RESPIRATION AND RESPIRATORY CHAIN PHOSPHORYLATION IN HOMOGENATES AND MITOCHONDRIA OF RABBIT CARDIAC MUSCLE DURING EXPERIMENTALLY INDUCED MYOCARDITIS

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(Presented by S. E. Severin, Active Member, Acad. Med. Sci. USSR)
Translated from Bylleten' Éksperimental'noi Biologii i Meditsiny, Vol. 51, No. 3, pp. 55-58, March, 1961
Original article submitted May 28, 1960

The process of oxidative phosphorylation has considerable significance for the normal activity of cardiac muscle, in that it leads to the accumulation of high-energy phosphate bonds in the form of adenosine triphosphate. Inhibition of tissue respiration disrupts the activity of the heart by slowing down the resynthesis of adenosine triphosphate [7].

The results of biochemical investigations, devoted to studying the processes of respiration and respiratory chain phosphorylation in cardiac muscle during various pathological states of the heart, are not unequivocal. For example, it was observed that the rate of oxidative phosphorylation in cardiac muscle does not decrease during diphtheria toxicosis [5] or thyrotoxicosis [6]. On the other hand, during myocarditis arising as a result of adrenaline injections, a decrease in oxidation-reduction reactions in the heart [4] and weakening of respiratory chain phosphorylation [3] were noted.

In the present investigation, the processes of respiration and respiratory chain phosphorylation in the tissue of rabbit cardiac muscle were studied during experimentally induced mycarditis.

EXPERIMENTAL METHODS

As experimental animals we chose male rabbits, weighing 2-2.5 kg. Myocarditis was produced in the animals by injecting a 1% solution of theophylline (20 mg per kg body weight), followed after two minutes by an injection of 0.2 ml of a 0.1% solution of adrenaline.

The processes of respiration and respiratory chain phosphorylation were studied in homogenates of cardiac muscle on the third or fourth day after injection of theophylline and adrenaline.* Experimental techniques did not differ from those used in a previous investigation [2].

The added respiratory substrates were pyruvic, α-ketoglutaric, succinic, malic, citric, and β-hydroxybutyric acids. The terminal concentration of these acids in the incubation tube was 0.025 M.

To isolate the mitochondria of cardiac muscle (after perfusion with cool 0.15 M KCl solution), the tissue was cut up with scissors and homogenized in the cold in two volumes of 0.25 M sucrose solution with 0.001 M ethylenediaminetetraacetate, and the pH of the solution was brought to 7.4 with 2 N KOH. After homogenizing

* From histological evidence, the characteristic changes of myocarditis are already detectable on the third day after injection of these compounds.

for 30 seconds, seven more volumes of the same solution were added to the minced tissue, and afte filtration through several layers of gauze, the homogenate was fractionated into cellular components by differential centrifugation in the cold.

Mitochondria, isolated at 7000 g, were subjected to a single rinse.

The isolated mitochondria were suspended in a 0.25 M sucrose solution at pH 7.4. A quantity of sucrose solution equal to the original weight of cardiac muscle was used. The mitochondrial suspension was rapidly poured out into Warburg vessels. Each vessel contained 1 ml of incubation mixture, consisting of 0.5 ml of mitochondrial suspension (corresponding to 0.9-1.2 mg of protein nitrogen), 0.3 ml of buffer solution, 0.1 ml of sucrose solution (0.25 M) with respiratory substrate, and 0.1 ml of hexokinase solution. The concentrations of the various components in the buffer solution (pH 7.4) were: K_2HPO_4, $3 \cdot 10^{-2}$ M; $MgSO_4$, $1.5 \cdot 10^{-2}$ M; adenosine triphosphate, $6 \cdot 10^{-3}$ M; glucose, $5 \cdot 10^{-2}$ M. The respiratory substrates were α-ketoglutaric acid (along with malonic acid), succinic acid, and β-hydroxybutyric acid. The terminal concentration of these acids in the incubation tube was the same as in the experiments with homogenates. Hexokinase was obtained from yeast [9].

