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Ubiquinone (coenzyme Q) and the regulation of basal metabolic rate by thyroid hormones

Many explanations of the calorigenic action of thyroid hormones have been based on their effects on the activity, or amount, of enzymes or other constituents involved in the electron transport and phosphorylation systems (see ref. 1). There is evidence now that ubiquinones or coenzymes Q are members of the mitochondrial respiratory chain[2] and experimental hyperthyroidism or thyrotoxicosis in the rat led to large increases in the ubiquinone content of the liver[3–5]. It is, however, not possible to tell from the latter studies whether it is the increase in ubiquinone content which causes an elevation of the basal metabolic rate or, conversely, that an increased level of ubiquinone results from a higher rate of various cellular processes, since thyroid hormones were administered "chronically" or over a relatively long period before measurement of the quinone. In other studies in our laboratory on the action of thyroid hormones at the cell level[6,7], primary actions could be dissociated from the secondary by studying changes in cellular functions or constituents as a function of time after the administration to thyroidectomized rats of a single, small dose of thyroid hormone. This communication describes experiments to determine whether changes in ubiquinone content in various tissues precede or accompany the initial stimulation of the basal metabolic rate by nearly physiological amounts of thyroid hormone.

"Chronic" hyperthyroidism was induced by the injection to normal male Wistar rats (120–150 g) of 25–30 μg of L-thyroxine or 3,5,3'-triiodo-L-thyronine every fourth or third day, respectively, over a period of 24 days. Rats were killed 4 days after the last injection. In the "acute" studies, a single injection of 25 μg of triiodothyronine was made to 150–180-g rats which were radiothyroidectomized (^{131}I) at least 6 weeks earlier. The basal metabolic rate of every animal was measured within 2 h of killing. The methods for the measurement of the basal metabolic rate as well as those of respiratory quotient and oxidative phosphorylation in mitochondria are described elsewhere[7]. Heart, liver and skeletal muscle from hind legs were removed and frozen immediately upon killing and the ubiquinone-45 (coenzyme Q_9) content, in tissue pooled from 3–6 animals per group, determined by one of the following two procedures.

Method I: Minced heart of liver was saponified with a mixture of KOH, methanol and pyrogallol before extraction with *n*-hexane according to CRANE *et al.*[8]. Ubiquinone was determined spectroscopically in the ethanol-soluble material of the extract from the difference in ultraviolet absorption at 275 mμ before and after the additon of KBH_4. The reducible material was considered as ubiquinone-45 (coenzyme Q_9) with $\Delta E_{1\,cm} = 154$.

Method II: The non-saponification procedure of BEYER, NOBLE AND HIRSCHFELD[9] was adapted for direct extraction of skeletal muscle and liver. Liver was homogenized in 6 vol. of water, and skeletal muscle in a mixture of 0.1 M KCl, 0.05 M Tris buffer (pH 7.4), 0.001 M ATP, 0.005 M $MgSO_4$ and 0.001 M EDTA, before extraction with acetone. The volume of the acetone extract was reduced under low pressure under nitrogen and the residue extracted repeatedly with iso-octane after the addition of methanol. The combined iso-octane extracts were washed with water and dried over anhyd. Na_2SO_4. The ubiquinone was then adsorbed on a silicic acid – celite (2:1)

TABLE I

THE EFFECT OF "CHRONIC" AND "ACUTE" ADMINISTRATION OF 3,5,3'-TRIIODO-L-THYRONINE ON THE UBIQUINONE CONTENT OF LIVER, HEART AND SKELETAL MUSCLE OF NORMAL AND THYROIDECTOMIZED RATS

Rats	*Basal metabolic rate (ml O_2/h/g)*	*Tissue*	*Mitochondrial activity**		*Ubiquinone** (μg/g wet tissue)*	
			(μatoms O/h/mg protein)	*P/O*	*Method I*	*Method II*
Normal	0.65	Liver	7.6	2.55	102 (93, 105, 107)	88 (84, 92)
		Heart	—	—	99 (91, 107)	—
		Muscle	11.3	2.60	—	37 (32, 39, 40)
Normal + "chronic" triiodothyronine treatment	0.84	Liver	9.5	2.54	146 (137, 155)	126 (118, 134)
		Heart	—	—	113 (104, 122)	—
		Muscle	18.3	2.56	—	46 (43, 84)
Thyroidectomized	0.45	Liver	5.8	2.47	—	106 (98, 114))
		Heart	—	—	94	—
		Muscle	8.2	2.51		27 (23, 25, 34)
Thyroidectomized, 42 h triiodothyronine treatment ("acute")	0.56	Liver	8.0	2.58	—	87 (77, 97)
		Heart***	—	—	94	—
		Muscle	12.4	2.70	—	23 (18, 20, 31)

* With glutamate, pyruvate and malate as substrates for liver and muscle mitochondria, respectively.
** The ubiquinone values in parentheses are from individual determinations made on different portions of the same tissue, pooled from 3–6 animals.
*** 36 h after injection of triiodothyronine.

mixture and eluted with chloroform – iso-octane (1:1). The ubiquinone content of the dried extract was determined spectroscopically in an ethanol solution. Added ubiquinone was recovered in 70–85 % yield in both procedures.

It is seen in Table I that repeated administration of thyroid hormone significantly increased, and thyroidectomy lowered, the basal metabolic rate and respiratory rates of liver and muscle mitochondria without affecting the efficiency of oxidative phosphorylation. Ubiquinone levels were at best 20–50 % higher in tissues of "chronically" hypermetabolic rats. This increase in liver ubiquinone after repeated thyroid-hormone treatment is considerably less than that reported by other authors[2–4] (who found the level to be increased by over 150 % in liver) but who found no change in heart ubiquinone levels, after thyrotoxicosis was induced. The smaller amounts of thyroid hormone used by us may account for this difference. In the "acute" experiments performed to detect any changes in ubiquinone level during the early phase of the stimulation of metabolic activity, values obtained 42 h after an injection of triiodothyronine to thyroidectomized rats have been selected. This is based on other work in our laboratory, which showed that initial stimulation of the basal metabolic rate and various cellular activities can be distinctly observed at this time interval[6,7]. It is therefore significant that the level of ubiquinone in all three tissues did not increase when the basal metabolic rate and liver and muscle mitochondrial respiratory activity had increased by 25, 55 and 52 %, respectively.

In conclusion, the use of low doses of thyroid hormones, administered repeatedly, partially confirms earlier reports on the increased ubiquinone levels in livers of rats made thyrotoxic[2–4]. However, our failure to observe any early change in ubiquinone concentration in the "acute" experiments at a time when the basal metabolic rate was increasing suggests that changes in total tissue ubiquinone level may be an adaptation to, rather than the cause of, increased metabolic activity. Furthermore, the exact role of ubiquinone in oxidative phosphorylation is not yet defined and the absence of an uncoupling effect after both "chronic" and "acute" stimulation of the basic metabolic rate by triiodothyronine should be considered before attributing a physiological meaning to changes in tissue ubiquinone levels. The above results should also be considered in conjunction with our recent work on the co-ordinate action of thyroid hormones on multiple cell functions[6,7].

The Wenner-Gren Institute, University of Stockholm, Stockholm (Sweden)

S. PEDERSEN
J. R. TATA*
L. ERNSTER

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* Present address: National Institute for Medical Research, Mill Hill, London. N.W. 7 (Great Britain).