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Adaptative decrease in expression of the mRNA for uncoupling protein and subunit II of cytochrome *c* oxidase in rat brown adipose tissue during pregnancy and lactation

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Uncoupling-protein (UCP) mRNA expression is decreased to 15% of virgin control levels between days 10 and 15 of pregnancy, and remains at these low values during late pregnancy and lactation. Abrupt weaning of mid-lactating rats causes a slight but significant increase in UCP mRNA. Expression of mRNA for subunit II of cytochrome *c* oxidase (COII) decreased to half that of virgin control in late pregnancy and during lactation. Whereas COII mRNA expression is in step with the known modifications of brown-fat mitochondria content during the breeding cycle of the rat, UCP mRNA expression appears to be diminished much earlier than the mitochondrial proton-conductance-pathway activity. On the other hand, the reactivity of brown fat to increase expression of UCP and COII mRNAs in response to acute cold or noradrenaline treatment is not impaired during lactation.

INTRODUCTION

Brown adipose tissue is the main site for non-shivering adaptative thermogenesis in response to different physiological situations or environmental stimuli of rodents. The adaptative changes in brown-fat thermogenic activity are achieved through modifications in the overall mitochondria content of the tissue, and specifically through changes in the concentration of the mitochondrial uncoupling protein (UCP). This specific component of brown adipose tissue has the ability to short-circuit the proton electrochemical gradient generated across the mitochondrial membrane during substrate oxidation, thus causing energy dissipation as heat (Nicholls *et al.*, 1986).

UCP synthesis appears to be mainly regulated at the transcriptional level through noradrenergic-mediated mechanisms (Ricquier *et al.*, 1986). In fact, different physiological or experimental situations associated with increased brown-fat thermogenesis (cold exposure, birth, noradrenaline treatment, cafeteria diet) have been reported to cause a specific increase in the UCP mRNA levels (Falcou *et al.*, 1985; Jacobsson *et al.*, 1986; Ricquier *et al.*, 1986). However, the role of UCP mRNA expression in physiological adaptations to diminished brown-fat activity have not been so extensively studied.

Late pregnancy, and especially lactation, are probably the best-characterized physiological situations associated with an adaptative decrease in brown-fat thermogenesis (Trayhurn *et al.*, 1982; Villarroya *et al.*, 1986). In rodents, the minimal thermogenic activity of brown fat is achieved in mid-lactation, when the mitochondrial content of the tissue is lowered and there is a specific decrease in the proton-conductance-pathway activity (Trayhurn *et al.* 1982; Villarroya *et al.*, 1986) and UCP content (Trayhurn & Jennings, 1987). The aim of the present work was to determine whether changes in brown-fat thermogenic activity during the breeding cycle of the rat are associated with specific modifications in UCP mRNA expression. It was compared with the mRNA expression of the mitochondrial-genome-encoded subunit II of cytochrome *c* oxidase (COII) during this period, used as a control for overall mitochondriagenesis. The study was extended to compare the capacity of brown adipose tissue from virgin and lactating rats to increase UCP mRNA expression in response to thermogenic stimuli such as cold exposure and noradrenaline treatment.

MATERIALS AND METHODS

Animals

Female virgin Wistar rats weighing initially 180–210 g were used. They were maintained under standard conditions of illumination (12 h-light/dark cycle) and feeding (A-03 type diet; Panlab, Barcelona, Spain). Environment temperature was kept at 21 ± 1 °C unless otherwise indicated. Female rats were mated with adult males, and the day of pregnancy was determined by the presence of spermatozoa in vaginal smears. When lactating rats were studied, litter sizes were adjusted at birth to ten pups. Pregnant (days 10, 15, 18, 20 and 21), lactating (days 15, 30), abruptly weaned (24 h after removal of 15-day-lactating pups) and virgin control rats were studied in basal conditions. Virgin, pregnant and abruptly weaned rats were caged in pairs, and lactating rats were caged singly. The effects of acute cold exposure (5 h, 4 °C) or noradrenaline administration (5 h after 3.3 μmol/kg body wt.; Arterenol, Sigma; subcutaneous) on virgin and 15-day-lactating rats were also determined. Rats were killed by decapitation, and the interscapular brown

Abbreviations used: UCP, uncoupling protein; COII, subunit II of cytochrome *c* oxidase.

