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Cold acclimation and oxygen consumption in the thymus

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Abstract

Mitochondrial uncoupling protein 1 is usually associated with brown adipose tissue but has recently been discovered in rat and mouse thymus. We wished to establish whether there was a thermogenic role for UCP 1 in thymus and thus examined the effect of 5 weeks cold-acclimation on rat thymus tissue abundance, thymocyte oxygen consumption, thymus mitochondrial abundance, uncoupling protein 1 expression and function. We found that thymocytes from cold-acclimated rats had oxygen consumption rates 8 times less than those from rats held at room temperature and that thymocytes from cold-acclimated rats or rats kept at room temperature were noradrenaline insensitive. In addition, we found that thymus tissue or mitochondrial abundance was not increased after cold-acclimation. However uncoupling protein 1 expression per unit mass of mitochondria was increased after cold-acclimation, as determined by immunoblotting (~1.7-fold) and GDP binding (~1.5-fold). Consistent with our protein expression data, we also observed an increased, state 4 (~1.5-fold), GDP-inhibitable (~1.3-fold) and palmitate activatable (~1.6-fold) oxygen consumption rates in isolated thymus mitochondria. However, extrapolation of our data showed that cold-acclimation only increased the amount of UCP 1 per gram of thymus tissue ~1.2-fold. Taken together, we conclude that UCP 1 does not have a thermogenic role in thymus.

Keywords: Uncoupling protein-1; Thymus; Thymocyte; Mitochondria; Purine nucleotide; Oxygen consumption

1. Introduction

Adaptation of rodents to cold environments involves a physiological process called non-shivering thermogenesis [1–4]. Brown adipose tissue (BAT) plays the principle role in this response through its capacity to produce heat. The stress response in BAT, in vitro, is manifest as (i) an increase in the abundance of BAT tissue, (ii) an increase in oxygen consumption rate by BAT cells from cold acclimated animals, an effect that is further activated by noradrenaline [1], (iii) an increase in the abundance of mitochondria in the BAT [1,4], (iv) an increase in the expression of mitochondrial uncoupling protein 1 (UCP 1) [1,4], (v) an increase in the activity of UCP 1 as manifest through an increase in fatty acid dependent/GDP-sensitive oxygen consumption rate by non-phosphorylating mitochondria [1,4], when compared with BAT tissue from animals kept at 37 °C or at room temperature.

Mitochondrial UCP 1 has always been considered specific to brown adipose [5–7]. However, we have recently demonstrated the presence of UCP1 transcripts in rat thymus and UCP 1 protein expression in mitochondria isolated from rat and mouse thymocytes/thymus [8,9]. Data were presented which showed that (a) UCP 1 message was associated with whole thymus and pure thymocytes (Thy-1-positive >99%) as determined by flow cytometry, (b) UCP 1 protein was detected using discriminatory antibodies in mitochondria isolated from whole thymus and isolated from pure thymocytes, (c) saturable radiolabelled GDP-binding was detected in mitochondria isolated from thymus to an equivalent extent to that observed for BAT mitochondria, (d) functional analysis demonstrated that nanomolar palmitate could overcome GDP inhibited oxygen consumption rates in thymus

Abbreviations: ATP, adenosine diphosphate; B_{MAX}, maximum binding capacity; BAT, brown adipose tissue; BSA, bovine serum albumin; DNP, 2,4-dinitrophenol; ECL, enhanced chemiluminescence; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether) *N,N,N′,N′*-tetraacetic acid; $F_1\beta$, the β subunit of the F_1 fraction of the ATP synthase; FBS, foetal bovine serum; GDP, guanosine diphosphate; HEPES, (*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid)); K_D, ligand binding affinity; PVDF, polyvinyldifluoride; ROS, reactive oxygen containing species; RPMI, Roswell Park Memorial Institute; SEM, standard error of the mean; UCP, uncoupling protein

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mitochondria giving an equivalent functional profile to that of BAT mitochondria and (e) UCP 1 was purified from thymus mitochondria and identified using mass spectrometry [8,9]. The role of UCP 1 in thymus is now a matter of investigation.

In this study we sought to discover the effect of cold acclimation on thymocyte oxygen consumption and whether UCP1 expression in rat thymus is sensitive to cold acclimation (as has been observed for UCP1 in BAT).

