Immunodetection of UCP1 in rat thymocytes

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Abstract

Thymi were dissected from rats and connective tissue was removed. Mitochondria were purified from isolated thymocytes and immunoblot analysis was performed using an antibody specific for uncoupling protein 1, which detected a 32.5 kDa protein associated with mitochondria from the thymocytes. This implies that rat thymocytes contain uncoupling protein 1.

Introduction

UCP1 (uncoupling protein 1, also known as UCP and thermogenin) has been exclusively associated with BAT (brown adipose tissue) [1,2] and is a prerequisite for non-shivering thermogenesis in mammals. UCP1 is known to transport protons and to dissipate the proton electrochemical gradient (Δp) across the mitochondrial inner membrane. UCP1 thus acts as a major regulator of metabolic flux in mitochondria and as a heat regulator in the whole animal [1–4]. Recent evidence obtained in vitro also suggests that UCP1 plays a role in regulating superoxide production by mitochondria [5–8]. Recent research also suggests that the Cidea (cell death-inducing DNA fragmentation factor a-like effector A) may be an endogenous regulator of UCP1 activity in BAT [9]. In the present study, we show evidence that UCP1 is present in rat thymus. The thymus is a primary lymphoid organ that plays a central role in creation of a fully functional immune system. The major function of the thymus is to provide the appropriate milieu within which cells of the T-lymphocyte lineage can develop, proliferate, mature and generate their antigen receptor repertoire. Apoptosis is prevalent in the thymus. It may be this latter role in which mitochondria and UCP1 have relevance in thymus.

Experimental

Thymocyte and mitochondria preparation

Wistar rats (Rattus norvegicus) (180–200 g) were provided by the BioResources Unit at Trinity College Dublin. All rats were housed either at room temperature (20 ± 2 °C) or in pairs at 8 °C for 5 weeks. Thymocytes were isolated as described by Buttgereit et al. [10]. The thymus was removed from the rat, trimmed clean of connective tissue and brown fat (if present) and transferred into RPMI 1640 medium containing fresh l-glutamine (final concentration, 2 mM). The thymus and medium were poured onto a nylon mesh in a Petri dish and, using a plunger of a 5 ml syringe, the thymus was teased into a suspension of thymocytes. Any fat cells present in the thymus were automatically separated from thymocytes as they floated to the surface of the isolation medium. Mitochondria were isolated by homogenization followed by differential centrifugation by the method of Chappell and Hansford [11].

PAGE and immunoblotting

SDS/PAGE, under reducing conditions, was used to separate mitochondrial proteins before immunoblot analysis. After SDS/PAGE, resolved proteins were transferred on to PVDF membranes (Immobilon-P; Millipore), as described by Cunningham et al. [12]. Commercial rabbit antiserum specific for UCP1 (amino acids 145–159) was purchased from Calbiochem (Merck Biosciences, Darmstadt, Germany). A rabbit antiserum specific for the β-subunit of F1-ATP synthase from Neurospora crassa was a gift from Dr M. Harmey (Department of Botany, University College Dublin, Ireland). The antisera were used at 1:1000 dilution. After blocking and a 1 h primary antibody incubation, the blots were incubated with a horseradish-peroxidase-conjugated goat anti-rabbit secondary antibody (1:10 000 dilution) in PBS/0.1% (v/v) Tween 20/5% (w/v) milk powder for 1 h at room temperature. Blots were developed using an ECL® detection system (Amersham Biosciences) and immunoreactions were visualized by exposure to Kodak X-Omat LS film.

Results

Figure 1 shows that the Calbiochem antiserum to the UCP1 peptide detects a 32.5 kDa band in BAT mitochondria from cold-acclimated rats, BAT mitochondria from rats kept at room temperature and thymocyte mitochondria from rats kept at room temperature. UCP1 was not detected in liver mitochondria from rats kept at room temperature.

Discussion

Our results provide evidence that rat thymus mitochondria contain UCP1. The presence of UCP1 associated with rat...
The thymus is not due to contamination of the preparation by BAT. In dissecting out the thymus any BAT is clearly visible and easily removed by dissection. We speculate that mitochondrial UCP1 would have a major bearing on metabolic flux in thymus and predict a role in regulating superoxide production by mitochondria. Recent research that appears to show a direct interaction between a protein of the apoptotic cascade (Cidea) and UCP1 protein in BAT [9] has implications for understanding the role of UCP1 in the thymus.

References

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