Experiments with mitochondria were conducted under an atmosphere of air, and oxygen absorption was measured in the Warburg apparatus. Incubation was carried out at 26° for 18 minutes. After incubation, inorganic phosphate [8] was determined in experiments with mitochondria, while inorganic phosphate and creatine phosphate [1] were determined in experiments with homogenates.

The rate of phosphorylation was computed from the disappearance of inorganic phosphate and formation of creatine phosphate. The quantity of protein in the incubation tubes was determined by the biuret reaction.

EXPERIMENTAL RESULTS

Figure 1 depicts results describing the rate of respiration and respiratory chain phosphorylation in homogenates of rabbit cardiac muscle under normal conditions and during experimentally induced myocarditis.

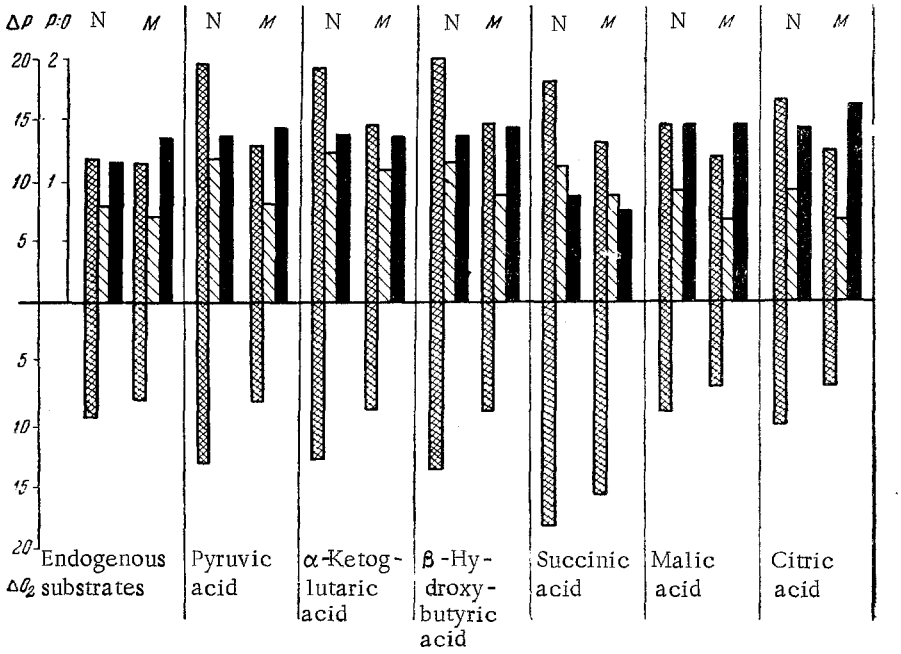

Fig. 1. Respiration and respiratory chain phosphorylation in homogenates of rabbit cardiac muscle under normal conditions (N) and during myocarditis (M). ΔO_2) Oxygen absorption (in micromoles); ΔP) binding of inorganic phosphate (in micromoles), cross-shaded columns. Obliquely shaded columns give formation of creatine phosphate (in micromoles of phosphorus); black columns give the P: O ratio.

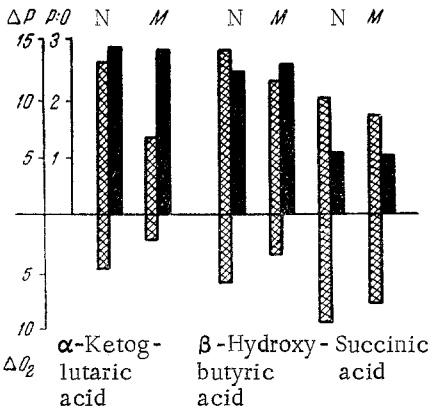

Fig. 2. Respiration and respiratory chain phosphorylation in mitochondria of rabbit cardiac muscle under normal conditions (N) and during myocarditis (M). ΔO_2) Oxygen absorption (in micromoles); ΔP) binding of inorganic phosphate (in micromoles). Black columns give P: O ratio.

