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In the rat, tumor necrosis factor α administration results in an increase in both UCP2 and UCP3 mRNAs in skeletal muscle: a possible mechanism for cytokine-induced thermogenesis?

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Abstract Since the discovery of the new members of the UCP (uncoupling protein) family, UCP2 and UCP3, very few studies have dealt with the regulation of their expression. Bearing this in mind, administration of a single intravenous injection of TNF-α (100 μg/kg body weight) to rats resulted in a significant increase in UCP2 (242%) and UCP3 (113%) gene expression in skeletal muscle. The results suggest a possible role for UCP2 and UCP3 in the increase of energy expenditure associated with cytokine treatment.

Key words: Thermogenesis; Tumor necrosis factor α; Skeletal muscle; Uncoupling protein 2; Uncoupling protein 3

1. Introduction

Until very recently, the UCP1 protein – present only in brown adipose tissue – was considered to be the only mitochondrial protein carrier that stimulated heat production by dissipating the proton gradient generated during respiration across the inner mitochondrial membrane and therefore uncoupling respiration from ATP synthesis. Recently, two new proteins sharing the same function, UCP2 and UCP3, have been described. While UCP2 is expressed ubiquitously [1,2], UCP3 is expressed abundantly and specifically in skeletal muscle in humans [3–6] and also in brown adipose tissue of rodents [7,3,4]. Concerning the regulation of their expression, UCP2 mRNA levels are upregulated in white adipose tissue and skeletal muscle in obesity-resistant mice but not in an obesity-prone strain [8], thereby suggesting a possible role as a defence mechanism against high-fat diet-induced obesity. Fasting increases UCP2 mRNA in skeletal muscle [9] while it does not seem to affect the expression of the gene in brown adipose tissue. In skeletal muscle UCP3 gene expression is also modulated by food intake: its expression is increased by fasting [10].

It has been known for a long time that cytokines, tumor necrosis factor α (TNF-α) in particular, are involved in wasting during pathological states such as cancer or infection due to their metabolic effects which result in an increased energy expenditure in the host [11]. However, the molecular mechanisms involved in the increased energetic inefficiency and heat production are still under discussion. In skeletal muscle, the cytokine has been shown to have important effects in promoting increased protein turnover and thus contributing to energetic inefficiency [12]. Concerning heat production, it was demonstrated that, in the young rat, a single intravenous TNF-α injection increased brown adipose tissue thermogenesis, measured as mitochondrial GDP binding [13]; a similar effect was observed when the cytokine was intracerebroventricularly injected [14]. Very recently, Faggioni et al. [15] have reported that UCP2 gene expression is induced by lipopolysaccharides (LPS) in several cell types, and therefore have suggested that it could represent a mechanism for the increased thermogenesis during infection.

Bearing all this in mind, and taking into account that skeletal muscle accounts for over 40% of total body weight in rodents, the aim of the present investigation was to see if an acute TNF-α administration resulted in the induction of the UCP2 or UCP3 genes in this tissue.

2. Materials and methods

2.1. Animals

Male Wistar rats (Interfauna, Barcelona, Spain) weighing about 200 g were used. The animals were maintained on a regular light-dark cycle (light on from 08.00 to 20.00 h) at an ambient temperature of 22 ± 1°C and had free access to food and water. The diet they were allowed to eat consisted of 54% carbohydrate, 17% protein and 5% fat (the residue was non-digestible material; Panlab, Barcelona, Spain). The animals were anesthetized with sodium pentobarbital and the gastrocnemius muscles were rapidly excised, weighed and frozen under liquid nitrogen.

2.2. Chemicals

All chemicals were either obtained from Boehringer Mannheim (Barcelona, Spain) or from Sigma (St. Louis, MO, USA). Human recombinant TNF-α was kindly given by BASF/Knoll AG (Ludwigshafen, Germany) and Dainippon Pharmaceuticals Co. (Osaka, Japan).

2.3. Cytokine administration

Animals were injected with an intravenous (tail vein) single dose of TNF-α (100 μg/kg body weight in 0.5 ml of 0.9% NaCl). Six hours after the administration of the cytokine they were killed and muscle samples were extracted.