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adipose tissue was rapidly dissected and frozen in liquid N_2.

RNA isolation and blot hybridization

Total RNA was prepared from each individual frozen tissue by a modified phenol/chloroform extraction procedure (Lomedico & Saunders, 1976) and routinely checked for purity. For RNA-blot hybridization both 'Northern' and 'slot-blot' methods were employed. For the former, equal amounts (10 μg) of total RNA were denatured at 65 °C in the presence of formamide and formaldehyde, electrophoresed on formaldehyde/agarose gels and transferred to a nylon membrane (Hybond N; Amersham) by standard procedures (Maniatis *et al.*, 1982). Blots were hybridized to the pUCP 36 insert, containing full-length cDNA for the rat UCP (Bouillaud *et al.*, 1985) or to the 0.5 kb pIL-7 insert corresponding to part of the cDNA for COII (Glaichenhaus *et al.*, 1986). Probes were previously labelled by the random oligo-priming method and [α-^{32}P]dCTP (Amersham). Prehybridization and hybridization conditions were as reported by Bouillaud *et al.* (1985). After being washed in stringent conditions (30 mM-NaCl/3 mM-sodium citrate/0.1% SDS; 55 °C, 30 min), blots were subjected to autoradiography, and the resulting autoradiographs were measured by densitometry (Chromoscan). For 'slot-blot' analysis, different amounts of total RNA of each sample were bound to the nylon membrane with a standard apparatus and according to the instructions of the manufacturer (Schleicher & Schuell). Prehybridization and hybridization conditions were as mentioned above. After autoradiography, only densitometric data in the linear range of the signal response were considered for quantification. Statistical comparisons between groups were performed by Student's *t* test.

RESULTS AND DISCUSSION

As shown in the Figures, Northern-blot hybridization of rat brown-adipose-tissue RNA using the cDNA probe for UCP resulted in the detection of the 1.5 kb and 1.8 kb mRNAs known to be present in rodents (Bouillaud *et al.*, 1985). During the present study separate densitometric analysis of the two UCP mRNA species indicated that all the observed changes in UCP mRNA levels during the breeding cycle occurred in parallel for both mRNAs (results not shown). Therefore results on UCP mRNA abundance are presented as the integrative data of both mRNA signals. Blot hybridizations using the cDNA of COII, coded by the mitochondrial genome, were also performed in order to check the specificity of changes in UCP mRNA expression with respect to overall changes in mitochondriagenesis. As shown in Figures below, this last probe detected a single band of mRNA at 0.8 kb in Northern blots of rat brown-fat RNA, equal to that originally detected in analysis of RNA from rat fibroblasts (Glaichenhaus *et al.*, 1986).

In a first approach we studied UCP and COII mRNA levels in brown adipose tissue of rats at mid-lactation (day 15) as well as after sudden or spontaneous weaning, and they were compared with data from virgin controls and late-pregnant animals. Results are depicted in Fig. 1, and indicate that 15-day-lactating rats show a significant decrease in COII mRNA expression, together with a much more pronounced lowering of UCP mRNA levels, when compared with virgin controls. After 24 h of abrupt

