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INDUCTION OF ELECTROGENIC PHOSPHATE TRANSPORT THROUGH THE MITOCHONDRIAL MEMBRANE BY THERMOSTABLE CYTOPLASMIC FACTOR IN HYPERTHYROIDISM

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KEY WORDS: hyperthyroidism; mitochondria; oxidative phosphorylation; electrogenic phosphate transport.

One of the main effects of thyroid hormones is stimulation of oxygen consumption in target cells [2]. The mechanism of stimulation of basal metabolism in hyperthyroidism is not yet known. It has been shown that mitochondria isolated from tissues of hyperthyroid rats perform oxidative phosphorylation in experiments *in vitro* with high P/O and ADP/O ratios and high respiratory control [6, 10]. In the intact cell the state of mitochondrial function is controlled by cytoplasmic regulators, whose action as a rule is to change activity of cation carriers and metabolites in the inner mitochondrial membrane [1, 5, 11].

It can be tentatively suggested that stimulation of oxygen consumption in hyperthyroidism is due to changes in activity of cytoplasmic regulators of mitochondrial metabolism. The aim of this investigation was to test this hypothesis.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 100-120 g. Mitochondria were isolated from the rats' liver by the method in [8]. The mitochondrial isolation medium consisted of 0.3 M sucrose, 5 mM Tris-HCl, 1 mM EDTA, pH 7.5. Oxidation of succinate by the mitochondrial suspension was measured polarographically. The kinetics of mitochondrial swelling was determined from the change in optical density at 540 nm on the LMF-69 photometer. Hyperthyroidism was induced by injection of thyroxine into the rats in a dose of 100 μg/100 g body weight daily for 5 days. The last injection of thyroxine was given 20 h before sacrifice. Cytoplasm was isolated from the liver and heart of satiated rats by centrifugation of the homogenate (1 g/ml of isolation medium containing 0.12 M KCl, 5 mM Tris-HCl, pH 7.65) for 20 min at 30,000g. Immediately after centrifugation in the cold the supernatant was heated to 97°C for 7 min, after which the denatured proteins and membrane fragments were centrifuged. The thermostable fraction of cytoplasm was kept in a refrigerator at −10°C. In the experiments with addition of thermostable cytoplasmic fraction to the mitochondrial suspension, 0.12 M KCl was added in the control experiment.

EXPERIMENTAL RESULTS

Preincubation of rat liver mitochondria for 2 min with thermostable cytoplasmic fraction of rat liver increased the rate of swelling of the mitochondria in iso-osmotic solution of potassium phosphate in the presence of valinomycin (Fig. 1). Under these conditions valinomycin is known to induce high permeability of the inner mitochondrial membrane for K^+ and swelling of the mitochondria is limited by the rate of transport of the anion through the mitochondrial membrane [5]. Consequently, in these experiments preincubation of the mito-

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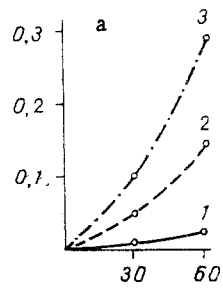

Fig. 1

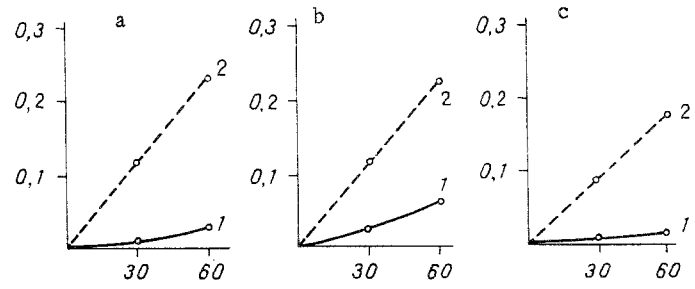

Fig. 2

Fig. 1. Effect of hyperthyroidism on activity of thermostable cytoplasmic inducer of phosphate tranport in rat liver (a) and heart (b). 1) Without addition of cytoplasm; 2) 0.1 ml cytoplasm of control rats; 3) 0.1 ml of cytoplasm of hyperthyroid rats. Mitochondria preincubated for 2 min in presence of thermostable cytoplasmic fraction (0.4 ml of preincubation medium + 0.1 ml cytoplasm) before addition of 3 ml incubation medium. Preincubation medium: 120 mM KCl, 10 mM Tris, pH 7.6. Incubation medium: 80 mM KH_2PO_4, 10 mM Tris, 10^{-4} M 2,4-DNP, 1 μg/ml rotenone, 1 μg/ml valinomycin, pH 7.6. Mitochondria — 0.4 μg protein/ml. Abscissa, time (in sec); ordinate, increase in optical density of suspension at 540 nm.

