Tissue-dependent upregulation of rat uncoupling protein-2 expression in response to fasting or cold

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Abstract The control of uncoupling protein-2 (UCP2) mRNA expression in rat brown adipose tissue (BAT), heart and skeletal muscles was examined. Cold exposure (48 h) increased UCP2 mRNA in BAT, heart and soleus muscle by 2.4-, 4.3- and 2.6-fold, respectively. Fasting (48 h) had no effect on UCP2 mRNA expression neither in BAT nor in heart, but markedly increased it in skeletal muscles. While the upregulation of UCP2 mRNA in response to cold exposure is in line with a putative uncoupling role for this protein in thermoregulatory thermogenesis, the unexpected upregulation of UCP2 in skeletal muscles in response to fasting seems inconsistent with its role as an uncoupling protein involved in dietary regulation of thermogenesis.

Key words: Uncoupling protein; Skeletal muscle; Heart; Brown adipose tissue; Fasting; Cold

1. Introduction

The uncoupling protein-1 (UCP1) gene encodes a unique mammalian mitochondrial protein carrier that stimulates heat production by uncoupling respiration from ATP synthesis in brown adipose tissue (BAT), an important site of non-shivering thermogenesis in rodents [1]. Recently, a new protein sharing a 55% amino acid identity with UCP1 was identified in human tissues and this UCP homologue was named UCP2. Whereas UCP1 is present only in BAT, UCP2 is ubiquitously expressed in tissues of both humans and rodents [2,3]. It has been reported that, in UCP2 transfected yeast, this protein can partially uncouple mitochondrial respiration [2]. Thus BAT thermogenesis could be mediated not only by UCP1 but also by UCP2 and, furthermore, thermogenesis in other organs, in particular in skeletal muscle, could be mediated by UCP2.

The aim of the present study was to investigate whether UCP2, in organs/tissues which highly express this protein (BAT, heart and skeletal muscles), is modulated by cold exposure or fasting – two well known conditions that increase or decrease, respectively, whole-body thermogenesis and UCP1 expression in BAT [1,4].

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Abbreviations: UCP, uncoupling protein; BAT, brown adipose tissue

2. Materials and methods

2.1. Rat treatments

Seven-week-old Sprague-Dawley male rats fed ad libitum standard laboratory chow with a 12-h light-dark cycle and either kept at 22°C or exposed individually to cold (6°C) for 48 h were used. Fasted rats were deprived of food with free access to water for 48 h. The rats were killed by decapitation, and soleus, tibialis anterior and gastrocnemius muscles, heart and interscapular BAT, carefully trimmed from white adipose tissue, connective tissue and muscle, were excised, immediately frozen in liquid nitrogen and stored at −80°C.

2.2. Northern blot analysis

Total RNA was purified by the method of Chomczynski and Sacci [5] and 12–20 μg was electrophoresed in a 1.2% agarose gel containing formaldehyde, as described by Lehrach et al. [6] and transferred to Electran Nylon Blotting membranes (BDH Laboratory Supplies, Poole, UK) by vacuum blotting. The probes used were a rat UCP1 cDNA probe of 766 bp obtained by RT-PCR on rat BAT total RNA using oligonucleotide primers corresponding to nucleotides 279–298 (UCPRF) and 1021–1044 (UCPRR) on rat UCP1 cDNA (GenBank Accession M11814), and a human UCP2 cDNA probe of 772 bp obtained by PCR from a cloned human UCP2 cDNA [3] using oligonucleotide primers corresponding to nucleotides 188–207 (UCP2HF) and 936–959 (UCP2HR) on human UCP2 cDNA (GenBank Accession U82819). The UCP2 signal had a size of 1.7 kb as already described [3]. The probe was labeled by random priming with [α-^{32}P]dCTP (Amersham, Bucks, UK) to a specific radioactivity of approximately 1×10^9 dpm/μg DNA. Northern blots were hybridized for 2 h at 65°C in QuikHyb solution (Stratagene, La Jolla, CA), then washed in a solution of 2×SSC (1×SSC is 150 mM NaCl, 15 mM sodium citrate, pH 7.0)/0.1% sodium dodecyl sulfate at 50°C twice for 5 min and in 0.1×SSC/0.1% sodium dodecyl sulfate at 50°C for 5 min. Blots were exposed to Hyperfilm ECL films (Amersham, Bucks, UK) at −80°C with intensifying screens. Size estimates for the RNA species were established by comparison with an RNA Ladder (Gibco BRL, New York, NY). The signals on the autoradiograms were quantified by scanning photodensitometry using ImageQuant Software Version 3.3 (Molecular Dynamics, Sunnyvale, CA). Hybridization of the blots with a [γ-^{32}P]ATP-labeled synthetic oligonucleotide specific for the 18S rRNA subunit was used to correct for differences in the amounts of RNA loaded onto the gel. Student's unpaired *t*-test was used to determine statistical significance.

