ALTERATIONS OF MITOCHONDRIAL PHOSPHOLIPIDS IN THE RAT BROWN ADIPOSE TISSUE AFTER CHRONIC TREATMENT WITH COLD OR THYROXINE

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Received 4 February 1975

1. Introduction

The discovery of the hypertrophy of the rat brown adipose tissue which is induced by prolonged cold exposure [1] has been followed by many ultrastructural and biochemical studies upon this tissue which presents the peculiarity to contain a great number of mitochondrial [2]. Chronic cold exposure increases the mitochondrial diameter [3], the number of cristae [4] or the concentration of inner membrane [5], correlatively to an increase of oxidative enzyme activities [6]. These modifications are associated with an increase of the phospholipid percentage [3,7,8] and important alterations of the phospholipid fatty acid pattern [7,8]. Chronic thyroxine treatment induces also a hypertrophy of the rat brown adipose tissue [9] but the phospholipid percentage is unchanged, while there is an increase of the total phospholipid content; some modifications of the phospholipid fatty acid pattern are also observed but they are less important than after cold treatment and in an opposite direction [7,8].

Since a great part of the phospholipids of brown fat belongs to the mitochondrial fraction we have studied the mitochondrial phospholipids of brown adipose tissue from rats treated chronically with cold or thyroxine. The most striking effect of cold exposure was a large increase of the mitochondrial phospholipid content of the tissue which was accompanied by a decrease of the phosphatidylcholine: phosphatidylethanolamine ratio (PC:PE), the cardiolipin percentage being unchanged; moreover the fatty acid composition of the different phospholipids was greatly modified after cold exposure. On the contrary thyroxine treatment had minor effects on mitochondrial phospholipids: the mitochondrial phospholipid content was not modified and only slight effects on the phospholipid and fatty acid composition were observed.

It seems therefore that cold exposure and thyroxine treatment have differential effects upon the mitochondrial development in the brown adipose tissue. The important modifications of the mitochondrial phospholipids which follow cold exposure suggest that the mitochondrial membranes are altered in this state where mitochondriogenesis is strongly stimulated.

2. Materials and methods

Young rats were bred at 22°C from the age of weaning (22 days) up to 6 weeks, thyroxine treated animals receiving everyday 50 μg L-thyroxine per 100 g body weight intraperitoneally; cold exposed rats were bred at 5°C. All animals were fed a normal semi-purified diet prepared in the laboratory. The mitochondrial fraction of the interscapular brown adipose tissue was isolated in a 0.3 M sucrose medium (EDTA Na_2 2 mM, Tris–HCl pH 7.2 10 mM) and washed twice under the same conditions (12 000 $g \times 10$ min). The lipids were extracted from this fraction by the procedure of Folch et al. [10] and its protein content estimated in the hydromethanolic phase by the Biuret method after 10% TCA precipitation.

The mitochondrial lipids were divided into two parts. In the first part the different lipid classes were

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separated on silica gel G plate with petroleum ether/diethy ether/acetic acid mixture as solvent (90/10/1 by vol); after spraying the plate with rhodamine 6 G (2°/₀₀ in water) the phospholipid fraction was scraped for a gas-liquid chromatography analysis (GLC). The second part of the mitochondrial lipids was chromatographed on silica plates using a single dimensional [11] or a two-dimensional procedure [12]; the phospholipids were identified with A grade reference standards (Calbiochem., Koch–Light or Sigma).

The methyl esters of the phospholipid fatty acids were prepared directly on silica gel with BF_3 in methanol (14% w/v) [13], and analysed by GLC with a varian Aerograph 1440 apparatus (BDS column and nitrogen as carrier gas at 195°C); reference fatty acid methyl esters were purchased from A.S.L. Quantitative results with the fatty acid methyl ester mixture No. 6 of the A.O.C.S. (A.S.L.) agreed with the stated composition data, with a relative error lower than 3.5% for major components and 12% for minor components (less than 10% of the total mixture). Quantitative results were obtained using methyl–heptadecanoate (A.S.L.) as an internal standard; a good correlation was found between the results obtained with this method and with colorimetric determination of phosphorus. Each determination was repeated 5–7 times (6 for control animals, 7 for cold exposed rats and 5 for thyroxine treated animals).

