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THERMOGENIC RESPIRATION IN MITOCHONDRIA OF SKELETAL MUSCLES IN WARM AND COLD-BLOODED ANIMALS

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ABSTRACT
In mitochondria of skeletal muscles of warm-blooded animals, two forms of respiration intensively function - coupled and uncoupled with ATP synthesis. In the cold-blooded animals, the uncoupled form of respiration is less developed. High-uncoupled respiration in mitochondria, considered as thermogenic, as well as efficiency, reducing the metabolic mechanism in warm-blooded organisms.

KEYWORDS: Thermogenesis, Skeletal muscles, Mitochondria, Warm and Cold-blooded Animals, Heat production, ATP-synthesis.

INTRODUCTION
Bioenergy system of mitochondria performs various functions in the body, including thermogenesis in warm-blooded organisms. There is no clear answer to the question of whether mitochondria are related to thermogenesis. In this regard, various assumptions have been made[1] that need further refinement. Therefore, since the XIX century, it was generally accepted among biologists that all biological processes in the body proceed with low efficiency and this is an integral property of life regardless of the type of animal world.[2-5] This viewpoint was adopted from physics and chemistry where heat was considered because of an entropic process. Therefore, many scientists did not associate the problem of warm-bloodedness and thermogenesis with the specifics of living things, but attributed it to one of the manifestations of the general laws of nature.[2-5]

Only in the process of certain studies, data were obtained in the direction that warm and cold-blooded organisms qualitatively, many times differ in the level of metabolism.[6-11] These results were a prerequisite for revising the nature of metabolism, in particular, for establishing the biological mechanism of thermogenesis, which can be responsible for the consumption of up to 80 - 90% of metabolic energy in the body, and only about 10-20% of the body energy of warm-blooded animals can be used for vital functions. Cold-blooded animals generate little heat, so little oxygen is consumed. Moreover, the efficiency of using the energy of metabolism in the latter is significantly higher than in warm-blooded animals.[12-15] It is possible that at the subcellular level, these groups of animals have different energy metabolic pathways. This question was not very popular at that time and was not widely considered in the literature. A comparative approach was also used at the mitochondrial level by studying their energetics in warm and cold-blooded organisms. Previous results showed that there is no qualitative difference between mitochondria of the compared animals, but only quantitative differences, which are not always clearly expressed.[16-20] In these works, the main way was studied phosphorylated ATP synthesizing respiration of mitochondria of tissues of different animal groups.

However, the study of this issue continued and in this regard, significant progress was made. The presence of uncoupling proteins in the inner mitochondrial membranes of various tissues was discovered.[20] However, thermogenic significance has been considered in brown adipose tissue.[21] In the mitochondria of other tissues, the uncoupling effect of these proteins was not specific, but an increase in proton leakage by these proteins into the inner mitochondrial membrane is indicated.[21-23] A comparison was carried out for membrane proton leakage in mitochondria of tissues in animals with different temperature status. It must be said that no large differences were found in the intensity of proton leakage in different groups of animals, although a lower level of this indicator was noted in cold-blooded animals.[24] It is believed that proton leakage is an important condition for the reduction of reactive oxygen

species formed in mitochondria during oxidative processes.

There are also other works devoted to the study of coupled (ATP-synthesis) and uncoupled respiration in tissue mitochondria in different groups of animals. These studies showed uncoupled mitochondrial respiration, which showed about a 10-fold difference between mitochondria of different groups of animals for this indicator. The obtained results gave grounds for the continuation of comparative studies in this regard. In the available works,[25-27] it is believed that the uncoupled form of respiration is associated with mitochondria of warm-blooded tissues. Their mitochondria are able to carry out not only coupled ATP synthesizing respiration, but also uncoupled respiration, which was the subject of additional research in this work using the mitochondria of skeletal muscles of warm and cold-blooded animals.