3. Results and discussion

3.1. Effect of cold exposure

In rats exposed for 48 h at 6°C, the well known effects on UCP1 in BAT were compared to those of UCP2.

As illustrated in Fig. 1, the level of UCP1 mRNA in BAT was increased (3.0-fold), a result in agreement with numerous previous reports [1]. Fig. 1 also shows that the level of UCP2 mRNA in BAT was increased by 2.4-fold, indicating that, in this tissue, UCP2 may be modulated by cold exposure in the same way as UCP1. This stimulatory effect of cold exposure on BAT UCP2 in the rat was not found by Fleury et al. [2] in

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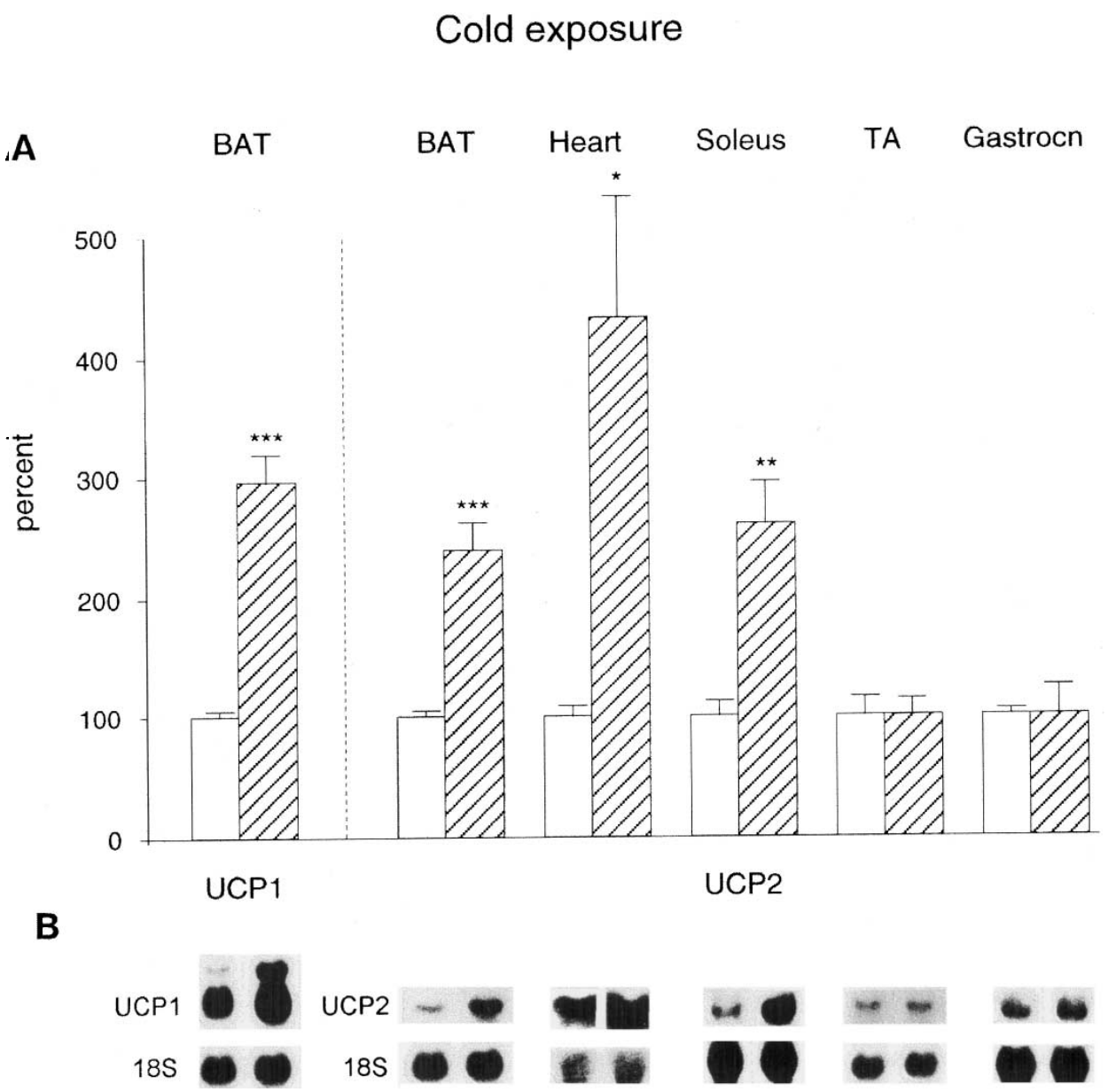

