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THE INFLUENCE OF THYROID HORMONES ON THE STRUCTURE AND FUNCTION OF MITOCHONDRIAL MEMBRANES

ANTHONY J. HULBERT[a], MICHAEL L. AUGEE[b] and JOHN K. RAISON[c]

[a]*Department of Biology, The University of Wollongong, N.S.W. 2500,* [b]*School of Zoology, University of N.S.W., Kensington, N.S.W. 2033 and* [c]*Plant Physiology Unit, CSIRO Division of Food Research and School of Biological Sciences, Macquarie University, North Ryde, N.S.W. 2113 (Australia)*

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Summary

In rat liver mitochondria, membrane lipid unsaturation increases, the temperature limits of the membrane phase transition decrease and the E_a of succinate oxidase increases following thyroidectomy. All three parameters change in the opposite direction within 12 h after thyroxine treatment. It is suggested that many effects of thyroid hormones can be explained by changes in membrane structure and function.

The interaction between membrane lipids and membrane function is clearly demonstrated by the increase in Arrhenius activation energy (E_a) of membrane-associated enzymes below the temperature limits of the membrane lipid phase transition [1]. Changes in membrane lipid composition alter membrane fluidity and also affect the E_a of membrane-associated enzymes [1]. The phase transition for membranes from rats starts at about 23°C and finishes at approximately 8°C [2,3]. Above 23°C the membrane lipids are in a liquid crystalline (fluid) phase. Between 23°C and 8°C, the lipids are in a transition consisting of mixed liquid-crystalline and gel phases and below 8°C they are in a predominantly gel (solid) phase.

Homeothermic (warm blooded) animals maintain their body temperature in the region where membrane lipids are fluid. Although hibernators reduce their body temperature during winter they also lower the upper temperature limit of the phase transition [4] by altering their membrane lipid composition [5], and thus maintain fluidity at low temperatures. It has been observed that thyroid hormone secretion in these hibernators stops just before the hibernating season [6] and it was suggested that this cessation of thyroid hormone secretion is responsible for the alteration of membrane composition and thus the maintenance of membrane fluidity and membrane function.

To determine the influence of thyroid hormones on membrane lipid structure and function we have investigated the effect of changes in thyroid status on the liver mitochondrial membrane of a non-hibernating mammal, the the rat. Liver mitochondrial membranes were chosen because it has been shown that with respect to the phase transition and the E_a of associated enzymes liver mitochondrial membranes are typical of mitochondria from kidney or heart and of the endoplasmic reticulum membranes from these tissues [2,7]

Wistar rats (male 200—250 g) were divided into a hypothyroid group (n = 9) and a control group (n = 3). The hypothyroid rats were produced by surgical thyroidectomy and were maintained on rat pellets (Allied Feeds, Sydney) and a 1% calcium gluconate drinking solution ad libitum for up to 15 weeks. None of the thyroidectomized rats showed a significant uptake of ^{131}I in the region of the thyroid before being killed. Three rats were sacrificed at each of 7, 10 and 15 weeks after thyroidectomy and liver mitochondria isolated by methods previously described [8]. Succinate oxidase activity was measured polarographically [9] by the simultaneous use of five oxygen electrodes each operating at a different temperature. Electron spin resonance (ESR) spectroscopy of the spin label 3-oxazolidinyloxy-2-butyl-2-pentyl-4,4-