MATERIALS AND METHODS

Isolation of mitochondria from various animal tissues and the study of their respiration. Mitochondria from skeletal muscles were isolated by differential centrifugation,[28, 29] After decapitation of the animals, the necessary tissues were removed from the body cavity of the animal and placed in a cooled isolation medium containing 300 mM sucrose, 10 mM Tris-HCl (pH 7.5). This medium also contained 2 mM EDTA and 1 mg/ml bovine serum albumin (BSA). After preliminary grinding with a micropress, the tissue was homogenized in a homogenizer with a Teflon pestle in a 10-fold volume of isolation medium,[28,29] The homogenate was centrifuged at 700×g for 7 min. Mitochondria were precipitated from the supernatant at 6000×g for 20 min. The mitochondrial sediment was suspended in the same isolation medium (about 30–40 mg protein/ml) and stored in the cold at 0–2°C. Mitochondrial protein was determined according to Lowry method.[30] Oxidation of various substrates in mitochondria was measured polarographically using a rotating platinum electrode.[30] The incubation mixture contained 120 mM KCl, 5 mM KH_2PO_4, 2 mM EDTA, 10 mM Tris-HCl, pH 7.5. The following substrates were used: 5 mM succinate, 1 mM NADH, NADH + cytochrome c 1 mg, 20 mM ascorbate + 2.5 mg cytochrome c per ml, ADP was added to the chamber in portions of 100 μM. The phosphorylation process in mitochondria was assessed according to Chance-Williams.[31] The following symbols are used: V3 - respiration during phosphorylation, V4 - respiration after phosphorylation, Polarographic recordings of mitochondrial respiration were made at 25°C.

RESULTS AND DISCUSSIONS

It must be said that mitochondria, as the energy system of the cell, has long been the subject of research by scientists and to our time they can present certain surprises, in particular, when comparing warm and cold-blooded organisms.

In this work, we have studied mitochondria of skeletal muscles of different animals. In relation to phosphorylated ATP-synthesizing respiration, Table 1 shows a certain difference between the compared animals. Thus, in warm-blooded rats, succinate oxidation occurs at an increased rate of V3 and V4. Under the same conditions, the rates of glutamate oxidation are slower. Therefore, according to the value of metabolic rates, it can be seen that here the respiration rates are much lower than on the succinate substrate, and the respiration control value (respiratory coupling) is noticeably higher on glutamate.

The obtained data on mitochondria of rat muscles show that they are characterized by higher metabolic rates for succinate (FAD-dependent substrate) than for glutamate (NAD-dependent substrate. Succinate also shows less coupling of oxidation (less RC value) with ATP synthesis than glutamate. In general, the difference between these oxidation substrates is quite large. Unequal coupling between these oxidation substrates can have a certain physiological meaning. The more uncoupled oxidation of succinate indicates its greater thermogeny than the oxidation of glutamate.

As previously shown, the oxidation of these two substrates occurs along two different respiratory chains[1] Along the coupled (glutamate) and partially along the uncoupled pathway (succinate).

Table 1: Mitochondrial respiration in skeletal muscles of marsh frogs, turtles and rats (substrates - succinate and glutamate, 4 mM each).

Oxidation substrates	V_3	V_4	RC	ADP/O
Rat tissue mitochondria				
succinate	117±12.1	57.0±5,8	2.1	1.6±0.3
glutamate	68.7±7.1	18.05±2.1	3.8	2.6±0.4
Mitochondria of marsh frog tissues				
succinate	41.6±3.4	11.9±2.1	3.5	1.8±0.21
glutamate	31.5±3.1	7.56±2.1	4.2	2.65±0.3
Turtle muscle mitochondria				
succinate	30.2±3.2	8.4±1.6	3.6	1.8±0.3
glutamate	24.4±2.1	5.54±1.1	4.4	2.7±0.4

V_3 V_4 – respiration rate of mitochondria in nanograms of oxygen atoms per minute per milligram of protein - (ng-at O/min mg of protein).

In the study of mitochondria of frogs and turtles skeletal muscles, we obtained certain important differences from the mitochondria of rats. Thus, the difference between succinate and glutamate in cold-blooded animals is less pronounced (Table 1). In cold-blooded animals, the rate of oxidation is lower and the coupling of respiration with the process of ATP synthesis is higher, since mitochondria have high RC and ADP/O values on both succinate and glutamate.

