Noradrenaline controls the concentration of the uncoupling protein in brown adipose tissue

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Received 15 December 1983

The importance of noradrenaline in the control of the level of the uncoupling protein responsible for the high thermogenic capacity of brown adipose tissue mitochondria was examined. It was observed that chronic infusion of noradrenaline through mini-osmotic pumps increased the mitochondrial concentration of this uncoupling protein to the same extent as chronic exposure to cold.

Noradrenaline *Brown adipose tissue* *Mitochondria* *Uncoupling protein* *Neurotrophism*

1. INTRODUCTION

Brown adipose tissue (BAT) is considered a major site of cold-induced [1] and diet-induced thermogenesis [2,3] in rodents. When rats are chronically exposed to cold, their BAT exhibits a complex trophic response. This response consists mainly of cell proliferation, mitochondriogenesis and a striking increase of one mitochondrial polypeptide of M_r 32 000 [4,5] which is the specialized uncoupling protein responsible for the high thermogenic capacity of BAT [6]. This trophic response is almost entirely suppressed by sympathectomy [7] and is mimicked by the presence of pheochromocytoma in animals [8]. Nevertheless, daily injections of catecholamines to rats induced mainly cell proliferation in BAT [9] without changes at the mitochondrial level [9,10]. Thus, as in some other cases of neurotrophism [11], the involvment of a chemical signal distinct from a classic neurotransmitter was suspected [7,8]. However, we report here that continuous administration of noradrenaline to rats through mini-osmotic pumps greatly increases the uncoupling protein concentration in BAT mitochondria.

2. MATERIALS AND METHODS

2.1. *Animals and treatments*

An ALZET mini-osmotic pump (model 2002) was implanted under the dorsal skin (just behind the interscapular BAT) in 40-day-old rats of the Wistar strain kept at 26°C. The pumps of noradrenaline-treated rats were filled with 0.8 M noradrenaline, 0.1 M ascorbic acid and 0.02 M 4,5-dihydroxy-1,3 benzene disulfonic acid as protectors against oxidation; they released 20 μg noradrenaline/h (from several pumping rates tested, 20 μg/h was chosen since this was a rate which caused a metabolic activation in BAT in conditions where no mortality of animals was recorded). Control animals reveived pumps containing the same solution without noradrenaline. Interscapular BAT was removed and studied after 9 days of treatment, as well as BAT of rats exposed for 9 days to cold (5°C).

2.2. *Biochemical and immunohistological determinations*

DNA, proteins and total lipids were determined as in [7]. Cytochrome *c* oxidase activity

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001457593/84/$3.00

(EC1.9.3.1) was measured polarographically [12]; the recovery of this activity in isolated mitochondria was used to calculate the yield of mitochondria extraction (see [7]). Mitochondrial protein values were corrected using this yield. GDP binding to isolated mitochondria was determined as in [13]. Uncoupling protein was separated by gel electrophoresis and its concentration in mitochondrial proteins measured by gel scanning [4]. Immunohistofluorescence visualization of uncoupling protein in tissue slices was carried out as in [14].

3. RESULTS AND DISCUSSION

Noradrenaline-treated animals weighed less than control animals and their BAT was slightly enlarged, although to a lesser extent than in cold-exposed rats (table 1). The DNA concentration of the tissue was unchanged (the total DNA content of BAT was 271 ± 17 μg, 313 ± 23 μg and 327 ± 27 μg, respectively, in the control, noradrenaline-treated and cold-exposed animals used for DNA analysis), a result which confirms our previous conclusions concerning the role of noradrenaline in the control of cell proliferation in BAT [7,9]. In noradrenaline-treated animals the lipid concentration was largely decreased, indicating that noradrenaline induced an intense lipid utilization. The total protein concentration was enhanced as in the cold-exposed rats: the mitochondrial protein concentration was also increased (+ 77.5%) but less than in cold-exposed rats (+ 127%). In noradrenaline-treated and in cold-exposed animals, similar high values were found for cytochrome *c* oxidase activity (per unit of tissue weight) and for the capacity of isolated mitochondria to bind GDP (the GDP binding to mitochondria is assumed to reflect the thermogenic capacity of the tissue and is due to the high affinity of the uncoupling protein for purine nucleotides, see [6]). Conversely, the most striking result in noradrenaline-treated rats as with cold-exposed rats was a large increase in the uncoupling protein concentration in BAT mitochondria (table 1, fig.1). In addition to gel electrophoresis analysis (fig.1), the same conclusion was reached following an immunohistofluorescence study (fig.2).

