Rapid increase of mitochondrial uncoupling protein and its mRNA in stimulated brown adipose tissue

Use of a cDNA probe

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The increase in mitochondrial uncoupling protein in brown adipose tissue during acute stimulation by exposure of animals to cold was examined. Uncoupling protein level increased during the first hours of tissue stimulation. Use of a cDNA probe shows that synthesis of uncoupling protein mRNA was quickly stimulated. Animals treated with propranolol exhibited neither increase in uncoupling protein mRNA nor increase in the protein itself.

Uncoupling *Mitochondria* *Cloned cDNA* *β-Adrenoreceptor* *Brown adipose tissue* *Cold exposure*

1. INTRODUCTION

The thermogenic function of brown adipose tissue (BAT) mitochondria is related to loose coupling of respiration due to the activity of a unique 32-kDa uncoupling protein (review [1]). During acclimatization to cold, chronic stimulation of rodent BAT leads to a striking increase in the amount of uncoupling protein [2–4] and of the corresponding mRNA [5,6]. The synthesis of uncoupling protein is due to continuous stimulation of brown adipocytes by sympathetic nerve endings ([6–8], reviews [9–11]). Recent investigations have shown that noradrenaline itself, or β-agonist drugs, when given continuously, stimulate the synthesis of a quantity of uncoupling protein similar to that observed in rats acclimatized to the cold [6,12–14]. Although the increase in uncoupling protein during prolonged stimulation of the tissue is well documented, it is unclear whether the increase occurs during the first hours of BAT stimulation. The uncoupling protein has often been assayed by measuring the GDP binding capacity of isolated BAT mitochondria [1] or by densimetric scanning of mitochondrial proteins separated by gel electrophoresis [2,3,15]. It is known that the GDP binding capacity of rat BAT mitochondria increases after 1 h exposure to cold [3] and that this change can be mimicked in animals by injecting noradrenaline or β-agonist drug [14,15]. However, according to authors in [3,15], the increase in the level of the protein, as determined by gel electrophoresis, becomes apparent only after 12–48 h exposure of animals to cold. These authors propose that initially cold stress induces the unmasking of a GDP binding site on BAT mitochondria but does not stimulate the synthesis of the uncoupling protein [15,16].

We report here that a small but significant increase in the proportion of uncoupling is observed in response to a very short exposure to cold. The synthesis of uncoupling protein is dependent on the activation of the β-adrenoreceptor.

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2. MATERIALS AND METHODS

2.1. *Chemicals*

[8-^{3}H]GDP and ^{32}P-labelled nucleotides were obtained from Amersham Radiochemical Centre (England). Enzymes for cDNA cloning were obtained from Boehringer-Mannheim (FRG). mAP-messenger activated paper was obtained from Orgenics (Yavne, Israel).

2.2. *Animals and treatment*

Male rats of the Wistar strain (IFFA-CREDO, France) weighing approx. 200 g were caged separately and kept at 26°C for 1 week. A group of rats was injected subcutaneously in the mid-dorsal region with 28 mg DL-propranolol·HCl (e.g., 12.5 mg L-propranolol base) per kg body wt. One h later several propranolol-treated rats, and several non-treated rats were kept at 26°C as controls while the remainder were exposed to 5°C for 1, 3 or 5 h. All experiments were made using interscapular BAT. Mitochondria were isolated by routine procedures [2,7,12]. Tissue used for mRNA extraction was rapidly dissected in the cold and frozen in liquid nitrogen.

