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Increase of uncoupling protein and its mRNA in brown adipose tissue of rats fed on 'cafeteria diet'

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The effect of 'cafeteria diet' on mitochondrial uncoupling protein in brown adipose tissue of rats was examined. 'Cafeteria diet' induced an increase of the 32 kDa uncoupling protein in electrophoresed proteins of brown-fat mitochondria. Use of a cDNA probe corresponding to uncoupling-protein mRNA indicated that this mRNA was increased in rats fed on the 'cafeteria diet'. Nevertheless, this effect was weak compared with that observed in rats adapted to cold.

INTRODUCTION

Both non-shivering and diet-induced thermogenesis are associated with brown-adipose-tissue heat production (Rothwell & Stock, 1984). Brown-adipose-tissue thermogenesis is related to the presence and activity of a 32000-M_r uncoupling protein in the mitochondrial inner membrane of brown adipocytes. Purine nucleotides such as GDP bind to this component (reviewed by Nicholls & Locke, 1984). Many authors have demonstrated that increased non-shivering thermogenesis in rodents is correlated with increased GDP-binding capacity and the amount of uncoupling protein (reviewed by Ricquier & Mory, 1984). It has also been clearly observed that 'cafeteria feeding' induces an increase in GDP binding to isolated brown-fat mitochondria of rodents (Brooks *et al.*, 1980; Himms-Hagen *et al.*, 1981; Himms-Hagen, 1983; Ashwell *et al.*, 1984; Nedergaard *et al.*, 1984; Rothwell & Stock, 1984). However, data concerning the effects of 'cafeteria feeding' on the amount of uncoupling protein are conflicting. Himms-Hagen *et al.* (1981), using sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of mitochondrial proteins, reported no increase in the proportion of uncoupling protein, whereas Nedergaard *et al.* (1984) and Ashwell *et al.* (1984), using immunological assays, detected a significant increase in uncoupling protein in 'cafeteria-fed' rats. In order to assess if the uncoupling protein is involved in diet-induced thermogenesis, young rats were submitted to a 3-week 'cafeteria diet'.

Here we present data supporting an increase in uncoupling protein in rats fed on a 'cafeteria diet'. These data were obtained after electrophoresis of brown-fat mitochondrial proteins. In addition, use of the cDNA probe corresponding to uncoupling-protein mRNA showed that 'cafeteria feeding' increases the amount of this mRNA. Nevertheless, this effect was weak compared with that observed in animals exposed to cold (Bouillaud *et al.*, 1984, 1985; Ricquier *et al.*, 1984).

MATERIALS AND METHODS

Animals and diets

Some 30 120 g male rats of the Wistar strain (Charles River, Saint-Aubin les Elbeuf, France) were kept for 1 week at room temperature (23 ± 1 °C) on laboratory chow. Then all animals were maintained on laboratory chow for 3 weeks. Among them, 15 were also offered 'cafeteria diet' *ad libitum* every day for 3 weeks, and 5 animals maintained on laboratory chow were kept at 5 °C for 3 weeks. The 'cafeteria diet' used in this study was described previously (Mandenoff *et al.*, 1982). The energy value of this diet was calculated from the chemical composition of foods stated by suppliers.

Brown-adipose-tissue dissection and isolation of mitochondria

After the rats were killed, interscapular brown adipose tissue was dissected out and weighed. This tissue was homogenized in ice-cold 0.25 M-sucrose/10 mM-Tris (pH 7.1)/1 mM-EDTA. A sample of each homogenate was taken for protein and cytochrome *c* oxidase activity assays. Mitochondrial pellets were sedimented and washed by a conventional procedure (Nedergaard & Cannon, 1979). The protein content and cytochrome *c* oxidase activity of mitochondrial pellets were also determined.

Biochemical determinations

Protein content was determined as described by Bradford (1976) and cytochrome *c* oxidase activity as described by Schnaitman *et al.* (1967). Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of mitochondrial proteins was carried out in 10%-polyacrylamide slab gels (Laemmli, 1970). [8-^{3}H]GDP-binding capacity of isolated mitochondria was measured as described by Desautels *et al.* (1978). Specific GDP binding was calculated as the excess of [8-^{3}H]GDP bound to mitochondria in the presence of a large excess (250 mM) of non-labelled GDP.

