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Stimulation of carnitine acylcarnitine translocase activity in heart mitochondria from hyperthyroid rats

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Abstract The effect of hyperthyroidism on fatty acid oxidation and on carnitine-acylcarnitine translocase activity in rat heart mitochondria has been studied. The rates of palmitoylcarnitine supported respiration as well as the carnitine-palmitoylcarnitine exchange reaction were both stimulated (approx. 36%) in heart mitochondria from hyperthyroid rats. Kinetic analysis of the carnitine-carnitine exchange reaction showed that thyroid hormone affects the V_{max} of this process, while having no effect on the K_m values. The level of cardiolipin was significantly higher (approx. 40%) in heart mitoplasts from hyperthyroid rats than from the control rats. It can be concluded that thyroid hormones produce a stimulation of heart mitochondrial carnitine translocase activity and that the basis of this effect is likely an increase in the cardiolipin content.

Key words: Carnitine translocase; Cardiolipin; Hyperthyroidism; Rat-heart mitochondria

1. Introduction

Thyroid hormones are known to play an important role in the regulation of cardiac function. Mitochondria are considered possible subcellular loci of thyroid hormone action in view of their crucial role in the regulation of energy production. Long-chain fatty acids represent, together with pyruvate, the major substrate for energy production in the normal working heart [1]. Fatty acid oxidation rate is proportional to the thyroid state [2]. The major site of control of fatty acyl-CoA oxidation is the mechanism that transports cytoplasmic fatty acyl-CoA to the fatty acyl-CoA dehydrogenase in the mitochondrial matrix adjacent to the electron transport chain. Carnitine is an absolute requirement for the transport of activated long chain acyl units (fatty acyl-CoA) into mitochondria [3]. The inner mitochondrial membrane contains a specific protein system for the transport of carnitine and acylcarnitine known as carnitine translocase [4–6]. This translocase exchanges cytosolic acylcarnitine or free carnitine for carnitine in the mitochondrial matrix. The properties of this carrier system have been investigated in intact mitochondria [5,7]. The carnitine carrier has been isolated and its activity reconstituted in artificial membranes as liposomes [8]. An essential requirement of cardiolipin for mitochondrial carnitine acylcarnitine translocase activity has been demonstrated [8,9].

We have recently reported that the activity of several anion carrier proteins is stimulated in heart mitochondria isolated from hyperthyroid rats [10–12]. Changes in the mitochondrial cardiolipin content have been considered as a possible factor responsible for this effect by thyroid hormone. In addition, it has been recently reported that mitochondrial cardiolipin biosynthesis is under thyroid hormone regulation [13,14].

To date, there has been no report assessing heart mitochondrial carnitine-acylcarnitine translocase activity relative to the hyperthyroid state. In this paper the effect of hyperthyroidism on fatty acids oxidation, carnitine-acylcarnitine translocase activity and phospholipid composition in rat heart mitochondria was examined.

2. Materials and methods

Male Wistar rats (180–200 g) were made hyperthyroid using 3,3′,5-triiodo-L-thyronine (30 μg/100 body wt), injected intraperitoneally with a single daily injection for 5 consecutive days [10]. Rat heart mitochondria were prepared as described previously [15].

Rat heart mitoplasts were prepared as described in [16].

Carnitine-acylcarnitine translocase activity was determined as described in [17]. Freshly isolated mitochondria were loaded with 2 mM 1-[*methyl*-^{14}C]carnitine, essentially as described by Pande and Parvin [6]. Efflux of radiolabeled carnitine was initiated at 0°C by addition of [^{14}C]carnitine-loaded mitochondria (approx. 0.5 mg protein) to 0.5 ml 200 mM mannitol and 50 mM Tris-HCl (pH 7.4) containing unlabeled carnitine or palmitoylcarnitine. The exchange reaction was stopped by the addition of 1 mM mersalyl followed by rapid centrifugation of the mitochondrial suspension and measurement of radioactivity in the supernatant and pellet. Mitochondrial inhibitors rotenone, oligomycin and antimycin were included throughout the loading and assay procedure to dissociate carnitine-acylcarnitine transport from electron transport and oxidative phosphorylation. Rates of exchange were calculated as described by Pande and Parvin [6].

