



INFLUENCE OF 6''-n-CUMAROILPRUNINE ON THE RESPIRATION AND ENERGETIC FUNCTION OF MITOCHONDRIA

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ABSTRACT

It was established 6''-n-cumaroilprunine does not influence on the speed of the oxidative phosphorylation of glutamate, but decreases the speed of mitochondrial respiration in state 4. In result the parameters of oxidative phosphorylation – coefficient ADP/O and rate of respiration control by Chance are increased. 6''-n-cumaroilprunine decreases speed of succinate oxidation in different metabolic states of mitochondria, without any significant changes in parameters of oxidative phosphorylation.

KEYWORDS: oxidative phosphorylation, mitochondrial respiration, ADP/O, mitochondria, apoptosis.

1. INTRODUCTION

In recent years, herbal medicinal products have an important place in the vast arsenal of medicinal preparations. This is because many synthetic drugs have significant side effects, the herbal medicinal agents are less toxic, and they tend to act sufficiently soft.

Wide spread occurrence and universal metabolic role of flavonoids in metabolism are the basis for the study of the influences of certain some new isolated flavonoids on various aspects of metabolism.^[1-5] In this connection, identification of mechanisms, relating to the relationship between structure and biological activity of flavonoids, decoding action of these substances on the basic mechanism of metabolic complexes of cells determine the relevance of their study in physiology and medicine. Research of the influence of flavonoids on the structure and function of mitochondrial membrane is of interest not only to clarify the position of physiological and biochemical mechanisms of regulation of vital activity of an organism, but also to establish the pathogenic significance of these parameters under different stress conditions and diseases. Solving these problems sets up new challenges to determine the mechanisms of regulation of the functional state of biological membranes and thereby establish new opportunities of influence the course of these processes under stress conditions and pathologies.

It is known that there are processes in the mitochondria, resulting in cells accumulate energy and that these organelles have all the basic functions of an independent organism: contractility, ions transport and heredity.

Moreover, mitochondria play a key role in apoptosis.^[6-9]

All the above mentioned facts suggest that mitochondria are sufficiently integral elements of living things, preserving its basic properties. If so, the responses of isolated mitochondria (their metabolic status) must comply with the laws of general physiological responses of living organisms to external stimuli. In other words, we can assume that there is a correlation between the metabolic states of isolated mitochondria and is often used to describe the systemic reactions of the whole organism. We use them in the sense that in which they are used in general physiology to identify of the basic laws of the response of living tissue to external influences.

In this regard, within the framework of the molecular mechanisms study of flavonoids action, in particular, flavonones, it seemed essential to investigate influence of one of the representatives of these compounds - 6''-n-cumaroilprunine on the respiratory system and mitochondrial oxidative phosphorylation.

2. MATERIALS AND METHODS

Mitochondria were isolated from the liver of animals by conventional differential centrifugation method^[10] with some modifications.^[11]

Polarographic installation was collected on the basis of CSR-4 potentiometer connected to it polarographic attachment, supporting 0.65 volt outlet. In the installation it was used a rotating platinum electrode and a thermostat (250°C) mesh at 1 mL.^[12] The incubation medium of the following composition was added to the

mesh: 250 μ M sucrose, 10 mM Tris-HCl, pH=7.4. 6''-n-cumaroilprunine as well as mitochondria could be added to it as needed. Oxidation substrates were 10 μ M succinate, 10 μ M glutamate. The mixture was intensively mixed in polarographic mesh by rotating platinum electrodes, sensitive to oxygen. The registration process of oxygen consumption in the incubation medium started by bringing in mitochondrial suspension into the mesh (0.05 - 0.06 ml).

Experiments carried out in the absence of flavonones and with addition of 6''-n-cumaroilprunine into the polarographic mesh under in vitro conditions, in a final concentration of 20, 40 and 60 micrograms/mg of mitochondrial protein, and have studied features of mitochondrial functional state changes. Studied flavonones were kindly provided by research officers of Plant Chemistry Institute of the Academy of Sciences of Uzbekistan prof. Hushbaktova Z.A. and Syrov V.V.

Calculations of respiration parameters and mitochondrial phosphorylation were carried out on Chance-Williams criteria.^[13] Meanwhile it was determined following rates of respiratory preparations: V2 - before ADP addition, V3 - active phosphorylated state, V4 - after the ADP exhaustion in the mesh; respiratory control on Chance (RC_{Ch} - V3 / V4 ratio), as well ADP/O ratio in the oxidation of various substrates (succinate, glutamate). Isolated mitochondrial respiration rate in all metabolic conditions expressed in nanograms of oxygen/min to 1 mg of protein. Mitochondrial protein was determined by the Lowry.^[14]