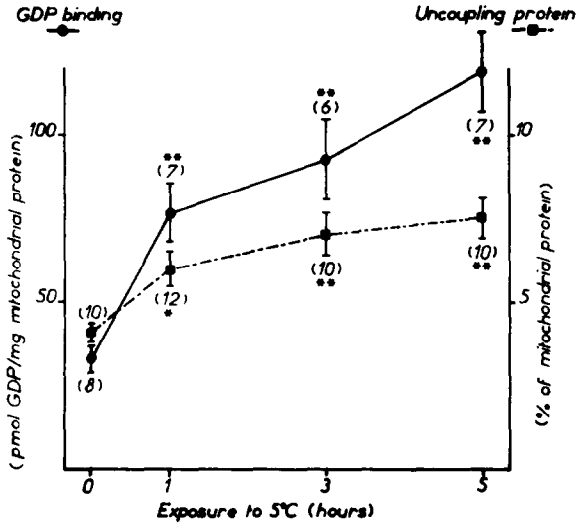

Fig.1. Time course of mitochondrial response in BAT of rats exposed to 5°C. Animals were caged individually and kept at 26°C before experiment. Results are expressed as means ± SE. The number of determinations is between brackets. Statistics: * $p \leq 0.05$; ** $p \leq 0.01$ *vs* control values (zero time). In animals kept at 5°C for 9 days the GDP binding value was 566 pmol per mg mitochondrial protein and the proportion of uncoupling protein was 10.3%.

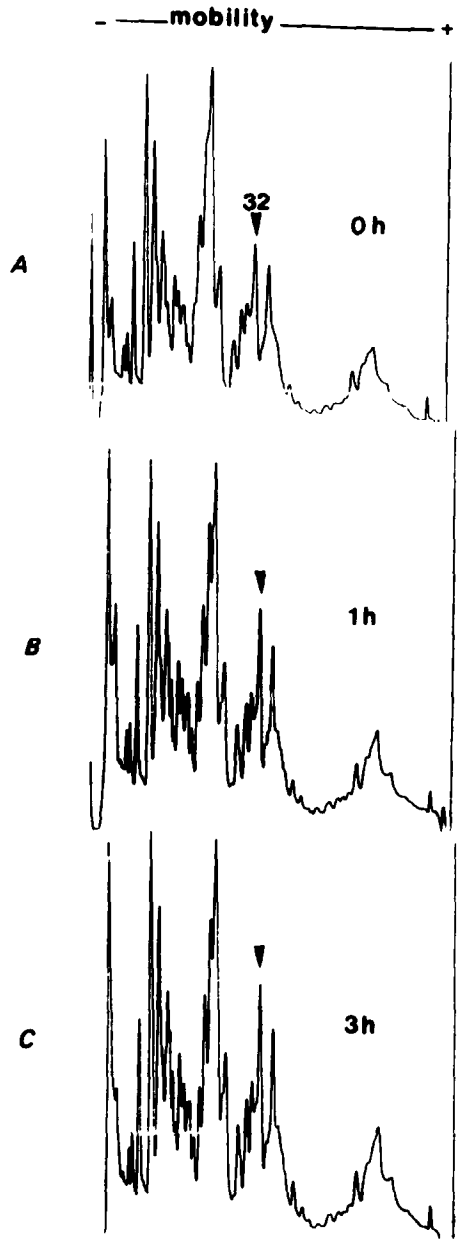

Fig.2. Densitometric scanning of BAT mitochondrial proteins separated by SDS gel electrophoresis. (A) Animals kept at 26°C; (B) animals exposed to 5°C for 1 h; (C) animals exposed to 5°C for 3 h. The '32 arrow' indicates the localization of the 32000 daltons uncoupling protein.

2.3. *Assay of uncoupling protein*

GDP binding to isolated mitochondria was measured in duplicate in the presence and absence

of 1 mM GDP as in [18]. The mitochondrial proteins were separated by SDS-gel electrophoresis as in [2] and [12]. The proportion of the peak corresponding to the 32 kDa was carefully measured by densimetric scanning at 560 nm. Purified uncoupling protein [19,20] was used as a standard.

2.4. *Assay of uncoupling protein mRNA*

BAT RNA was prepared as in [5]. Poly(A^+) mRNAs were obtained using mAP-paper, essentially as described by the supplier. A cDNA probe specific for uncoupling protein mRNA was prepared as described in [17]. The probe pUCP 36 was labelled by nick-translation to a specific activity of 10^8 cpm/μg. After electrophoresis the mRNAs were hybridized to the labelled probe (Northern blot method). The hybridization was carried out overnight at 42°C in 45% formamide. After washing (30 mM NaCl, 3 mM Na citrate), the blots were subjected to analysis by autoradiography.