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Table 1. Effects of 'cafeteria diet' on body weight and interscapular brown adipose tissue

Values are means ± S.E.M. The number of animals and determinations for each group was 10. ** and NS indicate the effects of 'cafeteria feeding' ($P < 0.01$ and $P > 0.1$ respectively; Mann–Whitney U-test).

	Control	'Cafeteria diet'
Initial body wt. (g)	177 ± 3	178 ± 3^{NS}
Final body wt. (g)	326 ± 4	334 ± 4^{NS}
Body-wt. gain (g)	149 ± 3	156 ± 4^{NS}
Energy intake (kJ/day)	376 ± 7	697 ± 19**
Interscapular brown-adipose-tissue mass (mg)	284 ± 19	605 ± 27**
Total interscapular brown-adipose-tissue protein (mg)	15.6 ± 3	36.9 ± 6**

Table 2. Effects of 'cafeteria diet' on mitochondria of brown adipose tissue

Results are means ± S.E.M. for five different mitochondrial preparations. Each mitochondrial pellet was obtained from two organs. *, ** and NS indicate the effects of 'cafeteria feeding' ($P < 0.05$, $P < 0.01$ and $P > 0.1$ respectively; Mann–Whitney U-test). Mitochondrial protein was corrected according to the yield of mitochondrial extraction. This yield was the ratio of cytochrome c oxidase activity in the tissue homogenate compared with that in the mitochondrial pellet.

	Control	'Cafeteria diet'
Total mitochondrial protein (mg/animal)	6.5 ± 0.06	14.0 ± 1.9*
Mitochondrial protein (% of tissue mass)	2.29 ± 0.15	2.32 ± 0.35^{NS}
Total cytochrome c oxidase activity (nmol of O_2/min per organ)	6425 ± 422	14790 ± 1141**
Mitochondrial cytochrome c oxidase activity (nmol of O_2/min per mg of mitochondrial protein)	990 ± 68	1070 ± 73^{NS}
[^{3}H]GDP bound (pmol/mg of mitochondrial protein)	68 ± 8	115 ± 10**
Uncoupling protein		
(μg/mg of mitochondrial protein)	55.0 ± 3	68.2 ± 3*
(μg/organ)	357 ± 19	954 ± 42**