Phospholipids were analysed by HPLC as described in [18].

Protein concentration was measured by the usual biuret method.

3. Results and discussion

Table 1 presents the results of experiments on fatty acid oxidation in heart mitochondria isolated from control and hyperthyroid rats. Rates of phosphorylating oxygen consumption with palmitoylcarnitine were significantly higher (approx. 35%) in mitochondria from hyperthyroid rats compared to the values obtained with normal rats. Similar results were obtained when palmitoylcarnitine was replaced by other fatty acid respiratory substrates such as palmitoyl-CoA plus carnitine or acetyl-L-carnitine (results not presented).

Palmitoylcarnitine-carnitine exchange represents the physiological expression of carnitine translocase activity in the pathway to oxidation of fatty acids. Since palmitoylcarnitine-supported respiration is increased in mitochondria from hyperthyroid rats (see Table 1), it seemed possible that increased rates of exchange of extramitochondrial palmitoylcarnitine for matrix carnitine may be responsible for the stimulation of palmitoylcarnitine oxidation. To assess this, the activity of carnitine-palmitoylcarnitine exchange was meas-

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Table 1
Rates of palmitoylcarnitine supported respiration in heart mitochondria isolated from control and hyperthyroid rats

Animals	Rates of O_2 consumption (ng atoms O/min per mg protein)		
	State 3	State 4	RCR
Control	284 ± 32	35 ± 4.2	8.1
Hyperthyroid	383 ± 38[a]	44 ± 5.1	8.7

Rates of mitochondrial respiration were measured polarographically with a Clark-type oxygen electrode. The respiration medium consisted of 120 mM KCl, 20 mM Na-HEPES, 1 mM $MgCl_2$, 1 mM EDTA, % mM phosphate. Final pH 7.4. When a steady state of oxygen consumption was obtained, 0.01 mM palmitoylcarnitine plus 2 mM malate were added and 1 min later respiration was stimulated by adding 1 mM ADP. Each value represents the mean ± S.E.M. for four separate experiments with three rats for each group.
[a] $P < 0.01$ vs. control.

ured in mitochondria from control and hyperthyroid rats. Due to the membrane-active properties of the palmitoylcarnitine micelles, palmitoylcarnitine exchange was measured at palmitoylcarnitine concentration below 20 μM. As shown in Fig. 1, rates of carnitine-palmitoylcarnitine exchange were significantly higher, at all external palmitoylcarnitine concentrations used, in heart mitochondria from hyperthyroid rats compared to the values of mitochondria from normal rats.

The kinetic parameters of the carnitine translocase in mitochondria isolated from control and hyperthyroid rats were determined by studying the dependence on substrate concentration of the rate of carnitine efflux by carnitine preloaded mitochondria. The data reported in Table 2 indicate that the maximal velocity of carnitine transport was significantly enhanced (around 38%) in mitochondria from hyperthyroid rats compared with that obtained in mitochondria from control rats, while the K_m value was unaffected.

It has been reported that cardiolipin, a phospholipid almost exclusively located in the inner mitochondrial membrane [19], is specifically required for optimal functioning of the isolated carnitine-acylcarnitine translocase reconstituted in liposomes [8,9]. Moreover, in intact mitochondria of rat liver and heart, the carnitine translocase activity was markedly inhibited by micromolar concentrations of doxorubicin [9], a specific cardiolipin binding agent [20]. Thus, changes in mitochondrial cardiolipin content may affect the carnitine translocase activity in situ. Since the carnitine translocase protein is located at the level of the inner mitochondrial membrane, the phospholipid composition of mitoplasts isolated from normal and hyperthyroid rats was analysed. The results reported in Table 3 demonstrate that the content of cardiolipin was significantly increased (around 40%) in mitoplasts from hyperthyroid rats, while there was no substantial change in the content of other

Table 2
Kinetic parameters of carnitine-carnitine exchange in mitochondria isolated from control and hyperthyroid rats

Animals	K_m (mM)	V_{max} (nmol/min per mg protein)
Control	1.74 ± 0.21	1.25 ± 0.18
Hyperthyroid	1.66 ± 0.34	1.72 ± 0.22[a]

The K_m and V_{max} values were calculated from double-reciprocal plots of the rates of intramitochondrial [^{14}C]carnitine efflux versus external added carnitine (ranging from 0.5 to 20 mM). Each value represents the mean ± S.E.M. obtained for four separate experiments with three rats for each group.