Fig. 1. Cold exposure increases UCP2 expression in BAT, heart and soleus. (A) Expression levels of UCP1 or UCP2 mRNA in brown adipose tissue (BAT), heart, soleus, tibialis anterior (TA) and gastrocnemius (gastrocn) muscles of control (empty columns) or 48 h cold exposed (hatched columns) rats. Photodensitometric comparison of signals obtained from total RNA hybridized with ^{32}P-labeled rat UCP1 and human UCP2 cDNA probes as described in Section 2. UCP1 signal had a size of 1.4 kb and UCP2 signal a size of 1.7 kb. The results are expressed as percentage ± S.E. of the mean respective control value taken as 100%. The number of experiments was 3–5. The signals were quantified by scanning photodensitometry and normalized using the corresponding 18S rRNA values. $^{*}p < 0.02$; $^{**}p < 0.01$; $^{***}p < 0.001$ vs. control. (B) Representative UCP1 or UCP2 mRNA and 18S ribosomal RNA signals in the various tissues studied.

the mouse. This discrepancy might be due to differences in species and/or in the duration of cold exposure. Under the conditions of our study in the rat, the levels of UCP2 mRNA in heart and soleus muscle were also increased 4.3- and 2.6-fold, respectively, by cold exposure, whereas in tibialis anterior and gastrocnemius muscles, they were not affected (Fig. 1).

The increase in UCP2 expression in BAT, heart and soleus muscle under conditions of stimulated thermogenesis is consistent with a role of UCP2 as an uncoupler of oxidative phosphorylation. Using tracer kinetic studies of noradrenaline turnover rates, a reliable index of sympathetic nervous system activity, it has been shown that the stimulation of thermogenesis in response to cold exposure is accompanied by a marked (several fold) increase in sympathetic activity in BAT as well as in heart [7,8], and to a lesser extent in soleus muscle (+48%), whereas increases in sympathetic activity in tibialis anterior (+22%) and gastrocnemius (+24%) were small and of borderline statistical significance [9]. The parallelism observed between the sympathetic nervous system activity and UCP2 expression in response to cold would therefore be consistent with the notion that the uncoupling activity of UCP2 (as for UCP1) is likely to be under the control of the sympathetic nervous system.

