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EFFECT OF THYROIDECTOMY AND HYPERTHYROIDISM ON ACTIVITY OF $Ca^{++}/2H^{+}$ ANTIPORTER ACTIVITY IN RAT LIVER MITOCHONDIRA

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Ca^{++} ions are a universal regulator of metabolic processes [9, 10]. One of the mechanisms regulating the concentration of ionized Ca^{++} in the cytosol is its transport Ca^{++} ions *in vivo* and *in vitro* [1, 2, 11]. It can be tentatively suggested that hormones can exert their action on metabolism by changing the distribution of Ca^{++} ions between mitochondria and cytosol. It has recently been shown that Ca^{++} transport in the the liver mitochondria is effected by two carriers: The Ca^{++} ion porter is responsible for electrophoretic transport from cytosol into mitochondria, and the $Ca^{++}/2H^{+}$ antiporter is responsible for removal of Ca^{++} ions from mitochondria in exchange for H^{+} ions [6, 7].

Since changes in activity of mitochondrial $Ca^{++}/2H^{+}$ antiporter during changes in the hormonal status of the body may affect the Ca^{++} concentration in the cytosol, the effect of thyroidectomy and hyperthyroidism on activity of $Ca^{++}/2H^{+}$ antiporter was investigated in rat liver mitochondria.

EXPERIMENTAL METHOD

Mitochondria from rat liver were isolated in 0.3 M sucrose containing 5 mM Tris-HCl, pH 7.4, at 5000g. Transport of Ca^{++} ions in the mitochondria was measured by an ion-selective Ca^{++}-sensitive electrode and pH-metric method based on the kinetics of Ca^{++}/H^{+} exchange in the presence of phosphate. The kinetics of swelling of the mitochondria was measured as changes in their optical density of 540 nm. Male rats weighing 100 g were used in experiments with thyroidectomy [3]. The thyroidectomized rats 4 months after the operation weighed 130-160 g, the controls 250-280 g. Hyperthyroidism was induced by intraperitoneal injection of thyroxine in a dose of 100 μg/100 g daily for 4 days.

EXPERIMENTAL RESULTS

After thyroidectomy the calcium capacity of rat liver mitochondria (the number of Ca^{++} ions accumulated by mitochondria before the beginning of spontaneous outflow of Ca^{++} from the mitochondria) was increased. Injection of physiological concentrations of thyroxine into thyroidectomized rats reduced the calcium capacity pratically to normal, but in hyperthyroidism the calcium capacity was significantly lower than in the control (Fig. 1). As Fig. 1b shows, adequate correlation exists between changes in the calcium capacity of the liver mitochondria and the rate of outflow of Ca^{++} ions from the mitochondria on additon of ruthenium red. Since the outflow of calcium ions is undertaken by $Ca^{++}/2H^{+}$ antiporter, which is insensitive to ruthenium red [6, 7], it can be concluded that after thyroidectomy manifestation of

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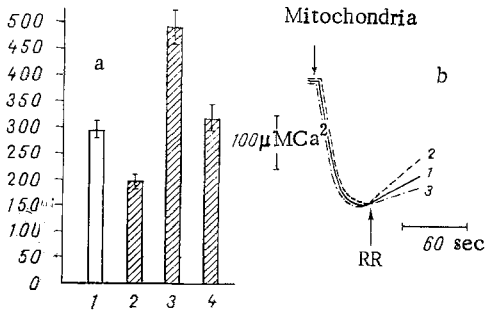

Fig. 1. Transport of Ca^{++} ions into rat liver mitochondria in hyperthyroidism and after thyroidectomy. Incubation medium: 0.1 M KCl, 3.0 mM Tris-buffer, 5 mM succinate, 1 mM phosphate, 0.7 μg/ml rotenone, pH 7.1. a) Calcium capacity of mitochondria (in nmoles/mg protein): 1) control, 2) hyperthyroidism, 3) thyroidectomy, 4) thyroidectomy + thyroxine (20 μg/100 g for 3 days). $CaCl_2$ was added to the cell in a dose of 10^{-4} M. Mitochondria equivalent to 1.5 mg protein in 1 ml; b) outflow of Ca^{++} ions from mitochondria on addition of ruthenium red (RR): 1) control, 2) hyperthyroidism, 3) thyroidectomy. $CaCl_2$ concentration in incubation medium 250 μM. Mitochondria equivalent to 1.7 mg protein in 1 ml, ruthenium red added in concentration of 10^{-5} M.

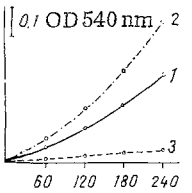

Fig. 2. Phosphate-induced swelling of rat liver mitochondria after thyroidectomy and during hyperthyroidism. Incubation medium the same as in Fig. 1, but phosphate concentration 5 mM, mitochondria equivalent to 0.4 mg protein in 1 ml. 1) Control, 2) hyperthyroidism, 3) thyroidectomy. Abscissa, time (in sec); ordinate, optical density (OD).

the activity of $Ca^{++}/2H^{+}$ antiporter is inhibited, whereas during hyperthyroidism it is stimulated, under the experimental conditions used. The decrease in the calcium capacity of the mitochondria associated with an increase in the plasma thyroid hormone concentration is evidently the result of increased realization of Ca^{++} ions in the mitochondria, due to increased activity of the $Ca^{++}/2H^{+}$ antiporter. It was shown previously that in thyrotoxicosis the calcium capacity of rat liver mitochondria is reduced [1, 2], at increase in calcium capacity after thyroidectomy was relatively small [1, 2] compared with the results of the present experiments (Fig. 1). The reason evidently was that our own experiments were carried out 4 months after thyroidectomy, whereas those cited above were carried out after only 3-4 weeks [1, 2].