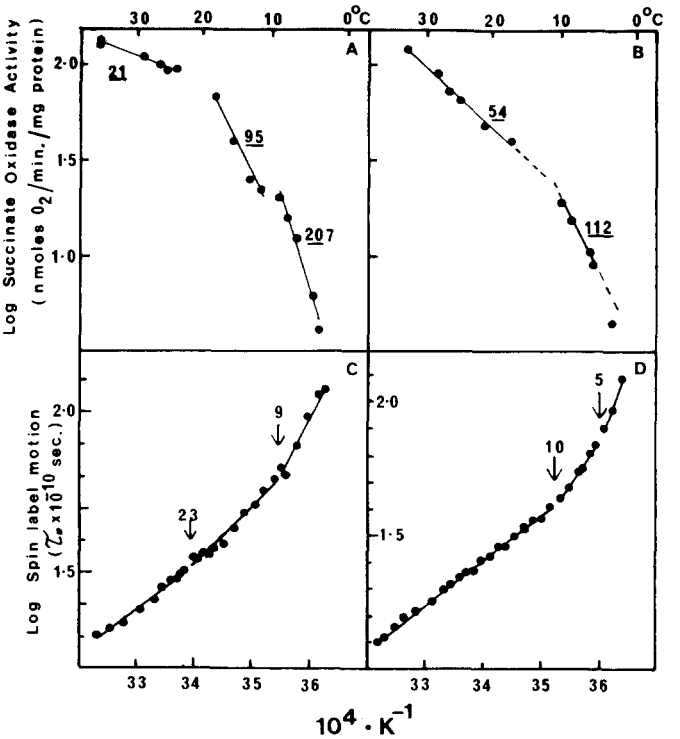

Fig. 1. Arrhenius plots of succinate oxidase activity (A and B) and spin label motion (C and D) for mitochondria from a control rat and a thyroidectomized rat as a function of temperature. For the oxidase plots the number underlined beside each line is the Arrhenius activation energy in kJ · mol^{-1}. The upper (T_f) and lower (T_s) limits of the phase transition, obtained from plots of spin label motion were determined by statistical analysis for determining the change in slope of the straight portions of the graph. The temperatures quoted have an estimated error of ±0.7°C and all the changes in slope are significant at a level of $P < 0.01$.

dimethyl (5N10), infused into mitochondrial membrane was carried out using a Varian E-4 spectrometer by the methods previously described [2]. Changes in the temperature responses of motion for spin labels infused into the lipid region of membranes have been used to indicate changes in molecular ordering of membrane lipids induced by changing temperature [10]. Fatty acid composition of the membrane lipids was determined as described previously [11].

Fig. 1 shows Arrhenius plots for both succinate oxidase and spin label motion with membranes of rat liver mitochondria. For the control rat (Figs. 1 Aand C) changes in molecular ordering, indicated by the changes in the temperature coefficient of spin label motion (change in slope of the plot of log τ_0 against the reciprocal of absolute temperature) and the E_a of succinate oxidase activity, occur at about 23 and 9°C as previously reported [2]. Results with thyroidectomized rats show that their mitochondria differ from the controls in two respects. As shown in Figs. 1B and D the upper temperature limit of the transition (T_f) is reduced from 23 to 10°C and the lower limit from 9 to 5°C, 15 weeks after thyroidectomy. Furthermore, in the temperature zone above T_f, the E_a of either state 3 or state 4 rates of succinate oxidation for the thyroidectomized rat was 54 kJ · mol^{-1} (Fig. 1B) compared to 21 kJ · mol^{-1} for the control rat (Fig. 1A).

Table I shows additional features of the changes in structure and function induced by thyroidectomy. Firstly, the decrease in T_f, was clearly evident after 7 weeks and was progressive up to 15 weeks. Secondly, the decrease in T_f was related to an increase in unsaturation of membrane lipids from 65% at 7 weeks, to 71% after 15 weeks. Although the change in the relative unsaturation of membrane lipids is small, it is sufficient to account for the large change in T_f [1]. The third important feature of Table I is the progressive increase in the E_a of succinate oxidation, in the temperature zone above T_f, following thyroidectomy. Such increases in E_a are indicative of an alteration

TABLE I

CHANGES IN MEMBRANE FATTY ACID COMPOSITION, TEMPERATURE-INDUCED PHASE TRANSITION AND THE ARRHENIUS ACTIVATION ENERGY FOR SUCCINATE OXIDASE ACTIVITY IN RAT LIVER MITOCHONDRIA FOLLOWING THYROIDECTOMY

	Time after thyroidectomy (weeks)	Unsaturated* fatty acids (% of total fatty acids)	Membrane** phase transition (°C)		Succinate*** oxidase E_a above T_f (kJ · mol^{-1})
			T_f	T_s	
Control	—	61	23	9	21
Thyroidectomized	7	65	19	9	30
	10	66	15	7	40
	15	71	10	5	54

*The total of palmitoleic (16:1), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidonic (20:4), as well as small amounts of 20:5 and 22:6 acids, expressed as a percentage of total fatty acids. The maximum variation in unsaturated fatty acids between individuals within a treatment was ±1.4%.

