EFFECT OF STRENUOUS PHYSICAL TRAINING OF RATS ON CONTENT AND BIOSYNTHESIS OF UBIQUINONE IN THEIR SKELETAL MUSCLES

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The ubiquinone content was determined in the skeletal muscles and muscle mitochondria of rats after undergoing strenuous physical training for 2.5-3 months. The incorporation of the labeled precursor acetate-1-^{14}C into ubiquinone and sterols by thin slices of skeletal muscles was investigated at the same time. An increase in the ubiquinone content and production of mitochondrial protein was observed in the muscles of the trained animals. Incorporation of the radioactive label into ubiquinone by thin muscle slices from trained rats was considerably increased, whereas its incorporation into sterols was unchanged.

KEY WORDS: ubiquinone; sterols; biosynthesis; mitochondria; skeletal muscles; physical exertion.

Prolonged strenuous physical training of rats leads to a marked increase in the concentration of mitochondrial protein and activity of mitochondrial enzymes in the limb muscles [6, 7]. Ubiquinone (coenzyme Q) transfers reducing equivalents from various mitochondrial dehydrogenases to the cytochrome system [8]. It synthesized directly in animal tissues [10].

The ubiquinone content was accordingly determined in skeletal muscles and muscle mitochondria of control and trained rats. The incorporation of the labeled precursor, acetate-1-^{14}C, into ubiquinone and sterols by thin slices of skeletal muscles also was investigated.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing initially 130-140 g. The animals of group 1 were trained by running on a treadmill; the animals of group 2 were the control and were kept under the same conditions and received the animal house diet ad lib. These animals were trained for 6 days a week. The intensity of the load was gradually increased [11]. To begin with the rats ran for 10 min at a speed of 22 m/min twice a day with an interval of 4 h. After 3 weeks the rats ran for 40 min a day at a speed of 22 m/min with an interval of 4 h. After 6 weeks the rats ran twice a day for 60 min each time, at the same speed and with an interval of 4 h. After 9 weeks they ran for 120 min daily at a speed of 33 m/min. The rats were used in the experiments 2.5-3 months after the beginning of training. Rats which did not complete their training program were excluded from the experiments.

About 24 h after the last training the rats were decapitated, the white and red muscles were removed simultaneously from their hind limbs, and their ubiquinone content was determined [4]. Some of the mice were used for isolation of the mitochondria [2]. The concentrations of ubiquinone and protein were determined in the mitochondria [9].

The biosynthesis of ubiquinone and sterols was determined by measuring the rate of incorporation of acetate-1-^{14}C by thin slices of the hind limb muscles. Four grams of slices were cut manually from one rat [3] and incubated in special vessels in 15 ml of Krebs-Ringer-phosphate medium, pH 7.4, for 3 h at 37°C with 40 μCi acetate-1-^{14}C [5]. The slices were then inactivated by placing the incubation vessels in boiling water

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TABLE 1. Effect of Training on Ubiquinone Content in Skeletal Muscles and Incorporation of Radioactive Label by Thin Muscle Slices into Ubiquinone and Sterols (M ± m)

Control of animals	Yield of mitochondrial protein, mg/g	Content of ubiquinone		Incorporation of ^{14}C into ubiquinone, counts/min/mg protein	Biosynthesis of sterols	
		μg/g wet weight of tissue	μg/mg mitochondrial protein		mg/g wet weight of tissue	counts/min/mg protein
Control	4,68±0,33 (7)	18,80±2,52 (7)	1,24±0,17 (4)	2964±1136 (4)	0,74±0,15 (4)	1218±554 (4)
Trained	6,42±0,47 (6)	30,50±4,91 (6)	0,88±0,13 (6)	9166±2597 (7)	0,68±0,06 (7)	853±69 (7)
P	1,1%	6%	11%	6%	>5%	>5%

Legend. Number of experiments given in parentheses.

for 3 min. The liquid was drawn off exactly. The sections were hydrolyzed with an alcoholic solution of alkali and the unsaponifiable lipids were isolated [3]. Ubiquinone was isolated from the unsaponifiable lipids by chromatography on a thin layer of silicagel [3] and purified to constant specific activity by chromatography on thin layers in the following system of solvents: 1) benzene; 2) 15% acetone in petroleum ether; 3) 95% aqueous solution of acetone; 4) N-hexane; 5) benzene. The isolated ubiquinone was placed on a target and its radioactivity and weight determined [3]. During the process of isolation and of obtaining radiochemically pure ubiquinone losses are inevitable; for that reason, the efficiency of incorporation of label into ubiquinone was judged from the specific radioactivity. After the first chromatography sterols were removed from the chromatogram, extracted with ether, and then precipitated with a 1% alcoholic solution of digitonin. The sterol-digitonins were washed and the residue suspended in methanol. One part was used for the determination of radioactivity, the other part for quantitative determination with acetic anhydride in sulfuric acid [5]. Crystalline cholesterol was used as the standard.