3. Results

3.1. *Total mitochondrial fraction*

As compared to control animals, the amounts of protein and phospholipid recovered in the mitochondrial fraction of the brown fat from cold exposed rats were largely increased, contrary to what was observed after thyroxine treatment (table 1). Cold treatment led to important changes in the fatty acid spectrum of mitochondrial phospholipids, the general trend being an increase of the degree of unsaturation; the effect of thyroxine was less important and in an opposite direction (table 2).

3.2. *Mitochondrial phospholipids*

In cold exposed rats the proportion of PE was increased (from 29 to 41%) while the proportion of PC was slightly decreased (from 42 to 37%). Whatever the treatment, the percentage of cardiolipin was not modified (16% of total mitochondrial phospholipids). The main effect of cold exposure was a strong increase of the amount of all analysed fractions and more especially of the PE fraction; this phenomenon was not observed after thyroxine treatment (table 3).

3.3. *Fatty acid composition*

Important alterations of the fatty acid distribution were observed in cold exposed rats, essentially in the

Table 1
Composition of the mitochondrial fraction of rat brown adipose tissue (mean ± S.E.M.)

	Tissue weight (mg)	Mitochondrial components (mg/g tissue)			
		Phospholipids		Proteins	
		Experimental	Corrected*	Experimental	Corrected*
22°C	123 ± 7	1.71 ± 0.22	8.4	8.5 ± 0.6	41.7
5°C	253 ± 20	4.08 ± 0.70	19.6	14.8 ± 2.0	71.0
T_4-22°C	232 ± 6	1.01 ± 0.15	7.8	5.1 ± 0.4	39.2

* Experimental values have been corrected according to the yield of the mitochondrial extraction determined by polarographic assay of cytochrome *c* oxydase activity and spectrophotometer estimations of succinodehydrogenase and α-glycerophosphate dehydrogenase activities in the homogenate and in the mitochondrial fraction. Yields of mitochondrial extraction were 20.5% for control rats, 21% after cold exposure and 13% after thyroxine treatment.

Table 2
Fatty acid phospholipid composition of the mitochondrial fraction (mole %)*.

	16:0	16:1	18:0	18:1	18:2	20:4	'Double bond ** number'
22°C	21.6	6.1	19.1	25.8	14.4	13.0	113
5°C	17.4	1.7	25.6	18.0	18.5	18.8	132
T_4–22°C	22.9	5.2	18.5	27.7	14.1	11.6	107

* S.E.M. are not indicated to simplify the presentation, in all cases it did not exceed 10% of the mean.
** 'Double bond number' means number of double bonds per 100 fatty acid molecules.

Table 3
Phospholipid composition of the mitochondrial fraction*.

	Amount of mitochondrial phospholipids (mg/g fresh tissue)					
	CL		PE		PC	
	(1)	(2)	(1)	(2)	(1)	(2)
22°C	0.30 ± 0.05	1.46	0.48 ± 0.06	2.35	0.73 ± 0.10	3.57
5°C	0.56 ± 0.08	2.67	1.75 ± 0.37	7.44	1.54 ± 0.29	7.37
T_4–22°C	0.15 ± 0.04	1.14	0.31 ± 0.05	2.42	0.41 ± 0.07	3.13

* Abbreviations: CL cardiolipin, PE phosphatidylethanolamine, PC phosphatidylcholine. (1) Experimental values; (2) corrected values (see legend of table 1). These values were obtained by the method of Lepage [11], similar results were obtained by the procedure of Rouser et al. [12]. The data concerning minor phospholipids (less than 10% total phospholipds: phosphatidylglycerol, phosphatidylinesitol and lysophosphatids) are not indicated.

Table 4
Fatty acid composition of major phospholipids of the mitochondrial fraction (mole %) (see legend of table 2).