2.4. RNA isolation and Northern blot analysis

Total RNA from gastrocnemius muscle was extracted using the acid guanidinium isothiocyanate/phenol/chloroform method as described [16]. RNA samples (20 μg) were denatured, subjected to 1.2% agarose gel electrophoresis containing 6.3% formaldehyde and transferred to Hybond N membrane (Amersham). The RNA in gels and filters was visualized with ethidium bromide and photographed by UV transillumination to ensure the integrity of RNA, to check the loading of equivalent amounts of RNA and to confirm proper transfer. RNA was transferred in 20× standard saline citrate (SSC; 0.15 M NaCl and 15 mM sodium citrate, pH 7.0). RNA was fixed to membrane by Gene Linker (Bio-Rad).

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Prehybridization was done in a phosphate buffer (250 mM, pH 6.8) containing 7% SDS, 1 mM EDTA and 1% BSA, during 1 h at 65°C. Membranes were hybridized in the same buffer with appropriate probes (approx. 28 Bq/ng) at 65°C for 18 h. Non-specifically bound probe was removed by successive washes in 2×SSC+0.1% SDS (10 min at 65°C) and 1×SSC+0.1% SDS (10 min at 65°C) and 0.1×SSC+0.1% SDS (15 min at 65°C, twice). Specific hybridization was then detected by autoradiography in Hyperfilm-MP film (Amersham) using intensifier screens for 1–4 days at −80°C.

Radiolabeled probes were prepared by the random priming method (Boehringer Mannheim). The probes used were the entire coding frame for mouse UCP2 [1], a cDNA clone containing the entire coding from for mouse UCP3 (D.S. Fleury, C.F. Bouillard and D. Ricquier, GenBank accession number AF032902), and a 18S rat ribosomal probe used as hybridization/quantification standard. Blots were quantified on a phosphoimager using the Phoretix 1D gel analysis (Phoretix International, UK).

2.5. Statistical analysis

Statistical analysis of the data was performed by means of Student's *t*-test.

3. Results and discussion

Heat production in brown adipose tissue – an important site of non-shivering thermogenesis in rodents [17] – has long been associated with UCP1, a mitochondrial protein carrier that stimulates heat production by uncoupling respiration from ATP synthesis. The recent discovery of new members of the mammalian mitochondrial uncoupling proteins (UCP2 and UCP3) has resulted in a considerable amount of information to elucidate the role of these proteins in pathophysiological conditions.

The role of cytokines, TNF-α in particular, in thermoregulation and in regulating energetic efficiency has been recognized for a long time. Thus, it was demonstrated that, in the young rat, a single intravenous TNF-α injection increased brown adipose tissue thermogenesis, measured as mitochondrial GDP binding [13]; a similar effect was observed when the cytokine was intracerebroventricularly injected [14]. It was therefore the main aim of the present investigation to see if TNF-α administration to rats resulted in any changes in UCP2 or UCP3 gene expression.

Starvation results in an important increase in UCP2 gene expression in skeletal muscle while it does not influence brown adipose tissue [9]. Cold exposure also results in an increase in UCP2 gene expression in skeletal muscle and brown adipose tissue [9]. Interestingly, UCP2 levels are upregulated in white adipose tissue in response to fat feeding, suggesting regulation by diet [1]. The results obtained in our study show that TNF-α treatment significantly increased UCP2 gene expression (242%) in skeletal muscle (see Fig. 1). This is consistent with previous observations in mice [15] showing an increase in