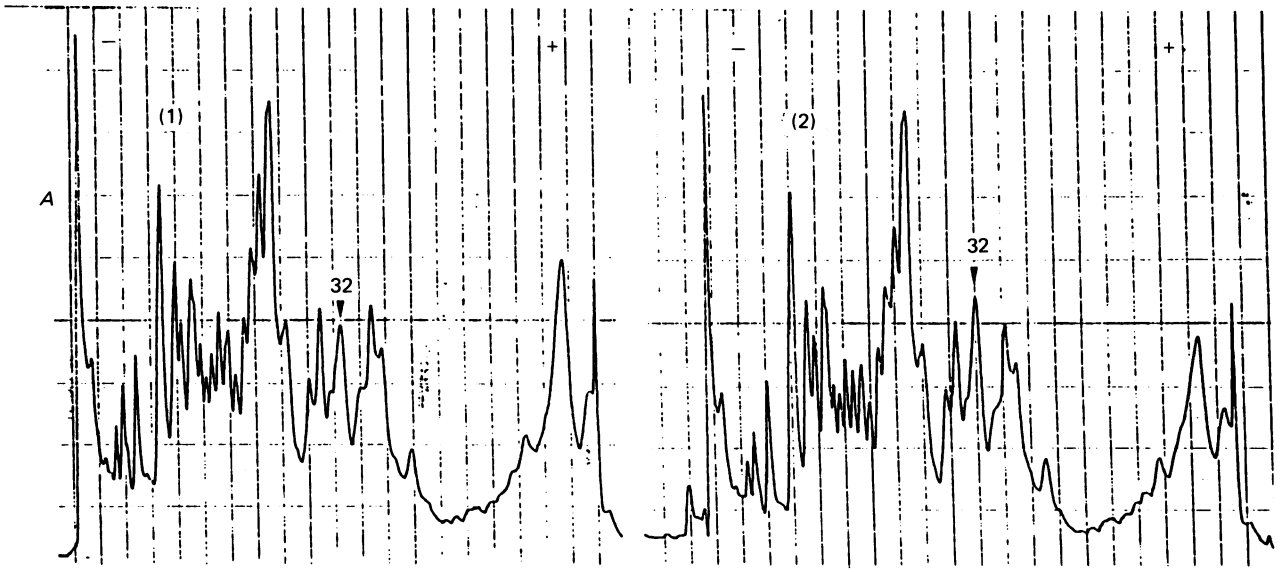

Fig. 1. Densitometric tracing of Coomassie-Blue-stained electrophoresis of brown-adipose-tissue mitochondrial proteins

Electrophoretic mobility is indicated by − and +. (1) Control animals. (2) Animals fed on a 'cafeteria diet'. The arrow '32' indicates the position of uncoupling protein. The increase in 32 kDa uncoupling protein was observed in five separate experiments.

Assay of uncoupling-protein mRNA

For this assay, interscapular brown adipose tissue was removed under sterile conditions at death and immediately frozen in liquid N_2. RNA was extracted as described by Ricquier *et al.* (1983), and poly(A)-containing RNA was prepared by paper affinity chromatography (Orgenics, Yavne, Israel) as described by Werner *et al.* (1984). Total RNA or poly(A)-containing RNA was electrophoresed in 1.5% agarose gel containing formaldehyde and then transferred to a nitrocellulose filter. pUCP 36 cDNA complementary to uncoupling-protein mRNA cloned in plasmid pBR 327 of *Escherichia coli* (Bouillaud *et al.*, 1985) was used as a probe after radioactive labelling. Hybridization of ^{32}P-labelled cDNA probes to RNA has been described by Bouillaud *et al.* (1985).

RESULTS AND DISCUSSION

'Cafeteria-fed' animals did not gain more weight than did control animals, although their energy intake was twice as great (Table 1). 'Cafeteria feeding' also induced a marked development of interscapular brown adipose tissue (Table 1), and resting oxygen consumption by 'cafeteria-fed' rats was higher (results not shown). All these data confirm the pioneer observation by Rothwell & Stock (1979). Brown-adipose-tissue growth was characterized by an increase in total and mitochondrial protein; nevertheless mitochondrial protein per tissue mass was not increased (Tables 1 and 2). Conversely, cytochrome *c* oxidase activity was increased per total organ, but not per mg of tissue nor per mg of mitochondrial protein (Table 2), and, as reported by others (Brooks *et al.*, 1980; Himms-Hagen *et al.*, 1981; Nedergaard *et al.*, 1984; Ashwell *et al.*, 1984), the GDP-binding capacity of isolated brown-fat mitochondria from 'cafeteria-fed' animals was significantly increased (Table 2).

The proportion of uncoupling protein was calculated as the relative area of the 32 kDa peak of electrophoresed mitochondrial protein. The exact location of this peak was obtained by comparison with electrophoresed brown-fat mitochondria from cold-acclimated rats, which are known to show a striking increase in uncoupling protein (for review, see Ricquier & Bouillaud, 1985). Using this technique, we showed that 'cafeteria feeding' also induces an increase in the proportion of uncoupling protein (Table 2, Fig. 1). This moderate relative increase corresponded to a marked increase in the total amount of uncoupling protein in brown adipose tissue (Table 2). These data were in agreement with those obtained by Nedergaard *et al.* (1984) and Ashwell *et al.* (1984), who used immunological titration. They are not in agreement with data obtained by Himms-Hagen *et al.* (1981), who used a technique similar to ours. Although sodium dodecyl sulphate/polyacrylamide-gel-electrophoretic assay of uncoupling protein is less sensitive than immunological assay, and probably overestimates the exact amount of the protein in control animals, as previously shown (for review, see Ricquier & Bouillaud, 1985), it can be used to detect alterations in the proportion of uncoupling protein.