3. RESULTS

Following acute exposure to cold both GDP binding to isolated mitochondria and the proportion of uncoupling protein increase (figs 1 and 2). The increase is very sharp during the first hour and

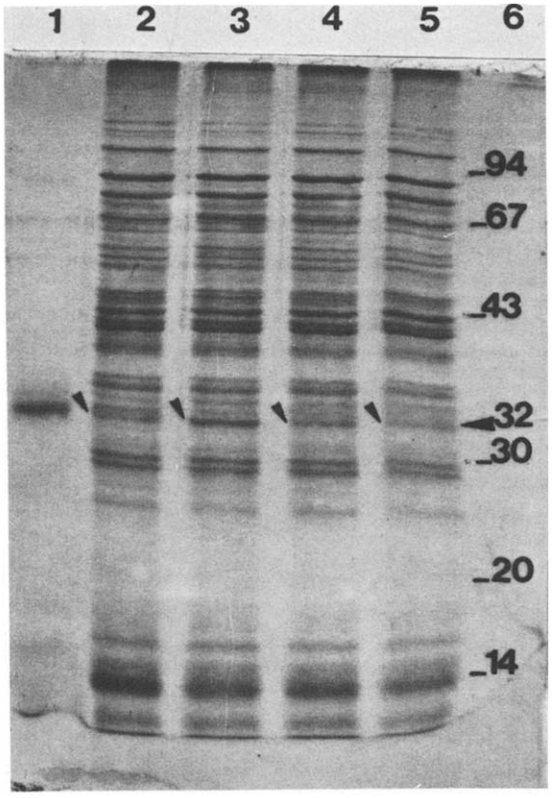

Fig.3. SDS polyacrylamide gel electrophoresis of BAT mitochondrial proteins of animals exposed at 5°C for 5 h and treated or untreated with propranolol. Lane 1, purified uncoupling protein. Lane 2, rats kept at 26°C receiving acetate buffer. Lane 3, rats kept at 5°C receiving acetate buffer. Lane 4, rats kept at 5°C receiving propranolol in acetate buffer. Lane 5, rats kept at 26°C receiving propranolol in acetate buffer. Lane 6, standard M_r markers $\times 10^{-3}$.

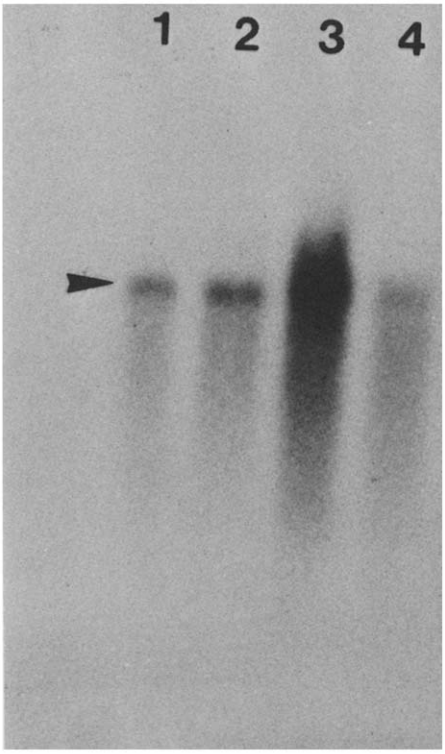

Fig.4. Northern blot hybridization of uncoupling protein mRNA in BAT of rats exposed to 5°C for 1 h treated or untreated with propranolol. The equivalent amount of poly(A^+) mRNA from brown adipose tissue was electrophoresed on a 1.5% agarose, 2 M formaldehyde gel and transferred to a nitrocellulose filter, for hybridization to the ^{32}P-labelled probe. (1) Rats kept at 26°C injected with acetate buffer. (2) Rats kept at 26°C injected with propranolol in acetate buffer. (3) Rats kept at 5°C injected with acetate buffer. (4) Rats kept at 5°C injected with propranolol in acetate buffer. Arrow indicates a value of 15 S.

Table 1

Effect of propranolol on BAT uncoupling protein in rats exposed to cold for 5 h

Animals	Temperature (°C)	GDP binding (pmol GDP · mg mitochondrial proteins^{-1})	Uncoupling protein (% of mitochondrial proteins)
Control	26	50 ± 5	3.8 ± 0.5
Propranolol	26	49 ± 8	3.0 ± 0.3
		n.s.	n.s.
Control	5	159 ± 18[b]	5.9 ± 0.3[b]
Propranolol	5	89 ± 12[a]	3.3 ± 0.4
			n.s.