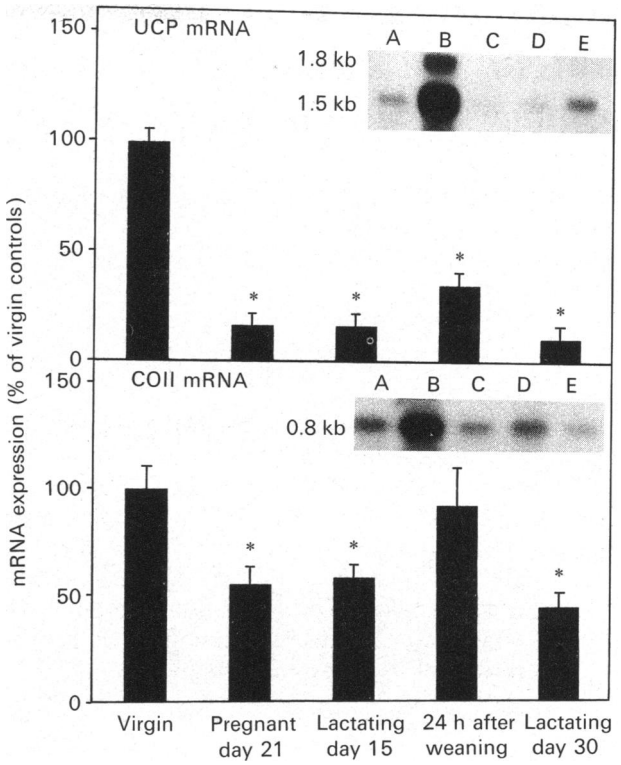

Fig. 1. Expression of mRNAs for UCP and COII during lactation

Results are means ± S.E.M. of densitometric scanning of 4–5 independent Northern-blot (UCP mRNA) or slot-blot (COII mRNA) analyses of RNA extracted from the interscapular brown adipose tissue of 4–5 different animals for each situation studied. Results are expressed as percentages of virgin control values. Inserts are examples of Northern blots (10 μg of RNA in each lane) probed with UCP cDNA or COII cDNA: A, 24 h after abrupt weaning of 15-day-lactating rat; B, virgin control; C, 21-day-pregnant rat; D, 15-day-lactating rat; E, 30-day-lactating rat. Statistical significance of comparisons with virgin control values is indicated by $*P < 0.05$.

weaning there was a slight but significant ($P < 0.05$) increase in UCP mRNA abundance, even though virgin control levels were not reached. COII mRNA expression was normalized after sudden weaning. Both COII and UCP mRNA levels were unchanged in 30-day-lactating rats as compared with 15-day-lactating ones.

These results are in agreement with previous reports on decreased GDP binding and mitochondrial content of rat brown fat during lactation (Isler *et al.*, 1984; Villarroya *et al.*, 1986) and on a lack of recovery of these parameters in suddenly weaned rats (Isler *et al.*, 1984) or in the first stages of spontaneous weaning of rats (F. Villarroya & T. Mampel, unpublished work) or mice (Trayhurn & Jennings, 1987). Thus it can be stated that the physiological adaptation of lactating rats to lowered brown-fat thermogenesis is associated with a decrease in mitochondrial gene expression and a specially marked inhibition of UCP mRNA expression.

Unexpectedly, results on 21-day-pregnant rats showed low UCP and COII mRNA levels similar to those found in mid-lactating animals. Therefore UCP and COII mRNA levels were studied at different days of gestation, in order to determine at which stage of pregnancy the