One possible explanation of the increase in $Ca^{++}/2H^{+}$ antiporter activity is that thyroid hormones increase the sensitivity of liver mitochondria to injury by Ca^{++} and phosphate ions

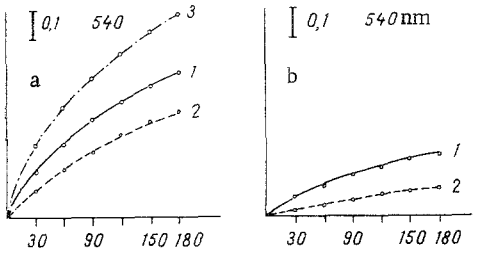

Fig. 3. Swelling of de-energized rat liver mitochondria in iso-osmotic $Ca(NO_3)_2$ solution. Incubation medium: 80 mM Ca $(NO_3)_2$, 1 μg/ml mitochondria. a)without ruthenium red, b) with 2×10^{-5} M ruthenium red; 1) control, 2) thyroidectomy, 3) hyperthyroidism. Remainder of legend as to Fig. 2.

in vitro, as a result of which activation of $Ca^{++}/2H^+$ exchange *in vitro*, which occurs in response to loading with Ca^{++} ions and addition of phosphate [12], is exhibited more strongly and rapidly in mitochondria from the liver of hyperthyroid rats. This explanation is supported indirectly by the results of experiments to study the effect of the thyroid status on high-amplitude phosphate-induced swelling (Fig. 2) and the increase in mitochondrial phospholipase A_2 activity during incubation of liver mitochondria *in vitro* [4]. Consequently, the increase in activity of $Ca^{++}/2H^+$ exchange by thyroid hormones may be due to labilization of the mitochondria toward the action of harmful factors *in vitro*. Nevertheless the connection between activity of the $Ca^{++}/2H^+$ antiporter and damage to the mitochondria by exongenous calcium and phosphate is not compatible with the injury, an increase in $Ca^{++}/2H^+$ exchange scheme, for inhibition of recycling of endogenous Ca^{++} by ruthenium red prevents damage to mitochondria under conditions of phosphate-inducded high amplitude swelling of mitochondria [14]. Consequently, the presence of correlation between activity of $Ca^{++}/2H^+$ exchange and resistance of the mitochondria to injury *in vitro* may be explained on the grounds that an increase in Ca^{++} recycling is a factor which stimulates activation of phospholipase A_2 and injury to mitochondria. If this is so, stimulation of phosphate-induced swelling by thyroid hormones must also be the result of increased activity of the $Ca^{++}/2H^+$ antiporter.

Evidence in support of the view that the increase in activity of $Ca^{++}/2H^+$ antiporter is primary *in vivo* and not injury to the mitochondria is given by the results of experiments to measure the kinetics of swelling of de-energized mitochondria in iso-osmotic $Ca(NO_3)_2$ solutions at pH 8.1 (Fig. 3). Under these conditions the rate of swelling is limited by permeability of the mitochondrial membrane for Ca^{++} ions [5]. In this connection inhibition of swelling after thyroidectomy and its stimulation during administration of thyroid hormones *in vivo* (Fig. 3a) can be regarded as an increase in the velocity of passive transport of Ca^{++} ions through the mitochondrial membrane. This effect is manifested particularly clearly in the presence of ruthenium red (Fig. 3b). Consequently thyroid hormones *in vivo* regulate activity of ruthenium-insensitive $Ca^{++}/2H^+$ antiporter in rat liver mitochondria. This effect cannot be linked with changes in phospholipase A_2 activity, for in the presence of uncouplers injury to the mitochondria due to activiation of phospholipase A_2 does not take place [14].

Regulation of activity of $Ca^{++}/2H^+$ antiporter by thyroid hormones may relate both to stimulation of basal metabolism [8] and also to activation of glyconeogenesis in the hepatocytes by thyroid hormones [13]. It is well known that an increase in the concentration of ionized Ca^{++} in the cytosol of hepatocytes leads to stimulation of gluconeogenesis through activation of phosphoenolpyruvate carboxylase and inhibition of pyruvate kinase [10]. In this connection the increase in activity of $Ca^{++}/2H^+$ antiporter by thyroid hormones ought to lead to an increase in the Ca^{++} concentration in the cytosol and stimulation of gluconeogenesis. Meanwhile an increase in the energy consumption for recycling of Ca^{++} in the mitochondria on account of activation of $Ca^{++}/2H^+$ antiporter may be one of the mechanisms of stimulation of oxygen consumption by the liver in response to an increase in the plasma thyroid hormones concentration [8].

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