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Uncoupling protein 1 dependent reactive oxygen species production by thymus mitochondria☆

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ABSTRACT

We have previously shown that uncoupling protein 1 is present in thymus and has a role in T-cell development. As reactive oxygen species have been implicated in T-cell development, we set out to determine whether uncoupling protein 1 had the potential to regulate reactive oxygen species production in mitochondria isolated from thymus. This was achieved by inhibiting proton leak through uncoupling protein 1 using the purine nucleotide GDP and through ablation of uncoupling protein 1, measuring the amplex red sensitive reactive oxygen species production by mitochondria. In this work we demonstrate, for the first time, that uncoupling protein 1 has the potential to regulate reactive oxygen species production in thymus mitochondria. We also show that reverse electron transport is possible in thymus mitochondria respiring on succinate and glycerol-3-phosphate. The implications of this regulatory role for uncoupling protein 1 are discussed in the context of thymus function.

This article is part of a Directed Issue entitled: Bioenergetic dysfunction, adaptation and therapy.

1. Introduction

Uncoupling protein 1 (UCP 1) is normally associated with brown adipose tissue (BAT) of mammals (Nicholls and Locke, 1984; Nicholls, 2001, 2006; Cannon and Nedergaard, 2004), with recent evidence confirming its presence in adult humans (Virtanen et al., 2009; Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Zingaretti et al., 2009). UCP1 is also present in thymus mitochondria (Carroll et al., 2004; Carroll et al., 2005; Porter, 2008). Evidence for the existence of UCP1 in thymus includes detection of RNA transcripts and confocal microscopy demonstrating UCP1 protein associated with mitochondria in situ in thymocytes (Adams et al., 2008a,b). The thymus is the site of T-helper (CD4+) and cytotoxic T-cell (CD8+) selection and maturation (Starr et al., 2003; Zúñiga-Pflücker, 2004; Hayday and Pennington, 2007) and the role for UCP1 in thymus would appear to be for the maturation and fate of developing T-cells (Adams et al., 2010). Evidence suggests that reactive oxygen species (ROS) play a role in T-cell maturation and selection in the thymus (Moon et al., 2004; Prasad et al., 2010).

Mitochondria are a constant source of ROS (Ježek and Hlvatá, 2005; Murphy, 2009). In vitro studies have estimated 1–2% of electrons are lost this way (Chance et al., 1979), with ROS production rate being greater when mitochondria have less ATP production demand, resulting in the redox couples of the electron transport chain being in a more reduced state (Turrens, 2003; Grivennikova and Vinogradov, 2006), and the driving force for release of these non-productive electrons the proton electrochemical gradient (Δp), being high (Miwa et al., 2003; Lambert and Brand, 2004; Andreyev et al., 2005; Boveris et al., 2006). The predominant sites of ROS production are NADH ubiquinone oxidoreductase (complex I) which directs electrons to the matrix (Miwa et al., 2003; Genova et al., 2003; Grivennikova and Vinogradov, 2006; Bell et al., 2007; Murphy, 2009), ubiquinol cytochrome c oxidoreductase (complex III) which directs electrons to the matrix and the inter-membrane space (Muller et al., 2004; Bell et al., 2007; Murphy, 2009) and glycerol-3-phosphate dehydrogenase situated on the outer surface of the mitochondrial inner membrane (Drahota et al., 2002; Vrbacký et al., 2007; Tretter et al., 2007). ROS production can also occur at complex I through reverse electron transport through complex I from substrates such as glycerol-3-phosphate, succinate and possibly fatty acids (Schönfeld et al., 2010; Lee et al., 2011; Shabalina and Mitochondrial, 2011). However, improved methodology is required to determine the extent of the occurrence of reverse electron flow in vivo on ROS production (Murphy, 2009; Shabalina and Mitochondrial, 2011).

All cells have antioxidants and antioxidation mechanisms which counteract ROS production by mitochondria to differing extents