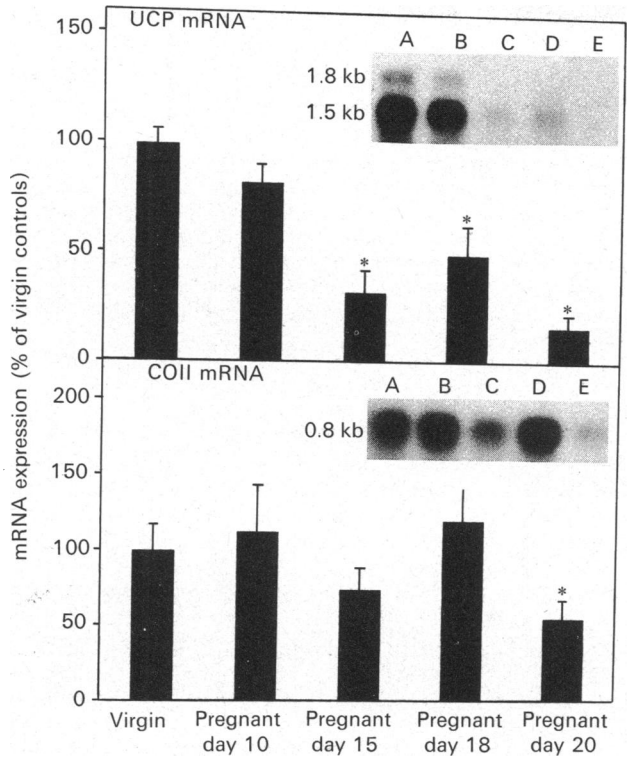

Fig. 2. Expression of mRNAs for UCP and COII during pregnancy

Results are means ± S.E.M. of densitometric scanning of 4–5 independent Northern-blot (UCP mRNA) or slot-blot (COII mRNA) analyses of RNA extracted from the interscapular brown adipose tissue of 4–5 different animals for each situation studied. Results are expressed as percentages of virgin control values. Inserts are examples of Northern blots (10mg of RNA each lane) probed with UCP cDNA or COII cDNA: A, virgin control rat; B, 10-day-pregnant rat; C, 15-day-pregnant rat; D, 18-day-pregnant rat; E, 20-day-pregnant rat. Statistical significance of comparisons with virgin control values is indicated by $*P < 0.05$.

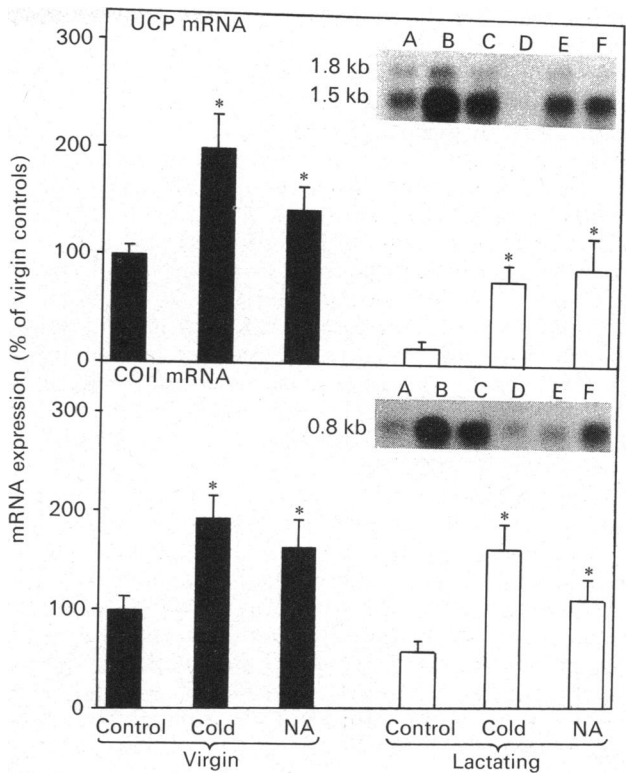

Fig. 3. Effects of cold exposure or acute noradrenaline treatment on expression of mRNA for UCP and COII in virgin and lactating rats

Results are means ± S.E.M. of densitometric scanning of 4–5 independent Northern-blot (UCP mRNA) or slot-blot (COII mRNA) analyses of RNA extracted from the interscapular brown adipose tissue of 4–5 different animals for each situation studied. 'Cold' means 5 h of exposure at 4 °C, and NA means 5 h after 3.3 mg of noradrenaline bitartrate/kg body wt., subcutaneous. Results are expressed as percentages of virgin control values. Inserts are examples of Northern blots probed with UCP cDNA or COII cDNA: A and D, virgin and lactating control respectively; B and E, cold-exposed virgin rat and lactating rat respectively; C and F, noradrenaline-treated virgin and lactating rat respectively. Results from cold-exposed or noradrenaline-treated virgin and lactating rats were compared with their respective controls, and statistical significance of differences is indicated by $*P < 0.05$.