We used another technique, derived from molecular cloning of uncoupling-protein cDNA (Bouillaud *et al.*, 1985), to confirm this finding. The cDNA probe can be used to assay specifically changes in the amount of uncoupling-protein mRNA. As previously shown (Bouillaud *et al.*, 1985), this probe hybridizes to two mRNAs (Fig. 2). Fig. 2 shows that 'cafeteria feeding' of animals generates a significant enhancement of uncoupling-protein mRNA, and probably triggers transcription of the corresponding gene. Densitometric measurement of the

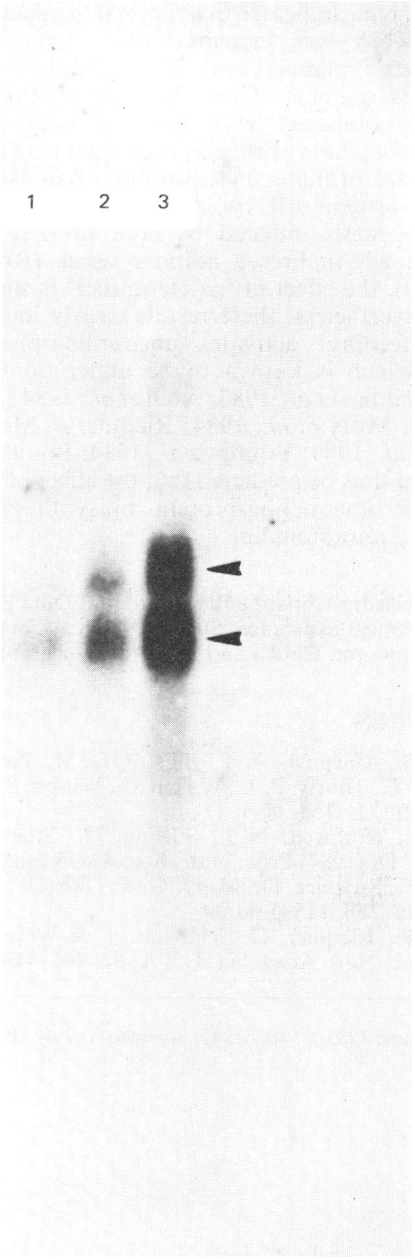

Fig. 2. Hybridization of uncoupling-protein cDNA to brown-fat RNA

Brown-fat RNA (10 μg) was electrophoresed on agarose gel in the presence of formaldehyde. The RNA was then transferred to nitrocellulose and probed with ^{32}P-labelled cDNA corresponding to uncoupling protein. Lane 1, RNA from animals fed on laboratory chow; lane 2, RNA from rats fed on 'cafeteria diet' for 3 weeks; lane 3, RNA from rats kept at 5 °C for 3 weeks and fed on laboratory chow. The upper and lower arrows indicate values of 1.9 and 1.6 kilobases respectively. The same data have been obtained with poly(A)-containing RNAs.

1.6-kilobase band indicated that the amount of uncoupling-protein mRNA was increased by a factor of 3 in 'cafeteria-fed' animals and by a factor of 10 in cold-adapted animals. Thus, the 'cafeteria-diet' effect was small compared with that of cold exposure. Moreover, the whole of the data reported here (total and mitochondrial proteins, cytochrome *c* oxidase activity, uncoupling-protein mRNA) demonstrate that, although the thermogenesis induced by cold or diet produces similar changes in brown adipose tissue (Rothwell & Stock, 1980), the effect of 'cafeteria diet' is significantly weaker. Nevertheless, these results clearly indicate that 'cafeteria feeding' activates uncoupling-protein biosynthesis, which is known to be under noradrenergic control (Ricquier *et al.*, 1983; Arch *et al.*, 1984; Bouillaud *et al.*, 1984; Mory *et al.*, 1984; Ricquier & Mory, 1984; Ricquier *et al.*, 1984; Young *et al.*, 1984; Bouillaud *et al.*, 1985). It can thus be predicted that the effect of 'cafeteria feeding' on uncoupling-protein biosynthesis is also mediated by noradrenaline.

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