Fig. 2. Effect of 2,4-DNP and N-ethylmaleimide on phosphate transport through mitochondrial membrane in presence of thermostable cytoplasmic fraction from rat liver. 1) Control; 2) 0.2 ml cytoplasm. a) Incubation medium; b) incubation medium + 2,4-DNP (10^{-4} M); c) incubation medium + 2,4-DNP (10^{-4} M) and N-ethylmaleimide ($1.5 \cdot 10^{-3}$ M). Preincubation medium: 120 mM KCl, 10 mM Tris-HCl, 1.5 mM EDTA, pH 7.6. Incubation medium: 80 mM KH_2PO_4, 10 mM Tris-HCl, 1 μg/ml rotenone, 1 mg/ml valinomycin, 1.5 mM EDTA, pH 7.6. Abscissa, time (in sec); ordinate, increase in optical density of mitochondrial suspensions.

chondria with thermostable cytoplasmic fraction increased permeability of the inner membrane for phosphate. The action of thermostable cytoplasmic fraction of rat liver on transport of phosphate ions through the mitochondrial membrane was strengthened in hyperthyroidism (Fig. 1). A similar effect also was obtained in experiments with thermostable fraction of cytoplasm isolated from the heart of control and hyperthyroid rats (Fig. 1). It can be concluded from these results that in hyperthyroidism, activity of the thermostable cytoplasmic factor increasing permeability of the inner mitochondrial membrane for phosphate is increased in rat liver and heart. This factor cannot be free fatty acids or calcium ions, for addition of 1 mM EDTA or of bovine serum albumin (2 mg/ml) did not weaken the action of thermostable cytoplasmic fraction on phosphate transport.

Addition of N-ethylmaleimide, an inhibitor of activity of the carrier responsible for P_i^-/OH^- exchange in the mitochondrial membrane [3], had no effect on stimulation of phosphate transport by thermostable cytoplasmic fraction (Fig. 2), although basal phosphate transport was inhibited by addition of N-ethylmaleimide. It could be concluded from this experiment that addition of thermostable cytoplasmic fraction induces phosphate transport by a mechanism unconnected with an increase in P_i^-/OH^- activity, namely an antiporter in the mitochondrial membrane.

The results of an experiment showing that on addition of thermostable cytoplasmic fraction electrogenic phosphate transport is induced through the mitochondrial membrane, are given in Fig. 2. Under these experimental conditions, when valinomycin induces electrogenic transport of K^+, swelling of deenergized mitochondria can take place in only two cases: 1) if there is electrogenic transport of the anion; 2) if P_i^-/OH^- exchange takes place. Addition of 2,4-dinitrophenol (2,4-DNP) induces H^+ (or OH^-) transport through the membrane. Since in the absence of 2,4-DNP a fall in pH of the mitochondrial matrix limits P_i^-/OH^- exchange [4], in the present experiments in the absence of 2,4-DNP, the basal rate of mitochondrial swelling was very low and was sharply increased on the addition of 2,4-DNP (Fig. 2). The reason is that in rat liver mitochondria without the addition of thermostable cytoplasmic fraction, mainly P_i^-/OH^- exchange takes place [3] and it is practically impossible to find electrogenic phosphate transport. Conversely, swelling of mitochondria induced by incubation with thermostable cytoplasmic fraction takes place even in the absence of 2,4-DNP in the incubation medium; the experiments showed, moreover, that under these conditions permeability of the inner mem-

TABLE 1. Effect of Thermostable Cytoplasmic Fraction from Liver of Control and Hyperthyroid Rats on Oxidative Phosphorylation of Rat Liver Mitochondria (M ± m)

Experimental conditions	V_3	V_4	RC
Control	57,6±3,7	25,5±1,2	2,26
Cytoplasm of control rats	61,0±4,2	33,4±1,7	1,82
Cytoplasm of hyperthyroid rats	58,0±3,8	41,5±1,8	1,39

Legend. Incubation medium for mitochondria: 0.15 M KCl, 1 mM phosphate, 1 mM EGTA, 0.7 μg/ml rotenone, 10 mM Tris-HCl, pH 7.4. Oxidation substrate 5 mM succinate. Reagents added: ADP ($1 \cdot 10^{-4}$ M), 2,4-DNP ($1 \cdot 10^{-4}$ M). Mitochondria – 4 mg protein/ml. Oxygen consumption in microatoms/min/mg mitochondrial protein. Cytoplasm added in concentration of 0.2 ml/ml incubation medium. In control, 0.2 ml of 0.12 M KCL was added. RC) Respiratory control.

brane for H^+ or OH^- is not increased. Consequently, the thermostable cytoplasmic fraction induces electrogenic phosphate transport through the mitochondrial membrane.