3. RESULTS AND DISCUSSION

As it can be seen from the above data, 6''-n-cumaroilprunine dose-dependently reduced the rate of glutamate oxidation in the state 4, but does not influence on the rate of oxidative phosphorylation (Table 1). For example, if after addition of 20 g of 6''-n-cumaroilprunine into polarographic mesh to 1 mg of mitochondrial protein respiration rate in mitochondria of state 4 is reduced to 7.0%, at concentrations of 40 and 60 micrograms / mg protein - 14.0 and 20.0% of the control level respectively. These changes lead to an increase in the parameters of mitochondrial oxidative phosphorylation. Increase the value of the coefficient RC_{Ch} and ADP / O depends on the concentration of 6''-n-cumaroilprunine, added into the polarographic mesh. If, after addition of 20 microgram/protein into 6''-n-cumaroilprunine RC_{Ch} value and ADP / O respectively increased only to 4.4 and 6.3%, after 40 microgram/protein - 18.7 and 12.6%, after 60 microgram/mg protein - 19.0 and 39.4%. This means that 6''-n-cumaroilprunine translates mitochondria to more economical use of molecular oxygen. Meanwhile parameters of oxidative phosphorylation significantly increased.

Introduction of 6''-n-cumaroilprunine into the suspension of mitochondria brings to reduction of succinate oxidation rate in state 3 and 4 and is dependent on the concentration of used flavanone.

Table 1: Influence of 6''-n-cumaroilprunine on respiration (respiration rate, nanograms of oxygen atoms/ min of mg protein) and mitochondrial oxidative phosphorylation (M $\pm$ m; n = 8-12).

Substrates	Indexes	6''-n-cumaroilprunine, microgram/mg of protein			
		0	20	40	60
Glutamate	V ₃	68,4 $\pm$ 2,6	66,6 $\pm$ 2,4	69,8 $\pm$ 2,6	65,4 $\pm$ 2,1
	%	100	97,3	102,0	95,6
	V ₄	20,0 $\pm$ 1,1	18,6 $\pm$ 1,3	17,2 $\pm$ 1,2	16,0 $\pm$ 1,1*
	%	100	93,0	86,0	80,0
	V _{DNP}	63,2 $\pm$ 2,6	53,4 $\pm$ 2,4**	48,0 $\pm$ 2,5***	42,8 $\pm$ 3,0****
	%	100	84,5	75,9	67,7
	RC _{Ch}	3,42 $\pm$ 0,13	3,57 $\pm$ 0,12	4,06 $\pm$ 0,11**	4,07 $\pm$ 0,12**
	%	100	104,4	118,7	119,0
	ADP/O	2,54 $\pm$ 0,06	2,70 $\pm$ 0,06	2,86 $\pm$ 0,07*	3,50 $\pm$ 0,08****
Succinate	%	100	106,3	112,6	139,4
	V ₃	116,8 $\pm$ 4,4	98,6 $\pm$ 4,2**	92,0 $\pm$ 4,8***	82,0 $\pm$ 4,4****
	%	100	84,4	78,8	70,2
	V ₄	26,0 $\pm$ 2,4	22,0 $\pm$ 2,2	20,0 $\pm$ 2,0**	16,0 $\pm$ 2,2****
	%	100	84,6	76,9	61,5
	V _{DNP}	168,7 $\pm$ 6,8	140,6 $\pm$ 5,6**	121,6 $\pm$ 4,8***	99,7 $\pm$ 4,6****
	%	100	83,3	72,1	59,1
	RC _{Ch}	4,49 $\pm$ 0,13	4,48 $\pm$ 0,14	4,60 $\pm$ 0,13	5,12 $\pm$ 0,12*
	%	100	99,8	102,4	114,0
	ADP/O	1,76 $\pm$ 0,08	1,76 $\pm$ 0,07	1,88 $\pm$ 0,07	1,95 $\pm$ 0,06*
	%	100	100	106,8	110,8

All analyses are the mean of triplicate measurements $\pm$ standard deviation. Means not sharing a common letter were significantly different at P $\leq$ 0.05.

So if after administration of 20 micrograms/mg of protein and 6''-*n*-cumaroilprunine succinate oxidation rate in the 3 and 4 is reduced to 15.6 and 15.4%, respectively concerning control level, after administration of 40 microgram/mg of protein - 20.2 and 23.1%, while at 60 micrograms/mg protein - 29.8 and 38.5%. It may be noted that at low concentrations of flavanone ADP / O ratio and the value RC_{Ch} were not changed. However, after addition of 60 µg of 6''-*n*-cumaroilprunine to 1 mg of protein mitochondrial RC_{Ch} value increased up to 14.0% and ratio of ADP/O - 10.8%. The parameters of mitochondrial coupling increased. 6''-*n*-cumaroilprunine decreases dinitrophenol-stimulated oxidation of glutamate and succinate and this effect depends on its used concentration. Thus, if after administration of 20 µg of 6''-*n*-cumaroilprunine to per mg of mitochondrial protein dinitrophenol-stimulated oxidation of glutamate and succinate oxidation decreased, respectively to 15.5 and 16.7% concerning control level, then after administration of 40 micrograms /mg of protein 24.1 and 27.9% , 60 µg /mg of protein - 32.3 and 40.9%. Thus, 6''-*n*-cumaroilprunine inhibits electron transport from substrates of oxidation through mitochondrial respiratory chain to molecular oxygen.

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