Studies on cold-blooded animals showed the possibility of other metabolic pathways in the oxidation of substrates; in particular, their mitochondria are more coupled during the oxidation of various substrates. In warm-blooded rats, mitochondrial respiration on succinate can be directly related to heat production, since, in addition to phosphorylating oxidation, it has a higher level of uncoupled oxidation. Earlier, a similar phenomenon was found on mitochondria of other tissue warm-blooded animals.[25] Mitochondria of cold-blooded organisms are characterized by a significantly lower severity of uncoupled oxidation of substrates that is confirmed in further studies.

Earlier, in previous works, it was shown that in mitochondria of warm-blooded organisms other substrates are also oxidized in addition to succinate in an uncoupled way, particularly NADH.[25,26] It was of interest to study the manifestation of such oxidation in mitochondria of such a massive body tissue as skeletal muscle. Table 2 shows the results of the studies carried out in a comparative way.

Table 2: Uncoupled oxidation of NADH and ascorbate in mitochondria of skeletal muscles of different animals.

Animals	NADH	NADH+ cytochrome c	Ascorbate +cytochrome c
Rats	82.6± 3,2	155.8±5,4	165.7±7,5
Marsh frogs	15, 21±1.4	17.81± 1,8	32,61±2,6
Steppe turtle	6,4±0,8	11,3±1,1	18,6±1.6

Mitochondrial respiration rate is presented in nanogram atoms oxygen in min of mg of protein (ng-atom O/min mg of protein).

As shown in the table, the NADH substrate is oxidized very intensively in the mitochondria of rat skeletal muscles, and in the presence of cytochrome c, its oxidation is further enhanced. This oxidation is uncoupled, since it does not change when ADP or the uncoupler - dinitrophenol is added to mitochondria. Consequently, this oxidation is not involved in ATP synthesis and can be directly related to heat production, as it proceeds intensively in a warm-blooded animal.

Use of NADH as a substrate for NADH oxidase in mitochondria of skeletal muscles of frogs and turtles has shown that its oxidation proceeds at a very low rate. Moreover, the addition of cytochrome c causes only a slight stimulation of oxidation. It can be said that uncoupled oxidation is poorly expressed in mitochondria of skeletal muscles of cold-blooded organisms and may be directly related to maintaining a low level of metabolism in these groups of animals.

Table 2 also shows the features of the oxidation of ascorbate + cytochrome c - as a substrate of cytochrome oxidase in mitochondria of skeletal muscles of warm and cold-blooded animals. It can be seen that this substrate is intensively oxidized in mitochondria of warm-blooded rats and is poorly utilized in mitochondria of cold-blooded animals. This oxidation is also uncoupled with ATP synthesis, since it is not affected by ADP and dinitrophenol (not shown).

Therefore, it is directly related to heat production.

Studies have shown that an uncoupled respiratory chain functions in mitochondria of skeletal muscles of warm-blooded animals that intensively oxidizes NADH and ascorbate + cytochrome c, and partially succinate. This respiratory chain is very weak in mitochondria of skeletal muscles of cold-blooded animals.

CONCLUSION

This uncoupled respiratory system is not the result of mitochondrial damage during homogenization of muscle tissue or by centrifugation, as previously suggested.[32] We have previously checked their nativeness by studying the nature of the manifestation of uncoupled oxidation of various substrates in a cell preparation.[33] It was confirmed that the uncoupled oxidation of the above investigated substrates occurs uncoupled and with high intensity even inside isolated cells. Therefore, comparative studies were carried out in mitochondria of warm and cold-blooded animals, which made it possible to show the relationship of uncoupled respiration with thermogenesis, as well as to establish a number of functional features of the mitochondrial system.

Author contributions

ARN designed the study, MEI, AGR, NBA, KVA conducted the research, ARN analyzed the data, NBA drafted manuscript, ARN and NBA participated in the write up and critical review of the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTERESTS

The authors declare that they have no any conflict of interests.

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