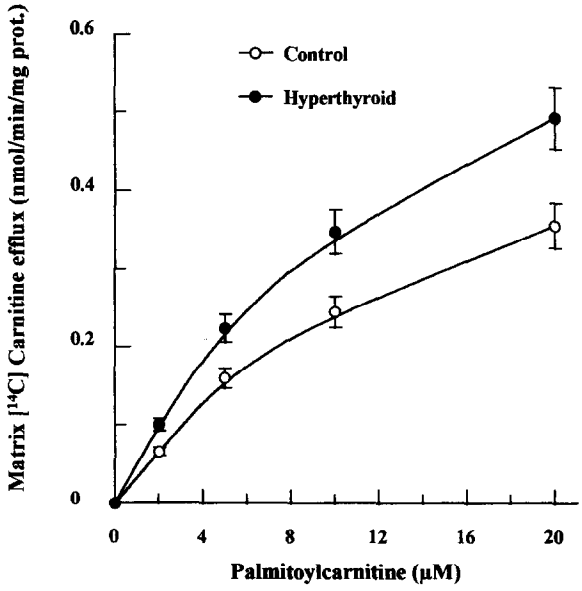

Fig. 1. Rates of carnitine-palmitoylcarnitine exchange in heart mitochondria isolated from control and hyperthyroid rats. Mitochondria loaded with [^{14}C]carnitine were added to a medium containing varying amounts of palmitoylcarnitine to initiate the exchange. Values are expressed as mean ± S.E.M. for four separate experiments with three rats for each group.

phospholipid species. These changes in mitochondrial cardiolipin level are quantitatively associated with parallel changes in the rates of palmitoylcarnitine-supported respiration (Table 1) and in the activity of carnitine-acylcarnitine exchange (Fig. 1 and Table 2). This suggests that the observed stimulation of carnitine translocase in mitochondria from hyperthyroid rats may be mainly ascribed to an increase in the cardiolipin content. Similarly to the carnitine translocase, other mitochondrial anion carrier systems have been shown to be stimulated by thyroid hormones such as the ADP/ATP carrier [21], pyruvate [10], citrate [11] and phosphate [12] translocators. Very recently, we have also reported stimulation of heart mitochondrial cytochrome oxidase activity by thyroid hormones [22]. All these mitochondrial inner membrane transporters and enzymes require cardiolipin for their optimal functioning [23]. Therefore, a unified mechanism responsible for thyroid hormone induced stimulation of all these membrane-associated transport systems and cytochrome oxidase activity can be postulated. This view appears particularly interesting in the light of recent reports which demonstrate enhancement of mitochondrial cardiolipin biosynthesis in animals treated with thyroid hormones [13,14].

Fatty acid oxidation provides a prime source of energy in heart tissue. The carnitine-acylcarnitine carrier may play a

Table 3
Phospholipid composition in heart mitoplasts isolated from normal and hyperthyroid rats

Phospholipid	Distribution (mol%)	
	Control	Hyperthyroid
Cardiolipin	22.2 ± 2.1	30.8 ± 3.5[a]
Phosphatidylethanolamine	35.8 ± 2.7	31.6 ± 3.2
Phosphatidylcholine	40.2 ± 2.8	36.2 ± 3.6
Phosphatidylinositol	0.4 ± 0.07	0.4 ± 0.06

[a] $P < 0.001$ vs. control.

central role, in addition to carnitine acyltransferases enzymes, in the regulation of heart energy metabolism by facilitating the entry of fatty acids through the mitochondrial inner membranes. Therefore, the observed stimulation of this translocase by thyroid hormones, may account, in addition to other factors, for the enhanced cardiac mitochondrial oxidative capacity, typical of the hyperthyroid state.

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