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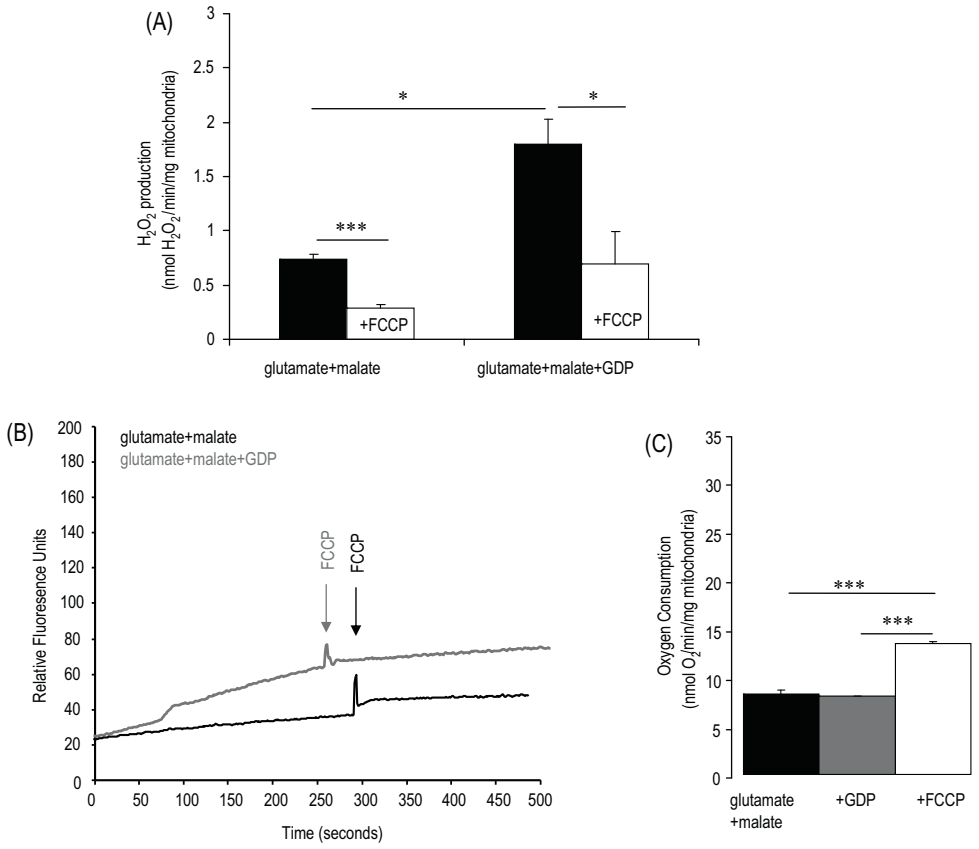

Fig. 1. ROS production (A and B) and oxygen consumption (C) by rat thymus mitochondria respiring on glutamate and malate [forward electron transport only]. Thymus mitochondria from rat (0.125 mg/ml) respiring on 5 mM glutamate plus 3 mM malate were incubated in 60 mM sucrose, 30 mM KCl, 20 mM Tris, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ethylene glycol-bis(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid (EGTA), 1 μM atractyloside, 1 μg/ml oligomycin, 0.1% defatted bovine serum albumin (BSA), pH 7.4 (with KOH), 5 μM Amplex Red, 10 U/ml of horseradish peroxidase, 30 U/ml superoxide dismutase (SOD), in the absence or presence of GDP (1 mM final). 0.5 μM protonophore carbonyl-4-(trifluoromethoxy)-phenylhydrazone (FCCP) was subsequently added. Fluorescence was detected by Perkin Elmer LS 55 Fluorometer with excitation set at 570 ± 8 nm and emission at 585 ± 4 nm. Oxygen consumption rates were measured using an Orborus Oxygraph Respirometer. The temperature throughout the experiment was maintained at 28 °C [$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$].

(Ernster, 1993; Andreyev et al., 2005; Ježek and Hlvatá, 2005). One potential physiological mechanism to alleviate ROS production by mitochondria is termed mild-uncoupling (Skulachev, 1996). Mild-uncoupling is based on the observations that uncoupling lowers Δp, decreases the degree of reduction in the electron transport chain and thus will reduce ROS production from the electron transport chain. Teleologically, uncoupling proteins can play a role in this mild-uncoupling process, or more accurately uncoupling proteins could potentially be used to regulate ROS production (Brand et al., 2004). The mitochondrial uncoupling proteins, UCP2 and UCP3 have been shown to be efficacious in alleviating ROS production in cells/tissue (Talbot and Brand, 2005; McLeod et al., 2005), and the lack of UCP2 has been associated with increased ROS production by cells (Arsenijevic et al., 2000; Duval et al., 2002; Bai et al., 2005). The investigations on the mitochondrial uncoupling protein, UCP 1, have predominantly focused on its key thermogenic role in brown adipose tissue, where it uncouples metabolism from ATP synthesis (Nedergaard et al., 2001). Recently, we (Dlaskovà et al., 2010) and others (Oelkrug et al., 2010) demonstrated that UCP1 can also regulate reactive oxygen species production by BAT mitochondria. In this study we set out to determine whether UCP1 can effect ROS production by thymus mitochondria.

2. Materials and methods

2.1. Wistar rats

Wistar rats were a mix of males and females aged between 10 and 12 weeks. They were housed at 25 ± 1 °C in individually ventilated cages. All rats were allowed free access to food [Harlan 2018 Teklad Global 18% (w/w) Protein Rodent diet] and water and a 12-h light/dark cycle was in place. All rats were killed by asphyxiation with carbon dioxide.

2.2. Wild Type (WT) and UCP1 knock-out (KO) mice

Wild Type (C57BL/6J) and UCP1 knock-out (C57BL/6J) [originally from the laboratory of Leslie P. Kozak, Pennington Biomedical Research Center, Baton Rouge, LA, USA] mice were housed in groups of 6 in a specific pathogen free environment. Mice were a mix of males and females aged between 6 and 10 weeks. They were housed at 25 ± 1 °C in individually ventilated cages. All mice were allowed free access to food [Harlan 2018 Teklad Global 18% Protein Rodent diet] and water and a 12-h light/dark cycle was in place. All mice were killed by CO_2 asphyxiation.