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Uncoupling protein is expressed in liver mitochondria of cold-exposed and newborn rats

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Uncoupling protein has been thought to be expressed only in the brown adipose tissue mitochondria of mammals. However, mRNA encoding mitochondrial uncoupling protein was detected in the liver of newborn rats and adult rats after cold exposure, although not in the liver of untreated adult rats.

Northern blotting; Uncoupling protein; Rat liver mitochondria; Brown adipose tissue

1. INTRODUCTION

Energy transducing membranes such as the mitochondrial inner membrane are impermeable to H^+, and the H^+ electrochemical gradient across these membranes generated by the electron transport is a driving force for the synthesis of ATP [1]. Therefore, a protonophore that dissipates the H^+ electrochemical gradient by causing it to become leaky to H^+ acts as an uncoupler, thus inhibiting ATP synthesis [2,3]. It has long been known that there is a protein that is responsible for heat production without energy conservation, acting as a H^+ conductor in the mitochondrial inner membrane. This protein is an intrinsic uncoupler, and hence it is called the uncoupling protein (for reviews, see [4–6]). As this uncoupling protein is thought to be mainly important for heat production in mammals and its action is regulated by GDP, it is also referred to as thermogenin or GDP-binding protein. This uncoupling protein is reported to be specifically expressed in brown adipose tissue in mammals and its expression is enhanced according to physiological demands under specified conditions such as in arousal from hibernation of rodents [6], in the postnatal period of mammals [7,8], and during cold-exposure [9,10]. As the uncoupling protein is an intrinsic regulator of energy transduction, it is important to know why it is expressed only in brown adipose tissue and why its expression is enhanced under specific conditions.

We were interested in whether the uncoupling protein can be expressed in tissues other than brown adipose tissue. In this study, we found that mRNA encoding the uncoupling protein is expressed in newborn rat liver and the liver of adult rats after cold exposure.

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2. MATERIALS AND METHODS

[α-^{32}P]dCTP (111 TBq/mmol) and nitrocellulose membranes (BA85) were purchased from New England Nuclear and from Schleicher and Schuell, respectively. Other materials and reagents were of the highest grade commercially available.

Adult male Wistar rats were kept at 4°C for 1 week before use as cold-exposed rats. One day postnatal rats of either sex were used as as newborn rats. Male Wistar rats without cold exposure were used as controls. The liver and interscapular brown adipose tissue of these rats were carefully separated from other tissues and their RNAs were promptly isolated.

Total RNA was extracted from each tissue with guanidine thiocyanate and centrifuged in a solution of cesium chloride according to the standard method [11]. The concentration of RNA was determined spectrophotometrically in a Shimadzu UV-160 apparatus. After electrophoresis on agarose gels containing formaldehyde, the RNA was transferred to nitrocellulose membranes. The cDNA of uncoupling protein (a gift from Dr. Ricquier; CNRS, Meudon, France), was radiolabelled by the multi-priming method [11], and used as a probe. The specific radioactivity of the probe was more than 10^8 cpm/μg. Pre-hybridization, hybridization and washing of the RNA on the membrane were carried out by the standard method [11] and the membrane was exposed to X-ray film with an intensifying screen at −80°C for 6 h.

3. RESULTS AND DISCUSSION

Samples of about 5.0 μg of total RNA from liver and brown adipose tissue were subjected to electrophoresis and transferred onto a nitrocellulose membrane. The membrane was then hybridized with ^{32}P-labeled cDNA of rat uncoupling protein. The quantity of RNA subjected to electrophoresis was reconfirmed by staining the RNA with Methylene blue after autoradiography (data not shown). As shown in lanes 1 and 2 in Fig. 1, a single RNA band hybridizing with the probe was observed

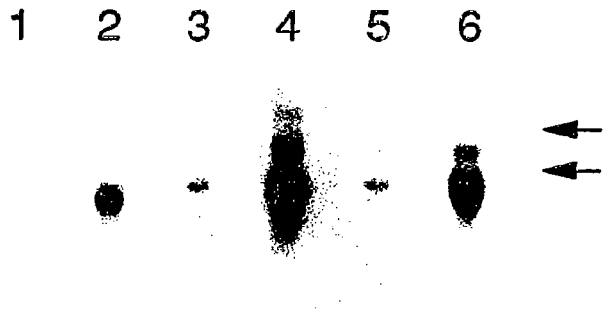

Fig. 1. Northern hybridization of total RNAs of brown adipose tissue and liver of rats under various conditions. After electrophoresis in the agarose gel, total RNAs were transferred to a nitrocellulose membrane. They were then hybridized with ^{32}P-labeled cDNA of rat uncoupling protein, and the membrane was exposed to X-ray film. The positions of 28S and 18S ribosomal RNAs are shown by arrows. Lanes 1 and 2, liver and brown adipose tissue, respectively, from a control adult rat (without cold exposure); lanes 3 and 4, liver and brown adipose tissue, respectively, from a newborn rat; lanes 5 and 6, liver and brown adipose tissue, respectively, from an adult rat after cold exposure.

only in the RNA of the brown adipose tissue of adult rats without cold exposure (control rats). This result confirms that uncoupling protein is expressed specifically in the brown adipose tissue under normal physiological conditions. In contrast, the RNAs of brown adipose tissues of both newborn rats (lane 4) and cold-exposed rats (lane 6), gave at least two major bands that hybridized with the probe, and the intensities of these bands were much greater than that of the band of control brown adipose tissue. These results are in line with reported observations by Northern blotting [10] of at least two dense hybridized bands in brown adipose tissue of newborn and cold-exposed rats.

Interestingly, RNA bands that hybridized with the probe cDNA were also clearly observed in the liver of newborn and cold-exposed rats (lanes 3 and 5, respectively), although their intensities were lower than those of the band of RNA from brown adipose tissues. The possibility of cross-hybridization of the probe with other mRNAs is excluded by the fact that the expression of mRNA of the uncoupling protein was completely restricted to the brown adipose tissue in control rats.

It is well established that expression of the uncoupling protein is normally strictly restricted to the brown adipose tissue [6]; even when poly(A)$^+$ RNA from normal rat liver was analyzed with cDNA of the uncoupling protein, no hybridized band was detected [10]. In studies on the expression of uncoupling protein, the RNA obtained from the liver of 'untreated' adult rats was always used as a negative control, as in the present study. But its expression in the liver under various conditions has not been examined. Recently, Valcarce et al. [12] reported that fetal liver mitochondria showed anomalous ohmic behavior in H^+ conductance, and suggested that this might be due to the existence of 'leaky mitochondria' (mitochondria leaky to H^+) in rat liver during development, as first postulated by Pollak and Sutton [13]. As adenine nucleotide translocator is structurally very homologous with the uncoupling protein [14] and is expressed constitutively, Valcarce et al. [12] concluded that adenine nucleotide translocator in the inner mitochondrial membrane is responsible for the 'leaky mitochondria', but they failed to consider the possible presence of the uncoupling protein in liver mitochondria.

From the present result, it is possible that the strictly restricted expression of uncoupling protein under normal conditions may change in such a way that the protein is expressed in tissues other than brown adipose tissue in response to physiological demand to produce more heat, such as during arousal from hibernation, postnatal development and cold exposure. Further study on the regulation of the expression of the uncoupling protein under various conditions is underway.

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