Animals were caged separately and kept at 26°C for 1 week. They received either propranolol in 25 mM acetate buffer pH 5 or acetate buffer 1 h before start of experiment. Then they were either kept at 26°C or exposed to 5°C for 5 h. Results are mean ± SE. Number of cases: 9 per experimental category. n.s., non-significant; [a] $P \leqq 0.05$; [b] $P \leqq 0.01$ (propranolol 26°C *vs* control 26°C, control 5°C *vs* control 26°C, propranolol 5°C *vs* propranolol 26°C)

then develops more slowly (fig.1). Although roughly similar, the kinetic variations of the two parameters measured were not exactly parallel. The proportional increase in GDP binding capacity is greater than that observed in the concentration of protein. The increased GDP binding measured in rats exposed 5 h at 5°C was largely, but not totally, reduced by propranolol treatment (table 1). In these animals, the cold-induced increase of uncoupling protein was no longer observable in polyacrylamide gels (table 1, fig.3).

Hybridization experiments using a cDNA pUCP 36 probe to uncoupling protein mRNA showed that the level of this mRNA increases during the first hour of BAT stimulation (fig.4, lane 3). Moreover, this change in mRNA content was abolished in propranolol-treated animals (fig.4, lane 4). As reported in [17] major species of mRNA (15 S) was detected by the cDNA probe in stimulated tissue (fig.4).

4. DISCUSSION

We report here that a short-time exposure to cold induces a small, but significant increase in the proportion of uncoupling protein in BAT mitochondria. The previously described increase of GDP binding within the first hour of exposure to cold [3] is confirmed, but in addition, contrary to what had been observed [3], we report here that an increase of the protein occurs in parallel. It is known that a process requiring protein synthesis does not obligatorily need more than 60 min. The two methods used here to assay the uncoupling protein (GDP binding and electrophoretic analysis) give similar but not identical data. The reason for such a discrepancy can be attributed either to unmasking of GDP binding sites during the first hours of exposure to cold [3], or to the fact that GDP binding capacity, just as it is determined here, is not always an accurate assay for the uncoupling protein [21]. Recently, authors in [22] found no evidence of rapid unmasking of nucleotide binding sites in BAT mitochondria of guinea pigs. Authors in [23] have described that a 1-h exposure to cold altered neither the GDP binding capacity nor the proportion of uncoupling protein in rat. This data can probably be explained by the low environmental temperature (21°C) of the control animals used [23]. We could not observe a significant increase in the uncoupling protein in rats exposed at 5°C for 1, 3 or 5 h when controls were kept at 21°C instead of 26°C. Here, the rapid increase of uncoupling protein biosynthesis during

stimulation of BAT is confirmed by the assay of the corresponding mRNA which increases significantly within the first hour. The cDNA probe is highly specific for uncoupling protein mRNA [17].

As the synthesis of uncoupling protein can be rapidly stimulated, it seems necessary to investigate possible control factors. Use of propranolol treatment prevents the normal increase of the protein and of its mRNA. The reason why the GDP binding enhancement is not fully abolished, as is the protein increase, is unclear. We conclude that uncoupling protein mRNA transcription and uncoupling protein biosynthesis are controlled by the sympathetic nervous system probably acting through activation of brown adipocyte β-receptors. Thus, as shown for the large increase of uncoupling protein in cold-acclimatized rats [6–8,12–14], we propose that stimulation of uncoupling protein synthesis during acute exposure to cold is controlled by noradrenaline released by nerve endings on β-receptors of brown adipocyte membrane. Interestingly, it has been observed that the increase of BAT lipoprotein lipase activity which also occurs during acute exposure of rats to cold is also a β-adrenergic mechanism [24]. Thus, in addition to its essential role in the rapid activation of brown adipocyte metabolism (lipolysis...), noradrenaline is probably able to trigger rapidly the synthesis of uncoupling protein mRNA, allowing cells to synthesize mitochondrial uncoupling protein and producing BAT capable of dissipating more energy as heat. It remains to be demonstrated whether if the nucleotide-sensitive proton conductance of the brown fat mitochondria increases in the first hours of cold exposure in correlation with the increased synthesis of the uncoupling protein.

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