Effect of acclimation temperature on the concentration of the mitochondrial 'uncoupling' protein measured by radioimmunoassay in mouse brown adipose tissue

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The effect of acclimation temperature on the concentration of the mitochondrial 'uncoupling' protein (M_r 32000) from brown adipose tissue of mice has been investigated. The uncoupling protein was measured by a specific radioimmunoassay. Between 33°C (thermoneutrality) and −2°C there was a progressive increase with decreasing environmental temperature in the amount of uncoupling protein. For mice at −2°C the mitochondrial concentration of the protein was 9-times higher than at 33°C, while the total amount of the protein in interscapular brown adipose tissue was estimated to be nearly 80-times greater at −2°C compared to 33°C.

Brown adipose tissue *Uncoupling protein* *Mitochondria* *Radioimmunoassay* *GDP binding* *Proton conductance*

1. INTRODUCTION

Brown adipose tissue is considered to be the main site of non-shivering thermogenesis in the newborn of many mammalian species, in arousing hibernators, and in adult cold-adapted rodents [1–4]. The principal mechanism for heat production in the tissue is through a proton conductance pathway across the mitochondrial inner membrane [5]. This pathway can be blocked by purine nucleotides, which bind to a specific 'uncoupling' protein of M_r 32000 [6]. Following adaptation of non-hibernating species to cold environments the capacity of brown adipose tissue mitochondria to bind purine nucleotides is increased [7–9], and this relates to an apparent increase in the amount of the uncoupling protein [6,7,10,11]. However, quantitation of the uncoupling protein at different temperatures has been made only on the basis of the amount of the band in the 32000 M_r region following separation of mitochondrial proteins by polyacrylamide gel electrophoresis in the presence of sodium dodecylsulphate (SDS). Since this is neither a very specific nor a very sensitive method of determining the amount of uncoupling protein, immunoassay procedures have now been developed [12,13].

In a preliminary illustration of the use of a radioimmunoassay for uncoupling protein, it was observed that the concentration of the protein was approximately doubled in cold-acclimated rats compared to rats at room temperature [13]. We have now examined in detail the effect of various acclimation temperatures, ranging from 33°C (which is thermoneutral for mice and therefore the most appropriate reference point) down to −2°C, on the amount of uncoupling protein from brown adipose tissue mitochondria of mice, together with parallel measurements of purine nucleotide binding. We show here that there are substantial dif-

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ferences in the concentration of the protein between mice acclimated at the temperature extremes.

2. MATERIALS AND METHODS

2.1. *Animals*

The animals used were male C57Bl 10ScSn mice from the Department of Pathology, University of Cambridge. At the start of the study they were 2.5 months old. Each mouse was caged individually in plastic cages with the minimum quantity of sawdust, and given free access to water and a commercial low fat/high carbohydrate diet (Spillers-Spratts Rodent Breeding Diet 1). The mice were divided into 5 groups of equal mean body weight and placed at one of the following environmental temperatures for 3 weeks; 33, 22, 13 and 4°C (2 groups). At the end of the first week one group at 4°C was transferred to wire-mesh cages, without bedding material, and then housed at −2°C for the remaining 2 weeks; this procedure was intended to further increase the requirement of the animals for thermogenesis. Temperatures other than 22°C were obtained by the use of temperature-controlled cabinets with the same 12 h light/12 h dark cycle (light period from 07.00 h) as the animal house.

2.2. *Brown adipose tissue*

The mice were killed by cervical dislocation and brown adipose tissue from the interscapular site was rapidly removed and dissected free of other tissues. The interscapular pad was then weighed, and homogenized in 250 mM sucrose/1 mM HEPES/0.2 mM EDTA, pH 7.2. For the mice at 33°C, homogenates from two animals were combined. A sample of the homogenate was removed for the assay of cytochrome oxidase activity [14] and total tissue protein [15]. The bulk of the homogenate was used for the preparation of mitochondria [16].