specific lowering of UCP mRNA expression first starts. Results presented in Fig. 2 indicate that COII mRNA expression in brown fat begins to be lowered in late pregnancy, in good parallelism with biochemical data on a progressive decrease in the mitochondrial content of the tissue during this period (Villarroya *et al.*, 1986). However, UCP mRNA expression was suddenly decreased between days 10 and 15 of pregnancy, whereas GDP binding is known not to be modified before parturition in the rat (Villarroya *et al.*, 1986). This early decrease in UCP mRNA expression raises the question of how the decrease in UCP mRNA abundance takes so much time to cause detectable changes in the thermogenic activity of brown-fat mitochondria. It is evident that GDP binding is not a direct measure of UCP concentration, but chronic adaptative modifications of brown-fat thermogenesis, such as the present situations, are known to result in synchronous changes in GDP binding and UCP levels. Therefore a substantial lag period seems to be necessary to achieve mitochondrial UCP concentrations that reflect the actual UCP mRNA abundance in an adaptation to lowered thermogenesis, such as the breeding cycle. This phenomenon would be similar to what has been reported in cold-deacclimation, another situation of adaptative lowering of brown-fat thermogenesis. A substantial decrease in UCP mRNA occurs in the first 24 h of cold-deacclimation (Reichling *et al.*, 1987), whereas mitochondrial UCP concentration has been described not be significantly decreased until 8 days later (Trayhurn *et al.*, 1987). Further research on the turnover of UCP and probably of the overall brown-fat mitochondria in adaptative situations of lowered thermogenesis would be necessary to explain these findings.

UCP mRNA expression is known to be mainly modulated by the action of the noradrenaline coming from the sympathetic innervation of the tissue (Ricquier *et al.*, 1986). Concerning the possible regulatory factors

responsible for the decrease in UCP mRNA during pregnancy, information on sympathetic activity upon brown fat during gestation in the rat is not at present available. However, parameters different from UCP gene expression but sharing a main noradrenergic modulation in brown fat, such as lipoprotein lipase or iodothyronine 5′-deiodinase activities, are already significantly lowered in mid pregnancy (Villarroya & Mampel, 1986; Viñas *et al.*, 1988). The decrease in this last enzyme activity must be specially considered if, as previously proposed (Bianco & Silva, 1987), the locally produced tri-iodothyronine has a role in the modulation of UCP mRNA expression. During lactation, when a decrease in noradrenaline turnover in brown adipose tissue is well established (Villarroya *et al.*, 1987; Trayhurn & Wusterman, 1987), a lowered sympathetic stimulation correlates with the lowered UCP mRNA and COII mRNA expression.

We extended the present study to determine whether, together with the known decrease in the sympathetic tone in brown fat during lactation, there is an adaptative impairment in the tissue sensitivity to increased UCP mRNA expression in response to thermogenic stimuli. Results comparing the effects of acute cold or acute noradrenaline treatment in COII and UCP mRNA levels in virgin and lactating rats are depicted in Fig. 3. Acute cold exposure and noradrenaline treatment resulted in a marked increase in UCP mRNA levels in virgin rats, in agreement with previous reports (Jacobsson *et al.*, 1986; Ricquier *et al.*, 1986). Interestingly there was also a significant increase in COII mRNA levels in response to the stimuli. It is well known that a cold environment or noradrenaline treatment causes a progressive increase in brown-fat mitochondriagenesis (Mory *et al.*, 1984), but to our knowledge this is the first report of a significant increase in a mitochondrial-genome-coded protein mRNA in response to a short-time thermogenic stimulus. However, these data are in agreement with previous suggestions indicating that UCP synthesis occurs in synchrony with the synthesis of other mitochondrial proteins in response to the thermogenic stimulus (Trayhurn *et al.*, 1987). Lactating rats showed a marked increase in both UCP and COII mRNA levels in response to cold or noradrenaline, despite the low basal values. Therefore, the physiological decrease in expression of brown-fat UCP and COII mRNA in lactation would be attributable to an adaptative lowering of the sympathetic stimulation of the tissue, whereas the sensitivity of the brown fat to increase expression of UCP and COII mRNA is essentially unaltered. This is consistent with noradrenaline turnover studies, which have indicated that sympathetic responsiveness is unaltered in cold-exposed lactating mice (Trayhurn & Wusterman, 1987). A similar pattern of response has been reported in different models of genetic or surgical obesity associated with pathologically decreased UCP mRNA expression (Ricquier *et al.*, 1986), thus suggesting a uniqueness in the mechanisms responsible for adaptative lowering of brown-fat thermogenesis.

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