2. Materials and methods

2.1. Tissue sources and isolation of mitochondria

Female Wistar rats (124.7±2.5 g) were used in this study. Animals were randomly assigned to either a control group (n=30) or to a cold acclimated group (n=30). Cold acclimated rats were housed in pairs and held in a temperature-controlled room set at 4 °C for 5 weeks. Control rats were housed in groups of 6 at 25 °C (room temperature) for 5 weeks. All animals were allowed free access to laboratory chow and water. All rats were killed by cervical dislocation.

Mitochondria from kidney, liver, thymus and spleen were isolated by homogenisation followed by differential centrifugation according to the procedure of Chappell and Hansford [10]. Mitochondria were isolated from rat skeletal muscle by mincing the muscle and treating the tissue with nagarse (protease VII, Sigma). Skeletal muscle mitochondria were separated from the homogenate by differential centrifugation according to the method of Bhattacharya et al. [11]. BAT mitochondria were prepared by homogenisation followed by differential centrifugation according to the method of Scarpace et al. [12]. Mitochondrial protein concentrations were determined by the reduction of Folins–Ciocalteau phosphomolybdic–phosphotungstic reagent according to the method of Markwell et al. [13].

2.2. Thymocyte isolation

Thymocytes were isolated from Wistar rats (180–200 g) essentially as described by Buttgereit and Brand [14]. The thymus was removed from the rat, trimmed clean of connective tissue and brown fat (if present) and transferred into RPMI-1640 (Gibco) medium containing 10% (w/v) foetal bovine serum (FBS), supplemented with L-glutamine (Gibco)(2 mM final concentration), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma). A single cell suspension was prepared by passage through a 70 μm nylon sieves (Falcon). The thymocyte suspension was washed extensively, firstly by using approximately 50 ml medium to wash the cells through the sieves. Cells were then centrifuged (250–300×g) and gently resuspended in fresh medium 3 times. Any fat cells present in the thymus were automatically separated from thymocytes as they floated to the surface of the isolation medium. The viability of cells was >99% by trypan dye exclusion. Oxygen consumption rates by thymocytes isolated from rats were measured in the aforementioned medium at 37 °C using an Oxygraph Respirometer (Oroboros™, Innsbruck, Austria).

2.3. Western blot analysis

Mitochondrial protein extracts were loaded onto polyacrylamide gels, separated by electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membrane as described by Cunningham et al. [15]. Blocking of the membrane was performed by incubating the blot in 5% (w/v) Marvel milk powder at room temperature for 1 h. Commercial anti-UCP1 (amino acids 145–159) rabbit antisera were purchased from Calbiochem. A rabbit antiserum specific for the β-subunit of F_1-ATP synthase from *Neurospora crassa* was a gift from Dr. Matt Harmey, Department of Botany, University College Dublin, Ireland. The antisera were all used at 1:1000 dilutions. Following blocking and 1-h primary antibody incubation, the blots were incubated with a horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:10,000 dilution) in phosphate-buffered saline, 0.5% (v/v) Tween-20, 5% 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. Following western blot analysis, the relative abundance of UCP1 was determined using densitometry. The band intensities of the exposed film were analysed using Scion Imaging software.

2.4. GDP binding assay

Endogenous residual bound nucleotides were removed from isolated mitochondria using an anion exchanger (Dowex 21K) according to the procedure described by Huang and Klingenberg [16]. Isolated mitochondria at a concentration of 2 mg/ml in buffer containing 250 mM sucrose, 20 mM HEPES, 1 mM EDTA (pH 8.0), were shaken with Dowex (120 mg/mg protein) at room temperature for 1-h. Measurement of binding of tritiated GDP was performed by a modification of the procedure described by Scarpace et al. [12]. Mitochondria (50 μg) were incubated with [^{3}H]GDP (0.1–6.0 μM, 11.0 Ci/mmol) and [^{14}C] sucrose (250 μCi/ml) for 15 min at 37 °C in the absence (total binding) and presence (non specific binding) of unlabeled GDP (1.5 mM). Specific binding was calculated from the difference between total and non-specific binding. The Michaelis dissociation constant (K_D) and the maximal binding capacity (B_{MAX}), describing the saturable binding of [^{3}H]GDP, were obtained by fitting mean values for specific binding sites on the y axis and free radioligand concentrations used on the 'x-axis using the program 'Sigma plot' version 5 (SPSS Inc. Chicago). The data were fitted to a rectangular hyperbola by non-weighted, non-linear least squares regression.