It is apparent that in the presence of endogenous substrates, respiration and phosphorylation are practically the same during myocarditis as under normal conditions, but when components of the tricarboxylic acid cycle are added to the medium, the rate of these processes decreases during myocarditis.

Since respiration and phosphorylation decrease proportionately, the P: O ratio remains the same as under normal conditions.

It should be noted that not only under normal conditions but also during myocarditis only 50-60% of the inorganic phosphate taken up from the medium appeared in the form of creatine phosphate. Moreover, under normal conditions a small part of the inorganic phosphate that disappeared was found in the form of fructose diphosphate, which could not be detected during myocarditis.

In the presence of added respiratory substrates, uptake of inorganic phosphate during myocarditis differed much more from the norm than did the formation of creatine phosphate, probably because creatine phosphate is formed more rapidly than other phosphorus compounds during myocarditis.

For further study of respiration and respiratory chain phosphorylation in cardiac muscle during myocarditis, experiments were continued on isolated mitochondria.

Figure 2 depicts results concerning the rate of respiration and respiratory chain phosphorylation (in the presence of various respiratory substrates) in mitochondria of rabbit cardiac muscle under normal conditions and during experimental myocarditis. It is evident that the rate of these processes in mitochondria decreases during myocarditis. When α-ketoglutaric acid is being oxidized, the rate of respiration as well as of phosphorylation decreases by 50%, in comparison with the normal. Because the rate of both processes decreases proportionately, the P: O ratio remains the same as under normal conditions. When β-hydroxybutyric acid is being oxidized, the rate of these processes decreases by 26% during myocarditis, but, just as in the case of α-ketoglutarate, the P: O ratio remains the same as under normal conditions. When succinic acid is being oxidized, an insignificant (~14%) decrease in the rate of respiration and phosphorylation is observed, without a change in the P: O ratio.

The results obtained from homogenates indicate that the rate of respiratory processes and formation of energy-rich phosphate compounds in the presence of endogenous respiratory substrates does not decrease in cardiac muscle during myocarditis. When the concentration of these substrates is increased by adding components of the tricarboxylic acid cycle to the medium, cardiac muscle makes less use of added substrate during myocarditis than under normal conditions, resulting in a lowered rate of oxygen absorption and associated respiratory chain phosphorylation.

Results obtained from mitochondria of cardiac muscle during myocarditis support this conclusion. As a result of proportional decreases in the rates of both processes, the values of the P: O ratio remain the same during myocarditis as under normal conditions.

The decreased rate of oxidation of α-ketoglutaric acid and β-hydroxybutyric acid provides a basis for supposing that during myocarditis, damage occurs to the dehydrogenase system connected with diphosphopyridine nucleotide in the heart. The greater decrease in the rate of oxidation of α-ketoglutaric acid is probably connected with the lower content of free and total thiamine in cardiac muscle during experimentally induced myocarditis [4]. As is well known, thiamine pyrophosphate, like diphosphopyridine nucleotide, is a component part of the dehydrogenase for α-ketoglutaric acid.

The insignificant decrease in oxidation of succinic acid may be interpreted as a consequence of the insignificant change in the system of flavinoid enzymes and the cytochrome part of the respiratory chain.

SUMMARY

In experimental myocarditis provoked in rabbits by intravenous injection of adrenaline and theophylline, the intensity of the respiratory processes and associated phosphorylation decreased in the homogenates of cardiac muscle and isolated mitochondria in the presence of respiratory substrates added to the medium. Reduction of respiratory intensity and of phosphorylation occurs gradually and evenly, the value of P:O coefficient in myocarditis thus equalling that in normal conditions. Experiments with mitochondria show that the system connected with the oxidation of the α-ketoglutaric acid is the one to be most markedly disturbed in myocarditis.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
