EFFECT OF HEPARIN ON THE MITOCHONDRIAL ATPase SYSTEM OF RABBIT WHITE MUSCLES

E. E. Dubinina and T. Ya. Nadirova

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Heparin, as a factor stabilizing the structure of mitochondrial membranes, helps to preserve the level of DNP-activated ATPase of aging mitochondria of rabbit white muscles and leads to a higher level of activation of Mg^{++}-ATPase by 2,4-DNP in intact preparations.

Investigation of mitochondrial ATPase is an interesting part of the study of the bioenergetics of muscle tissue. Data on the effect of fatty acids on the activity of the mitochondrial ATPase system are given in the literature [6, 7].

It was decided to study the effect of heparin, a phosphorylase inhibitor, on the ATPase activity of the mitochondrial fraction of skeletal muscles.

EXPERIMENTAL METHOD

Adult chinchilla rabbits weighing 2.5-3 kg were used. Mitochondria were isolated from the white muscles of the thigh by the usual method with the medium of Chappell and Perry [5]: 0.1 M KCl, 0.005 M $MgCl_2$, 0.05 M Tris-HCl, 0.001 M ATP, and 0.001 M EDTA (pH 7.4). The isolation medium also contained 0.5% heparin. The freshly isolated mitochondria were suspended in 0.25 M sucrose.

The integrity of the mitochondria was determined by studying the ratio between the marker enzyme cytochrome-c-oxidase (CCO) in the anuclear homogenate, the mitochondrial fraction, and the post-mitochondrial supernatant. CCO activity was measured on the SF-4 spectrophotometer by the method of Potter and Albawn in the modification of Cooperstein and Lazarow [8]. The concentration of oxidized cytochrome c was calculated from the difference between the coefficients of molar extinction for reduced and oxidized cytochrome c at λ 550 nm [10]. Activity of the enzyme was determined in preparations treated with 0.5% sodium deoxycholate made up in 0.1 N NaOH solution and expressed per milligram protein and per gram tissue per minute.

The ATPase activity was measured by the inorganic phosphorus set free during incubation for 15 min at 37°C in a medium containing 0.075 M KCl, 0.05 M Tris-HCl (pH 7.4), 0.0015 M $MgCl_2$, 0.0001 M 2,4-DNP 0.006 M Na_2ATP, and 0.2-0.8 mg mitochondrial protein; total value 1.5 ml. The enzymic reaction was stopped by the addition of 6 ml 3.3% TCA. The value of P_{in} was determined by the method of Lowry and Lopez [13] in Skulachev's modification [2]. Enzyme activity was expressed in μmoles P_{in}/mg mitochondrial protein/h. Protein was determined by Lowry's method [12].

EXPERIMENTAL RESULTS AND DISCUSSION

During isolation of the mitochondria 11 ± 1.4% of CCO activity of the anuclear homogenate, the activity of which was taken as 100%, was found in the post-mitochondrial supernatant, indicating that only slight solubilization of the enzyme had occurred [1].

The freshly isolated, intact mitochondria had low endogenous ATPase activity. Mg^{++}, in a concentration of $3 \cdot 10^{-3}$ M, induced an equal degree (fourfold) of activation of the mitochondrial enzyme obtained in

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TABLE 1. ATPase Activity (in moles P_{in}/mg protein/h) of Intact and Aging Mitochondria of Rabbit White Muscles (M ± m)

Experimental conditions	Chappell–Perry medium				Chappell–Perry medium and heparin			
	no. of exper.	freshly isolated mitochondria	no. of exper.	aging mitochondria	no. of exper.	freshly isolated mitochondria	no. of exper.	aging mitochondria
No activators added	11	2,94±0,40	11	2,94±0,40	7	1,54±0,32	7	1,42±0,52
Activation by 2,4-DNP ($5 \cdot 10^{-5}$ M)	11	11,16±2,14	11	4,68±0,42	7	5,32±0,82	7	5,74±1,14
Activation by Mg^{++} ($3 \cdot 10^{-3}$ M)	9	10,16±0,78	19	31,10±4,67	7	6,54±1,78	7	13,26±1,68
Activation by Mg^{++} ($3 \cdot 10^{-3}$ M) and 2,4-DNP ($5 \cdot 10^{-5}$ M)	9	13,04±0,83	8	23,92±3,53	7	14,78±2,78	7	13,44±1,94

the isolation medium in the absence and in the presence of 0.5% heparin (Table 1). Aging of the mitochondria at 20°C for 2-2.5 h led to a sharp increase (by 9-10 times) in Mg^{++}-ATPase activity. 2,4-DNP ($5 \cdot 10^{-5}$ M) caused activation (fourfold) of endogenous ATPase in the "heparin" and "nonheparin" mitochondria.

Aging of the "nonheparin" mitochondria was accompanied, as might be expected, by a decrease in DNP-activated ATPase activity. Heparin helped to maintain the level of DNP-ATPase in the aging mitochondria and increased their Mg^{++}-ATPase activity. The degree of activation of the Mg^{++}-ATPase of the freshly isolated mitochondria by 2,4-DNP was much higher (twice) if heparin was added to the isolation medium.

Heparin had no effect on the distribution of CCO activity in the subcellular fractions, but its addition to the isolation medium prevented the total liberation of the enzyme from mitochondrial preparations treated with deoxycholate.

The experimental results suggest that heparin is a factor stabilizing the structure of the mitochondrial membranes of skeletal muscles.

Heparin is known to be a competitive inhibitor of phosphorylase [4]. Addition of heparin to the isolation medium probably slows the rate of liberation of fatty acids which inhibit DNP-ATPase [3, 11]. This is a possible cause of the preservation of DNP-ATPase in aging "heparin" mitochondria.

Fatty acids are known not to affect Mg^{++}-ATPase activity of the mitochondria of skeletal muscles unlike the liver mitochondria [9]. It is therefore interesting to note the increased activity of Mg^{++}-ATPase in both the "heparin" and the "nonheparin" aging mitochondria observed in the present experiments. However, in the aging "heparin" mitochondria DNP did not stimulate Mg^{++}-ATPase activity. The results of these experiments suggest that the stabilizing action of heparin is not universal.

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