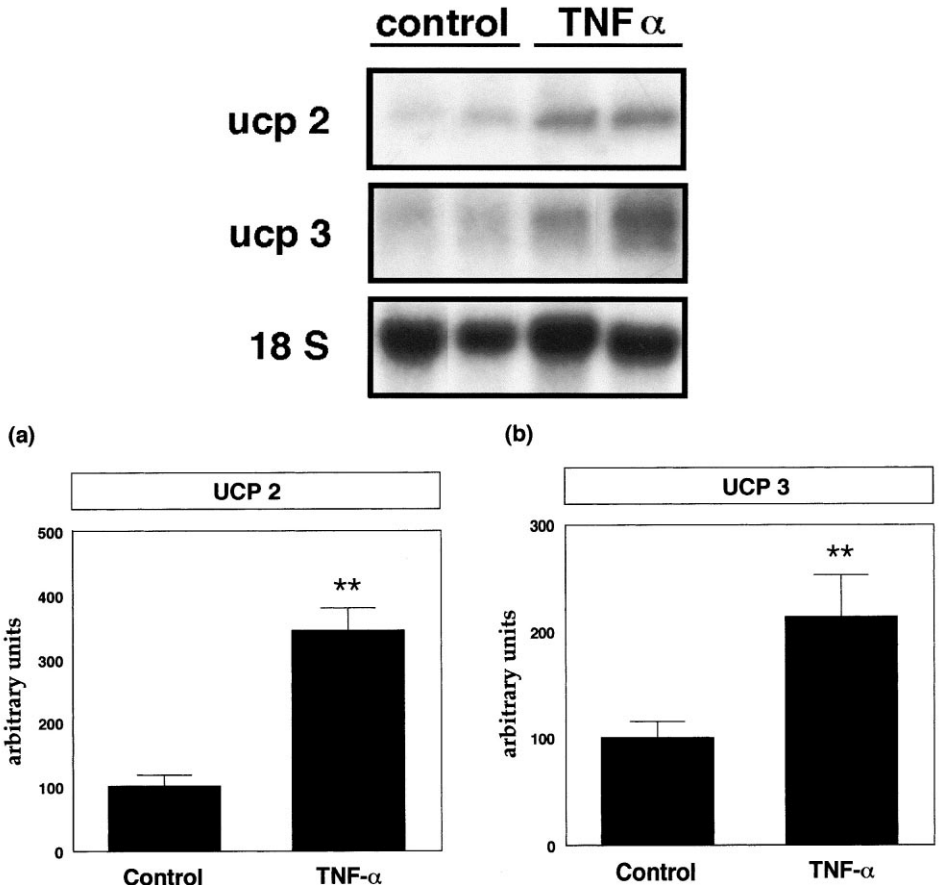

Fig. 1. Upper panel: Northern blots of skeletal gastrocnemius muscle from control and TNF-treated rats. Expression of both UCP2 and UCP3 mRNA was detected after hybridization with cDNA probes (see Section 2). Autoradiographs were quantified by scanning densitometry and the results are expressed as arbitrary units. Lower panel: Quantification of UCP2 (a) and UCP3 (b) mRNA in gastrocnemius from control and TNF-treated rats. (a) $n=3$ in each group. Significance of the differences: $^{**}P<0.01$. (b) $n=7$ control group; $n=5$ TNF-treated group. Significance: $^{**}P<0.01$.

UCP2 mRNA in liver, muscle and adipose tissue following either LPS or cytokine injection. It can therefore be suggested that the cytokine effects on energy expenditure could be partially mediated by activation of UCP2 in skeletal muscle. It is interesting to point out here that the induction of insulin resistance by a high-fat diet is associated with increased gene expression of TNF-α in white adipose tissue [18], thereby suggesting that TNF overexpression in white adipose tissue could be the trigger for the increased UCP2 expression induced by a high-fat diet.

Interestingly, TNF treatment also increased UCP3 gene expression in skeletal muscle (113%), compared with the non-treated animals (Fig. 1). This gene is abundantly expressed in skeletal muscle and is highly tissue-specific [6], its presence having been detected also in brown adipose tissue of rodents [3,4,7]. However, the regulation of its expression is still poorly known. In muscle it is not affected by cold acclimation [10] although it is increased following thyroid hormone treatment [19]; starvation increases UCP3 mRNA levels in skeletal muscle while it decreases them in brown adipose tissue [3]. UCP3 expression is also decreased in the soleus muscle of obese Zucker rats [10] suggesting that it could possibly have a role in obesity. The fact that TNF-α treatment increases its expression suggests that UCP3 may have a role in those pathophysiological states where the cytokine is overproduced, leading to a significant degree of energetic inefficiency and wasting such as infection or tumor growth (see [11] for review).

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