One consequence of induction of electrogenic phosphate transport through the mitochondrial membrane must be uncoupling of oxidative phosphorylation, for simultaneous functioning of the P_i^-/OH^--antiporter and of electrogenic phosphate transport in energized mitochondria must lead to the appearance of a phosphate cycle, by-passing $\Delta\mu H^+$. On the addition of thermostable cytoplasmic fraction uncoupling of oxidative phosphorylation was indeed found, and in hyperthyroidism this effect of thermostable cytoplasmic fraction was manifested more strongly than in the control (Table 1).

Three conclusions can be drawn from the experimental data described in this paper: 1) Addition of thermostable cytoplasmic fraction induces electrogenic phosphate transport through the mitochondrial membrane; 2) hyperthyroidism increases activity of thermostable inducer of phosphate transport in the cytoplasm; 3) functioning of the phosphate cycle may be the cause of uncoupling of oxidative phosphorylation in hyperthyroidism.

One of the effects of induction of electrogenic phosphate transport from mitochondria into cytoplasm must be an increase in the phosphate concentration in the cytosol in hyperthyroidism. An increase in the phosphate concentration in the cytosol under conditions of hyperthyroidism with no change in ATP/ADP ratio has been described [6]. Moreover, stimulation of the phosphate cycle in target cells of thyroid hormones explains well the toxicity of inorganic phosphate for hyperthyroid dogs, in which intravenous injection of phosphate induces strong stimulation of respiration, hyperthermia, and death [7, 9].

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EFFECT OF TRIHYDROXYOCTADECADIENE ACIDS FROM *Bryonia alba* L. ON ACTIVITY OF GLYCOGEN METABOLISM ENZYMES IN ALLOXAN DIABETES

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Enzyme systems involved in glycogen synthesis and breakdown are sensitive to the action of insulin and are under the control of cyclic AMP [14], with which is associated the ability of certain prostaglandins (PG) to affect secretion of insulin and glucagon, and also various stages of carbohydrate metabolism that are most vulnerable in diabetes [4, 10]. PGE_1, for instance, which has an insulin-like action, also acts on the cAMP level in various tissues [12], whereas PGE_2 has an antigluconeogenetic action through regulation of the cAMP level [11]. PGE_2 is known to stimulate the pentose cycle and glycolytic pathway of glucose metabolism [15] and PG are also known to have an influence on glycogen synthetase and glycogen phosphorylase in the liver and heart [6, 7]. It is natural to suppose that other oxidation products of polyenic fatty acids and, in particular, metabolites of the lipo-oxygenase pathway, can play a definite role in the processes described. Trihydroxyoctadecadiene acids (THDA), isolated from the root of *Bryonia alba* L. (Cucurbitaceae), which exhibit prostaglandin-like activity [3], are C_{18}-homologs of aliphatic trihydroxy acids (containing a 1,2,5-trihydroxy-trans-3-pentaenic group), and are oxidation products of arachidonic acid, whose physiological role has received little study [5]. Previously, in experiments on albino rats with alloxan diabetes, the writers found that THDA have a hypoglycemic action [2].

The aim of this investigation was to study the effect of THDA on activity of glycogen phosphorylase (the a- and b-forms — GP_a and GP_b respectively), phosphoprotein phosphatase (PPP), and hexokinase (HK) in liver and muscle tissues of albino rats with alloxan diabetes.

EXPERIMENTAL METHOD

Diabetes was produced by the method described previously [1]. From the 7th day of the disease the animals received daily intramuscular injections of an aqueous solution of THDA in a dose of 0.05 mg/kg body weight. GP, PPP, and HK activity was determined by known methods [8, 9, 13]. The animals were killed on the 21st day after injection of alloxan.

EXPERIMENTAL RESULTS

The results (Tables 1 and 2) indicate a marked increase in GP_a activity in the skeletal muscles of albino rats with alloxan diabetes, accompanied by relative stability of total phosphorylase activity measured in the presence of cAMP, and by an increase in the GP_a/GP_b ratio. In the liver of the diabetic animals, GP_a activity was reduced almost by half, GP_b activity was increased, and as a result there was a marked decrease in the GP_a/GP_b ratio.

Under the influence of THDA considerable restoration of GP_a activity and of the GP_a/GP_b ratio was observed in muscle tissue and correlated with an appreciable tendency toward normalization of PPP activity. Total phosphorylase activity and GP_a activity in liver tissue not only were not restored by THDA, but they were considerably higher than the control values. Since under these conditions there were no appreciable shifts of PPP activity it can be postulated that THDA participates in normalization of glycogen metabolism in the liver through

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