**T_f and T_s for each individual can be determined to within ±0.7°C. The maximum variation in T_f and T_s between individuals within one treatment was ±1°C of the value shown above.

***Arrhenius activation energy for each individual can be determined to within approximately ±2%. The maximum variation for any individual within a treatment was ±4 kJ · mol^{-1} of the value shown above.

in the catalytic efficiency of oxidative activity and since this is coupled to phosphorylation, indicates an alteration in the efficiency of energy production.

In a separate series of experiments all parameters were measured in liver mitochondria isolated from rats (n = 3) 12 h after the intraperitoneal injection of thyroxine (3 μg/g body weight, Sigma Chemicals). After the injection of thyroxine the changes in all parameters were in the opposite direction to those observed after thyroidectomy. Membrane fatty acid unsaturation decreased 7%, the upper phase change temperature increased by 6°C and the E_a of succinate oxidation above this temperature decreased by approximately 12 kJ/mol. The oxidative activity of mitochondria from the thyroxine-treated animals is adversely affected by high temperatures. Since the phase change is elevated by thyroxine treatment determination of the E_a of succinate oxidation was restricted to the temperature range of 28 to 33°C. Therefore the value for the E_a change after thyroxine treatment could vary by ±10%. A significant decrease in the E_a of succinate oxidation, compared to control rats, was however observed in all mitochondrial preparations from thyroxine-treated rats.

The dramatic changes, in all parameters measured, only 12 h after thyroxine injection, indicate that the action of this thyroid hormone on mitochondrial membranes is very rapid. This suggests a direct effect on membrane lipids especially in view of the relatively lengthy periods necessary for other thyroid hormone effects to become evident [12]. The difference between the time course for the changes induced by thyroidectomy and by thyroxine treatment, suggests that whereas the effect of thyroxine on the membrane lipids is relatively rapid, the change observed in the absence of thyroid hormones represents a considerably slower reversion to a more unsaturated state, a state which is more typical of the membrsnes of poikilotherms [13]. It may be that in the normal rat the thyroid hormones are responsible for maintenance of the "homeothermic" (less unsaturated) nature of the membrane lipids.

As well as influencing mitochondrial membrane lipids the thyroid hormones similarly affect the relative unsaturation of microsomal membrane lipids [14]. A direct relationship between changes in membrane structure and function on the one hand and thyroid activity on the other provides a rational explanation for a number of previously observed but apparently unconnected changes associated with thyroid hormones. In addition, it provides a new conceptual approach to the primary mechanism of action of the thyroid hormones. Many of the enzyme reactions known to be influenced by thyroid hormones, such as succinate dehydrogenase, cytochrome c oxidase [15], adenine nucleotide translocation [16], ($Na^+ + K^+$)-ATPase [17] and protein synthesis [12, 13] are membrane-associated enzyme systems. It is therefore likely that these enzyme systems are affected by changes in membrane fluidity. Such effects have been shown for the mitochondrial oxidative enzymes (Table I) adenine nucleotide translocation [19], ($Na^+ + K^+$)-ATPase [7] and amino acid incorporation [20,21]. Thus, by altering the lipid composition and hence fluidity of membranes, it is possible to alter the activity of a number of metabolic processes simultaneously.

An action of thyroxine on mitochondrial membrane structure might also explain the swelling of mitochondria that can be induced by thyroxine [22]. If, as we suggest, the thyroid hormones act on other cellular membranes then it is possible that the multiplicity of metabolic and clinical changes attributed to thyroid hormones will come to be explained as secondary events, resulting from alterations in membrane function caused by thyroid-hormone induced changes in membrane structure.

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