The results were subjected to statistical analysis [1].

EXPERIMENTAL RESULTS

As Table 1 shows, the yield of mitochondrial protein from muscle homogenates during differential centrifugation and concentration of ubiquinone in the hind limb muscles of the trained rats was 37 and 62% higher, respectively, than in the control animals. The specific concentration of ubiquinone in the mitochondria of the trained rats was a little lower (not significantly) than in the controls. Since more than 50% of the ubiquinone was in the mitochondrial fraction, the specific concentration of mitochondrial protein per gram wet weight of tissue evidently increased considerably during training [6, 7].

Incorporation of the radioactive label into ubiquinone by thin slices of skeletal muscles of the trained animals was three times higher than in the controls. However, marked fluctuations in the rate of incorporation of label in individual animals must be noted. The content and biosynthesis of sterols in the trained rats were virtually indistinguishable from those in the controls. It will also be noted that the specific radioactivity of ubiquinone in the animals of both groups was greater than the specific radioactivity of the sterols. By contrast with the liver, in muscles ubiquinone is evidently synthesized more rapidly than sterols [10]. The main reactions of ubiquinone biosynthesis are known to take place in the inner mitochondrial membrane [12]. An increase in the rate of incorporation of radioactive label into ubiquinone by thin muscle slices of trained rats is evidently attributable to an increase in the capacity of the enzyme system for its biosynthesis. In addition, an important cause of the increased activity of the enzyme system for ubiquinone biosynthesis in the muscles is activation of thyroid gland function under the influence of physical exertion [13].

Thyroid hormones are known to stimulate ubiquinone biosynthesis in animal organs [12].

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EFFECT OF DEXAMETHASONE ON RNA SYNTHESIS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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The action of dexamethasone on lymphoid tissue in a culture of partially purified peripheral blood lymphocytes was biphasic in character. After incubation of lymphocytes in vitro with the hormone for 6 h stimulation of RNA synthesis was found. Sedimentation analysis of labeled RNA fractionated on a column containing poly-V-sepharose indicated an increase in the mRNA content and enrichment of cytoplasmic RNA with polyA sequences. Meanwhile Mn^{++}-dependent RNA-polymerase, sensitive to α-amanitine, was activated. After cultivation of the lymphocytes with the hormone for 24 h, RNA synthesis was inhibited. The biphasic character of the action of the steroid also was observed in the rosette-formation test.

KEY WORDS: lymphocytes; RNA synthesis, dexamethasone.

It was in 1967 that Kidson [9] postulated and obtained experimental evidence for the relatively biphasic character of the action of glucocorticoids on lymphoid tissue. Munck [11] suggested that one of the first manifestations of the action of glucocorticoids on lymphoid tissue should be stimulation of the synthesis of "specific forms of RNA. However, the view is still held that the mechanisms of hormonal regulation of lymphocytes and of a parenchymatous organ such as the liver are in principle different, if not opposite. Under the influence of glucocorticoids, metabolic processes in the liver are stimulated, whereas in lymphoid tissue the synthesis of RNA and protein, on the contrary, is depressed.

In the investigation described below various stages of RNA synthesis were studied during exposure of small human lymphocytes to dexamethasone.

EXPERIMENTAL METHOD

Small lymphocytes were separated from the total leukocyte pool of blood by sedimentation on a nylon column by the writers' modification of the method of Shapot and Gorozhanskaya [3]. The leukocytes were retained on the nylon column for 30 min at 37°C in an atmosphere containing 5% CO_2. The suspensions studied consisted to the extent of 95-98% of small lymphocytes. The concentration of cells in the suspension was adjusted to 6×10^6 cells/ml. The lymphocytes were incubated in medium No. 199 with 20% autologous plasma. Dexamethasone was added simultaneously with the beginning of incubation up to a final concentration of 60 μg/ml. The nuclei were isolated from the lymphocytes in a medium of 0.32 M sucrose with 0.01 M $MgCl_2$ and Triton X-100 in a final concentration of 0.5%, followed by disintegration of the cells in a Potter-Elvehjem homogenizer. The

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