02 ± 0,21*	33,0 ± 0,02**
GR μmol NADPH/g/min	32,13 ± 1,78	35,31 ± 1,39	20,06 ± 2,31**
GPO μmol ox. glutathione/g per minute	2,69 ± 0,30	3,12 ± 0,21*	1,23 ± 0,04**
Catalase μat/g per min	69,85 ± 7,28	81,58 ± 3,08*	53,76 ± 5,77**
MDA nmol/g	0,04 ± 0,001	0,05 ± 0,01*	0,01 ± 0,001**
DC beats unit/g	0,02 ± 0,001	0,02 ± 0,001	0,01 ± 0,001**

Table 4: The effect of the combined action of the administration of epinephrine (4 mg/kg) and metoprolol (25 mg/kg) on the activity of enzymes of purine nucleotide metabolism and antioxidant defense enzymes in the heart.

Note: * - p < 0.05 in comparison with control

** - p < 0.05 in comparison with adrenaline.

Thus, β1-adrenergic blockade with metoprolol reduces the process of peroxidation and, accordingly, the activity of antioxidant defense enzymes decreases, thereby reducing the severity of oxidative stress caused by the administration of adrenaline.

In hepatocytes (Table 5), administration of epinephrine at the same dosage induces changes similar to the heart in the activity of cytosolic enzymes and the antioxidant defense system, which are correlated with the administration of metoprolol.

Index	Control n = 20	Adrenalin n = 15	Adrenaline + Metoprolol n = 15
AD μmol/mg per min	0,29 ± 0,21	0,40 ± 0,02*	0,50 ± 0,001**
AMPD μmol/mg per min	0,20 ± 0,01	0,27 ± 0,01*	0,31 ± 0,001**
5'H μmol/mg per min	0,04 ± 0,001	0,05 ± 0,001*	0,05 ± 0,001
AD+AMPD/5'H	12,25 ± 0,38	13,4 ± 0,5	16,2 ± 0,001**
GR μmol NADPH/g/min	24,69 ± 2,16	22,01 ± 1,01	19,85 ± 2,28
GPO μmol ox. glutathione/g per minute	2,86 ± 0,37	3,37 ± 0,26	1,25 ± 0,04**
Catalase μat/g per min	60,57 ± 4,58	81,61 ± 4,68*	50,23 ± 5,09**
MDA nmol/g	0,04 ± 0,001	0,05 ± 0,01*	0,01 ± 0,001**
DC beats unit/g	0,02 ± 0,001	0,03 ± 0,001*	0,02 ± 0,001**

Table 5: Influence of the combined action of the administration of adrenaline (0.4 mg/100g) and metoprolol (25 mg/kg) on the activity of purine nucleotide metabolism enzymes and antioxidant defense enzymes the hepatocytes.

Note: * - $p < 0.05$ in comparison with control

** - $p < 0.05$ in comparison with adrenaline.

Conclusion

The activating effect on the SDH of the mitochondria of the heart and liver, caused by the administration of the β-adrenoreceptor agonist isadrin, is prevented by β-adrenergic blockade. The activating effect of dopamine, adrenaline and isadrin on the CCO and the inhibitory effect of dopamine, norepinephrine, adrenaline and isadrin on Mg-activated ATP-ase is not mediated by β-adrenergic receptor.

The hormones-mediators of the sympathoadrenal system, adrenaline, dopamine, norepinephrine, izadrin, and catecholamine metabolites (adrenochrome and adrenoxyl), by changing the activity of the enzymes of the respiratory chain of the mitochondria, regulate the processes of tissue respiration, transferring mitochondria into a state of "loose" conjugation of respiration and phosphorylation.

In cardiomyocytes and in hepatocytes, adrenaline, as an agonist of β-adrenergic receptors, increases the activity of enzymes of metabolism of purine nucleotides AD and AMPD, and enzymes of antioxidant protection GPO and catalase, increases the level of MDA and DC. The blockade of β-adrenergic receptors with metoprolol removes the activating effect of adrenaline on GR, GPO and catalase, reduces the level of MDA and DC.

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