Mitochondrial GDP binding was determined by incubating the mitochondria with 10 μM [^{3}H]GDP at pH 7.1 for 7 min [17,18]. [^{3}H]GDP and [^{14}C]sucrose were obtained from Amersham International (Bucks).

2.3. *Radioimmunoassay*

The concentration of the uncoupling protein in mitochondrial preparations was determined by solid-phase radioimmunoassay [13] using a mouse protein standard isolated from cold-acclimated (4°C for 3 weeks) mice as in [19,20].

The statistical significance of differences between groups was assessed by Student's *t*-test.

3. RESULTS

The results in table 1 show that the mice gained a small amount of weight at each of the 5 temperatures to which they were acclimated, and the gain was greatest at 33°C and least at −2°C. There was no clear effect of acclimation temperature in these particular mice on the amount of interscapular brown adipose tissue. There was, however, a marked effect of temperature on the total protein content of the tissue, which was lowest at 33°C and rose progressively at each temperature down to −2°C. There was a similar trend with temperature in the cytochrome oxidase activity of the tissue, which was measured as an index of mitochondrial mass; cytochrome oxidase activity at −2°C was 8.6-times higher than at 33°C.

The extent to which brown adipose tissue mitochondria bind purine nucleotides, such as GDP, has become the standard method of assessing the activity of the proton conductance pathway [7–9,18,21]. Table 2 shows the GDP binding results obtained for the mice at each acclimation temperature. GDP binding was lowest at 33°C and increased progressively at each temperature down to −2°C, in a similar manner to the changes in protein content and cytochrome oxidase activity of the whole tissue. At −2°C the amount of GDP bound was just over 8-times that at 33°C.

Table 2 also shows the results obtained in the measurement of the mitochondrial uncoupling protein by radioimmunoassay. The lowest values were again obtained at 33°C, where the uncoupling protein was 0.9% of total mitochondrial protein. There was a substantial increase in the concentration of the protein between 33 and 22°C, and a further significant increase between 22 and 13°C. However, between 13 and 4°C there was only a small increase, which was statistically insignificant ($p > 0.05$), and no change in concentration occurred between +4 and −2°C. At the lowest temperatures the uncoupling protein amounted to nearly 10% of total mitochondrial protein.

Table 1

Weight, protein content and cytochrome oxidase activity of interscapular brown adipose tissue from mice acclimated at different environmental temperatures

	Acclimation temperature (°C)				
	33	22	13	4	−2
Initial body wt. (g)	28.5 ± 0.4 (14)	28.3 ± 0.6 (7)	28.1 ± 0.6 (7)	28.2 ± 0.6 (7)	28.1 ± 0.7 (6)
Final body wt. (g)	33.5 ± 0.7 (14)	30.8 ± 0.7[a] (7)	30.6 ± 0.5[b] (7)	30.4 ± 0.8[b] (7)	28.4 ± 1.1[c] (6)
Interscapular brown adipose tissue wt. (mg)	167.1 ± 7.7 (14)	124.4 ± 5.0[b] (7)	149.4 ± 6.6 (7)	179.9 ± 13.2 (7)	161.2 ± 5.3 (6)
Protein content (mg)	4.1 ± 0.2 (7)	13.0 ± 0.7[c] (7)	20.2 ± 0.6[c] (7)	25.0 ± 1.1[c] (7)	32.1 ± 1.1[c] (6)
Cytochrome oxidase activity (μmol cytochrome *c* oxidized/min	6.5 ± 0.4 (7)	21.9 ± 1.3[c] (7)	34.2 ± 1.2[c] (7)	39.7 ± 2.6[c] (7)	56.0 ± 3.0[c] (6)

Mice were acclimated at the various temperatures, as described in the text. The results are given as mean values ± SEM with the number of animals shown in parentheses. [a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$ compared with mice at 33°C

Table 2

GDP binding and the concentration of 'uncoupling' protein from brown adipose tissue mitochondria of mice acclimated at different environmental temperatures