		16:0	16:1	18:0	18:1	18:2	20:4	'Double bond number'
PE	22°C	15.9	4.3	26.9	24.2	5.4	23.3	132
	5°C	12.3	0.8	37.3	12.0	6.6	31.0	154
	T_4–22°C	14.4	3.1	27.8	24.7	5.9	24.1	136
PC	22°C	28.9	4.9	16.8	29.1	12.3	8.0	91
	5°C	22.2	1.4	26.1	21.1	18.1	11.1	103
	T_4–22°C	29.7	3.6	17.8	28.6	13.2	7.1	87
CL	22°C	10.0	9.4	6.3	34.5	36.2	3.6	131
	5°C	12.5	3.0	4.7	25.8	51.2	2.8	142
	T_4–22°C	10.4	9.8	6.4	30.5	40.1	2.8	132

PE and PC fractions (table 4). These modifications were similar for PE and PC which represent the bulk of phospholipids and agreed well with those reported for total mitochondrial phospholipids (table 2).

The decrease of 16:0 and the increase of 18:0 which were observed in both PE and PC fractions from cold exposed rats have not been found in the cardiolipin fraction where the main effects of cold were a decrease of 18:1 and an increase of 18:2. Very slight alterations were observed after thyroxine treatment.

4. Discussion

The qualitative and quantitative modifications observed in the mitochondria of brown adipose tissue from chronically treated rats are in good agreement with our previous studies about the total phospholipids of this tissue [7,8]. The phospholipid composition of the mitochondrial fraction from control animals is similar to that reported for other tissues [14,15]; the cardiolipin content is comparable to the values reported in the literature for the brown adipose tissue of the bat [16] and in the mitochondria of several other tissues [14,15,17]. The fatty acid composition of phospholipids we observed agrees well with those which had been published for other tissues [14,15]. Recently Cannon and Polnaszek [18] have studied the mitochondrial phospholipids of brown adipose tissue from adult rats chronically exposed to cold: the modifications they observed were in the same direction but not significant.

4.1. *Mitochondrial proteins and phospholipids*

Corrected values of mitochondrial phospholipids and proteins indicate clearly that cold exposure increased largely the amount of mitochondrial components while thyroxine treatment had no effect. It must be pointed out that the small experimental quantities of phospholipids and proteins observed in thyroxine injected rats can be explained by the lower yield of the mitochondrial extraction which is probably correlated with the high fat content of the tissue [7–9].

4.2. *PE and PC*

The increase of the percentage of PE and decrease of the percentage of PC in the mitochondrial lipids from cold exposed rat are difficult to understand; we do not know whether the plasmalogen content was altered because we did not distinguish between ethanolamine phosphoglycerides diacyl species and monoacylmonoalkenylether species. Furthermore the PC:PE ratio which is reduced in cold exposed rats (1.0) as compared to control animals (1.5) is generally smaller for inner mitochondrial membranes than for outer membranes [19]. Our data could indicate a stimulation of the internal mitochondrial membrane development and would therefore confirm the conclusions of other authors based upon ultrastructural studies [4–6].

4.3. *Cardiolipin*

Cardiolipin is considered as a good indicator of the mitochondrial mass [17,20] and particularly of the amount of inner mitochondrial membranes [19,21,22]. Accordingly the increase of the absolute amount of mitochondrial cardiolipin observed in cold exposed rats, should indicate that the mitochondrial mass in brown adipose tissue is approximately doubled, while thyroxine treatment does not change it. Moreover the important changes of the fatty acid composition of cardiolipin which are induced by cold exposure suggest that the metabolism of this characteristic component is altered.

Several workers have shown with *S. Cerevisiae* and *E. Coli* that alterations of the fatty acid moiety of phospholipids in mitochondria and membranes are associated with changes in many enzymatical and other membrane dependent functions (glycoside transport, respiration . . .) [23]. The increase of the mitochondrial mass in brown adipose tissue from cold exposed rats is accompanied by important modifications in mitochondrial fatty acid and phospholipid composition: the brown adipocyte from cold exposed rat could be of a special interest for studying mitochondriogenesis.

Acknowledgements

The advice of Professor P. Mazliak and his collaborators in the course of this research is gratefully acknowledged. This work has been supported in part by the Délégation Générale à la Recherche Scientifique et Technique (France), contract No. 71.7.3250.01).

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