Table 1

Effects of continuous administration of noradrenaline or exposure to cold during 9 days on interscapular brown adipose tissue

	Control	Noradrenaline-treated	Cold-exposed
Body weight (g)	191.6 ± 6.2 (18)	159.8 ± 6.1[b] (19)	184.6 ± 8.1[b] (10)
Interscapular brown fat mass (mg)	126.9 ± 2.7 (18)	156.7 ± 9.3[a] (19)	221.2 ± 17.2[b] (10)
DNA (% of fresh tissue wt)	0.16 ± 0.01 (5)	0.17 ± 0.01 n.s. (5)	0.16 ± 0.01 n.s. (5)
Total lipids (% of fresh tissue wt)	57.2 ± 1.5 (5)	33.9 ± 3.5[b] (5)	46.1 ± 1.4[a] (5)
Total proteins (% of fresh tissue wt)	10.1 ± 0.4 (11)	14.4 ± 0.6[b] (12)	14.2 ± 0.5 n.s. (10)
Mitochondrial proteins (% of fresh tissue wt)	4.0 ± 0.7 (6)	7.1 ± 0.9[a] (7)	9.1 ± 0.6[a] (5)
Cytochrome *c* oxidase activity (nmol O_2 consumed · min^{-1} · mg of $tissue^{-1}$)	20.5 ± 2.4 (6)	35.6 ± 2.2[b] (7)	39.6 ± 4.0 n.s. (5)
GDP binding (nmol GDP bound · mg mitochondrial proteins)	0.08 ± 0.007 (6)	0.26 ± 0.05[b] (7)	0.28 ± 0.03 n.s. (5)
Uncoupling protein (% of mitochondrial proteins)	6.3 ± 0.5 (6)	10.8 ± 0.4[b] (7)	10.3 ± 0.3 n.s. (5)

Results are means ± SE and the number of determinations are in parentheses; each point for mitochondrial values was obtained by pooling interscapular BAT of two rats. Statistical significance of differences (noradrenaline-treated *vs* control, cold-exposed *vs* noradrenaline-treated) was assessed with the non-parametric U-test of Mann-Whitney

[a] $P \leqslant 0.05$; [b] $P \leqslant 0.01$; n.s., non-significant

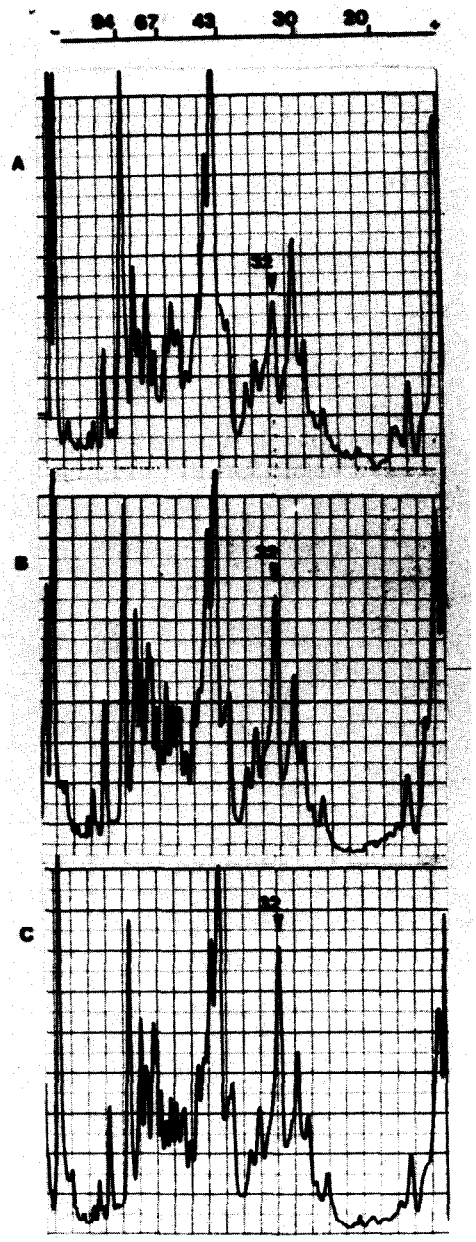

Fig. 1. Gel scanning after separation of interscapular BAT mitochondrial proteins by sodium dodecyl sulfate electrophoresis. Animals used were the same as in table 1. Migration occurred from − to + and the scale is in kDa. The '32 arrow' indicates the localization of the 32-kDa uncoupling protein. In all the mitochondria of the noradrenaline-treated rats analysed, the 32 000 peak was higher than the corresponding peak in the control rats. (A) Control, (B) noradrenaline-treated, (C) cold-exposed.

Previous experiments had shown that daily injections of noradrenaline or isoproterenol to rats partly reproduced the cold-induced trophic response of BAT : increase of BAT weight [9,10,15,16], of DNA content and of protein concentration [9,10,16], and an increase (slight) of the cytochrome *c* oxidase activity per unit tissue weight [9,15]; however, several other aspects of BAT response to chronic cold exposure such as the increase of uncoupling protein concentration [9,10], were not observed in these conditions.