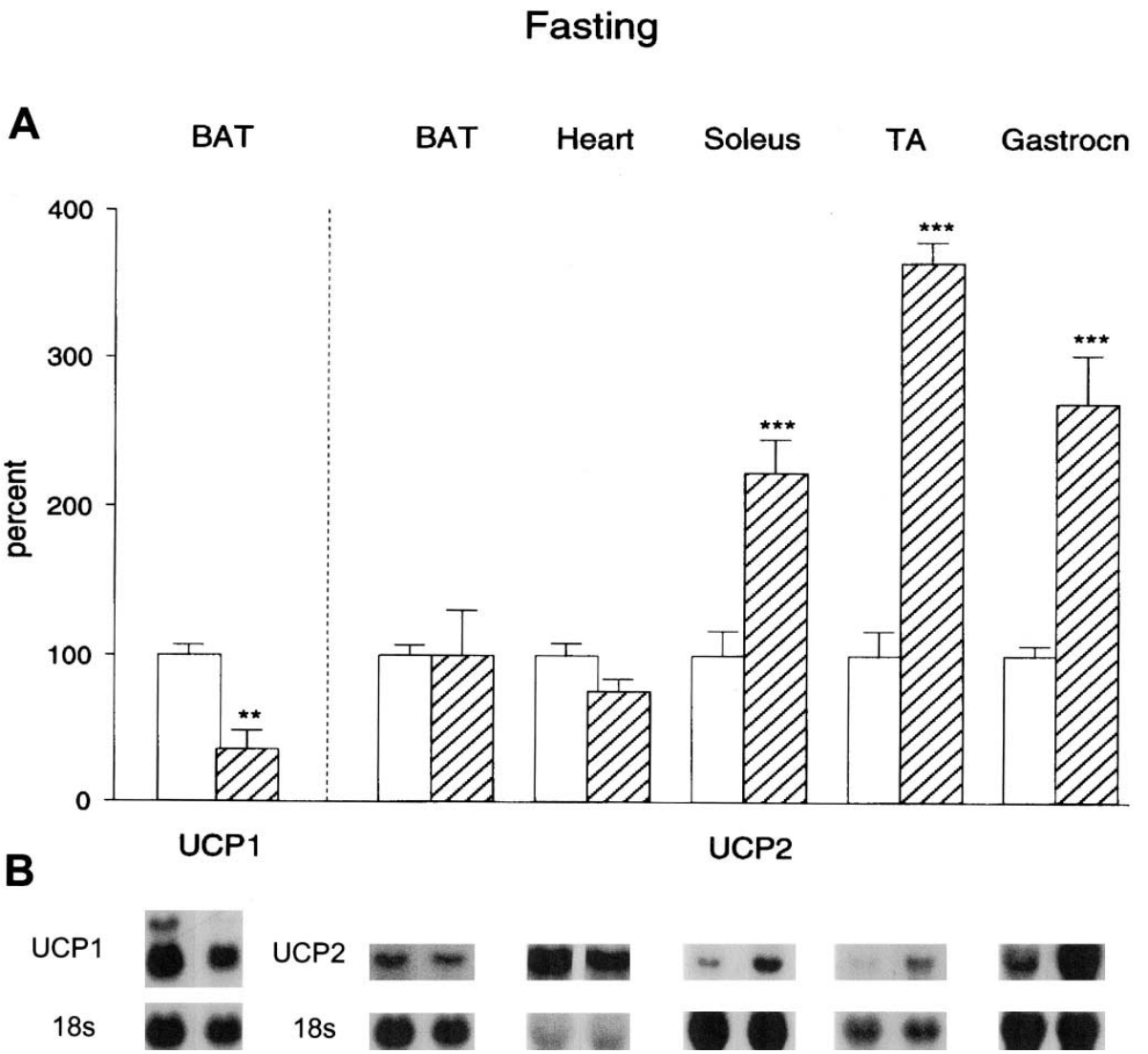

Fig. 2. Fasting increases UCP2 expression in skeletal muscles. (A) Expression levels of UCP1 or UCP2 mRNA in brown adipose tissue (BAT), heart, soleus, tibialis anterior (TA) and gastrocnemius (gastrocn) muscles of control (empty columns) or 48 h fasted (hatched columns) rats. For methodology, see Fig. 1. The number of experiments was 3–5. $^{**}p < 0.01$; $^{***}p < 0.005$. (B) Representative UCP1 or UCP2 mRNA and 18S ribosomal RNA signals in the various tissues studied.

Support for this hypothesis of a sympathetic modulation of UCP2 can be derived from preliminary studies indicating that administration to rats of the β_3-adrenoceptor agonist Ro-168714 for 32 h increased UCP2 mRNA by 2.1-fold in BAT (results not shown). Therefore, similarly to BAT UCP1 [10], BAT UCP2 is modulated by β_3-adrenoceptor stimulation in vivo.

3.2. Effect of fasting

Fasting is known to decrease UCP1 expression and hence the thermogenic activity of BAT [4]. As expected, the level of UCP1 mRNA in BAT was decreased (64% inhibition) by 48 h fasting (Fig. 2). However, as also shown in Fig. 2, the level of UCP2 mRNA in BAT was unchanged by fasting, thereby indicating differential modulations of UCP1 and UCP2 in response to fasting in this tissue. Fig. 2 also shows that in response to fasting the level of UCP2 mRNA was not modified in the heart whereas it was markedly increased in soleus, tibialis anterior and gastrocnemius muscles by 2.2-, 3.6- and 2.7-fold, respectively.

The marked stimulatory effect of fasting on UCP2 expression in skeletal muscles is unexpected, and contrasts with the demonstration, by blood flow studies coupled with regional arteriovenous O_2 differences [11], of an important contribution by the skeletal muscles to starvation-induced energy conservation.

Taken together, while our results indicating an upregulation of UCP2 mRNA in certain tissues (BAT, heart and soleus) in response to cold exposure is in line with a putative uncoupling role for this protein in thermoregulatory thermogenesis, the unexpected upregulation of UCP2 in skeletal muscles and no change in BAT and heart in response to dietary deprivation (fasting) seems inconsistent with its role as an uncoupling protein involved in dietary regulation of thermogenesis.

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