	Acclimation temperature (°C)				
	33	22	13	4	−2
GDP bound (pmol/mg mitochondrial protein)	69.1 ± 23.2 (6)	199.7 ± 27.1[a] (7)	378.1 ± 17.7[b] (7)	482.3 ± 28.8[b] (7)	572.1 ± 19.3[b] (6)
Uncoupling protein (μg/mg mitochondrial protein)	9.0 ± 1.3 (5)	43.4 ± 1.7[b] (7)	74.7 ± 4.4[b] (7)	85.0 ± 4.7[b] (6)	81.0 ± 2.5[b] (5)

Mice were acclimated at the various temperatures, as described in the text. The results are given as mean values ± SEM with the number of animals shown in parentheses: [a] $p < 0.01$; [b] $p < 0.001$ compared with mice at 33°C

4. DISCUSSION

This study shows that there are substantial increases in the concentration of the mitochondrial uncoupling protein induced by cold-acclimation in brown adipose tissue of mice. Between 33 and 4°C the concentration of the protein increased 9-fold, a change which approximately parallels the increase in GDP binding. Although this represents a

considerable rise in the amount of uncoupling protein, it does not reflect the full extent of the quantitative changes in the protein between thermoneutral and cold-acclimated conditions. In order to do this the increase in mitochondrial mass with decreasing temperature must be taken into account. Since the specific activity of cytochrome oxidase in the final mitochondrial suspensions was similar for the mice acclimated at each temperature (not shown), the ratio of the cytochrome oxidase activities obtained in the whole tissue for each group of mice reflects the relative changes with temperature in mitochondrial mass. On this basis, a relationship for the total amount of uncoupling protein in interscapular brown adipose tissue over the full temperature range of 1:16:44:57:77 (for 33, 22, 13, 4 and −2°C, respectively) is obtained.

These calculations indicate that there is a progressive rise with decreasing acclimation temperature in the total tissue content of uncoupling protein. With the exception of the increase between +4°C and −2°C, these increases result from a combination of a rise in the concentration of the protein per mg mitochondrial protein and a rise in mitochondrial mass. The increase in uncoupling protein between 4 and −2°C was, however, due entirely to an increase in mitochondrial mass. The further adaptation that occurred in the −2°C group could be the result of either the lower temperature or the change in caging conditions (see section 2), but other work has suggested that the caging is the important factor (S.W. Mercer and P. Trayhurn, unpublished).

Overall, the present results have shown a much greater capacity for adaptation in the amount of uncoupling protein than has been implied from studies where mitochondrial proteins have been separated by gel electrophoresis [6,7,10,11]. The large changes with temperature reported here are, however, consistent with the very substantial alteration in the thermogenic activity of brown adipose tissue which occurs between warm and cold environmental conditions [2,3].

Since both GDP binding measurements and the radioimmunoassay of the uncoupling protein were performed on the same mitochondrial preparations, the amount of GDP bound/mol of uncoupling protein has been calculated. It should be noted that Scatchard analysis of GDP binding to mouse and rat mitochondria has shown that maximum binding occurs with concentrations of GDP well below the 10 μM used here [9,18,22]. There was no clear effect of acclimation temperature on the molar binding ratio, which ranged from 0.25 mol GDP/mol protein at 33°C, to 0.15 mol GDP/mol protein at 22°C. Recent evidence has indicated that the uncoupling protein forms a dimer, and that each mol of dimer binds 1 mol of GDP [19,20]; this would give a ratio of 0.5 mol GDP bound/mol monomer at maximum binding. Our results suggest, therefore, that less than half the maximum number of GDP binding sites may be occupied in the fully acclimated mouse, assuming all the protein is available for binding, and that the proportion does not increase even at temperatures as low as −2°C. Thus, at all environmental temperatures there would appear to be 'spare' binding capacity in the acclimated animal, and this is consistent with the observation of rapid increases in GDP binding following either acute cold exposure or the administration of noradrenaline [7,8]. Spare GDP binding capacity, insofar as it reflects latent potential for thermogenesis, has a clear survival value.

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