Although it is not possible to rule out completely the possibility that the observed effect of noradrenaline may not be direct, the reported data indicate that noradrenaline, the natural neurotransmitter of BAT sympathetic nerves can stimulate mitochondriogenesis and specifically induces the synthesis of the uncoupling protein. The failure of previous daily injections of catecholamines to stimulate uncoupling protein synthesis [9,10] was the result, most probably, of the lack of duration of BAT stimulation (even when the amine injected was suspended in oil). Otherwise, we were recently informed that other authors [17] observed that mice treated per os with large doses of ephedrine (α and β agonist drug which also induces noradrenaline release from nerves) or fenoterol (a long action β-2 agonist) exhibited a slight increase in the uncoupling protein percentage in BAT mitochondria.

We can conclude that noradrenaline which is known to be the physiological activator of thermogenesis in BAT, is also the trophic factor responsible for to the most striking aspects of BAT response to chronic cold exposure, including cell proliferation, mitochondriogenesis and the specific synthesis of the characteristic thermogenic uncoupling protein located in mitochondrial inner membranes. Further, BAT seems to provide a good example of sympathetic-nervous-system-induced neurotrophism.

ACKNOWLEDGEMENTS

We thank Christine Blanchard for excellent technical assistance, the CNRS for financial support and Betty Talks for reading the manuscript.

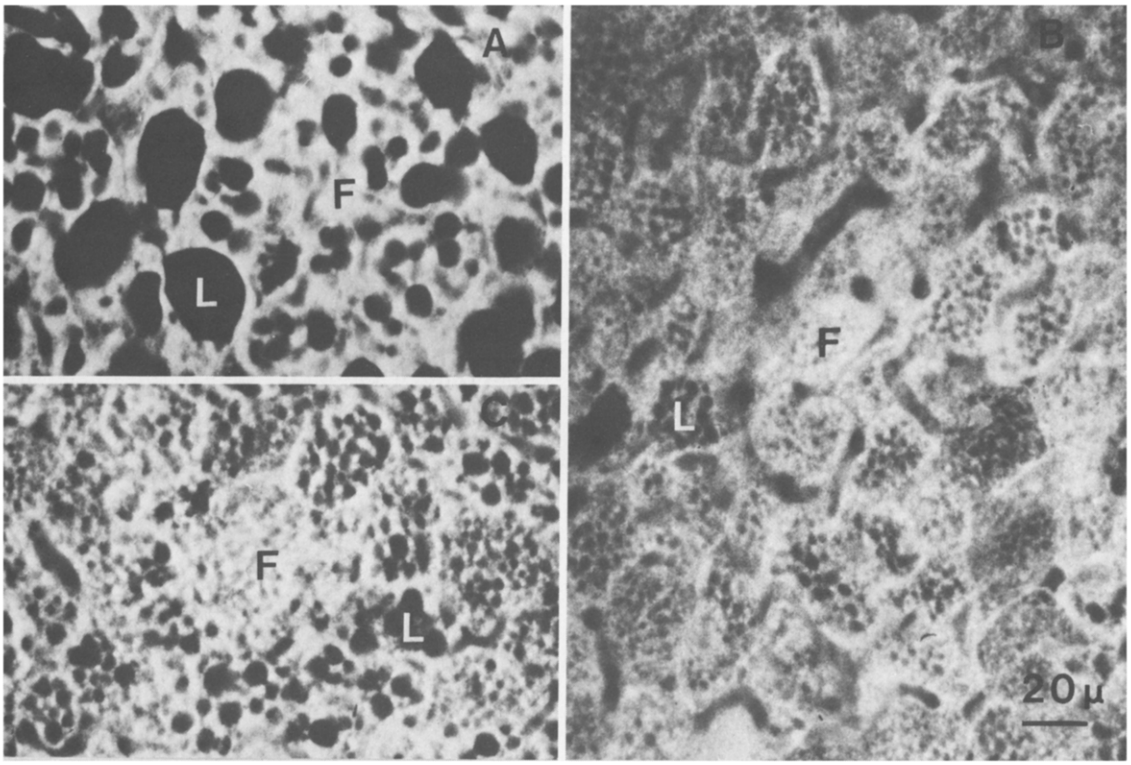

Fig. 2. Micrography of interscapular BAT treated with uncoupling-protein antibodies and then with fluorescent-conjugated double antibodies. Animals were the same as in table 1. Tissues were fixated, cut into 10-μm slices and incubated with uncoupling protein antibodies as in [14]. (A) Control, (B) noradrenaline-treated, (C) cold-exposed. Magnification, × 430; F, fluorescent area; L, lipid droplet. Note that both noradrenaline treatment and exposure to cold increased the multilocular aspect of the brown adipocytes and decreased the size of lipid droplets. The fluorescent area corresponding to the zone containing uncoupling protein was smaller in the control animals. Four tissues from each category were studied.

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