2.5. Oxygen consumption by non-phosphorylating mitochondria

Oxygen consumption rates were measured using a Clarke-type oxygen electrode as described by González-Barroso et al. [17]. Mitochondria (1 mg/ml) were incubated at 37 °C in a medium containing 120 mM KCl, 5 mM HEPES, 1 mM EGTA (pH 7.4), 16 μM fatty acid free bovine serum albumin (BSA), 5 μM rotenone and 1 μg/ml oligomycin, 5 μM atractyloside. Non-phosphorylating (state 4) oxygen consumption rates were measured as the steady-state rate achieved on addition of 7.5 mM succinate (succinate–KOH, pH 7.4). The sensitivity of this state 4 oxygen consumption rate to GDP (1 mM) was then determined. The sensitivity of the resulting oxygen consumption rate to palmitate (64 μM) (~40 nM free) was also determined. Finally, the mitochondrial uncoupler 2,4-dinitrophenol (DNP) (30 μM) was added to the chamber to determine the maximum oxygen consumption rate attainable due to uncoupling and to confirm that the isolated mitochondria were coupled. The Clarke oxygen electrode was calibrated according to the procedure of Reynafarje et al. [18] assuming 406 nmol O dissolved in 1 ml of incubation medium held at 37 °C.

2.6. Statistical analysis

All results are expressed as mean±SEM (n) unless otherwise indicated, where SEM in standard error of the mean and n is the number of determinations, performed in triplicate. Means values were compared using an unpaired Student's t-test. Unless otherwise stated a p value of ≤0.05 was taken to indicate significance.

3. Results

Thymocytes were isolated from cold-acclimated rats and rats kept at room temperature. Table 1 shows that oxygen consumption rates for thymocytes from cold-acclimated rats and those kept at room temperature. As can be seen, thymocyte oxygen consumption rates were 8 times less (p=0.002; p=0.0005) in thymocytes from cold-acclimated animals (0.22±0.05(3); 0.21±0.04(5)) when compared to those from rats kept at room temperature (1.6±0.05(3); 1.7±0.2(5)), whether in the presence or absence of noradrenaline (1 μM), respectively (units are nmol/O_2/min/10^7 cells). It is also important to note that there was no increase in oxygen consumption rates following

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Table 1
Summary of the effects of cold acclimation on thymocyte oxygen consumption rates

	Thymocytes from animals at room temperature	Thymocytes from cold-acclimated animals	Result of Student's *t*-test
Oxygen consumption (nmol O_2/min/10^7 cells)	1.7±0.2(5)	0.21±0.04(5)	$p=0.0005$
Oxygen consumption (nmol O_2/min/10^7 cells) +1 μM noradrenaline	1.6±0.2(5)	0.22±0.05(3)	$p=0.002$

addition of noradrenaline to thymocytes isolated from cold-acclimated rats or rats kept at room temperature.

We have previously shown that the Calbiochem anti-UCP1 is specific for UCP 1 over other UCPs and mitochondrial transporters [9]. Fig. 1A shows the Calbiochem anti-UCP1 peptide antibody detecting UCP1 protein in thymus mitochondria isolated from rats housed at room temperature and in thymus mitochondria isolated from cold-acclimated rats. UCP1 was also detected in BAT mitochondria isolated from cold acclimated rats. UCP1 protein was not detected in liver mitochondria isolated from cold acclimated rats. Fig. 1B shows $F_1\beta$ expression in liver, BAT and thymus mitochondria isolated from cold acclimated rats and in thymus mitochondria isolated from rats housed at room temperature. Table 2 illustrates the relative abundance of UCP1 protein expression as a ratio of $F_1\beta$ of the ATP synthase as determined by densitometry. Cold exposure induced a significant ($p=0.0205$; $n=3$) ~1.7-fold increase in UCP1 protein expression per unit mass of thymus mitochondria when compared to thymus mitochondria isolated from room temperature rats. We also compared UCP 1 expression in mitochondria isolated from BAT of cold acclimated rats and rats kept at room temperature. We observed a significant ($p\leq0.05$) ~1.5-fold increase in UCP 1 expression per unit mass of mitochondria as a result of cold acclimation (Table 3).

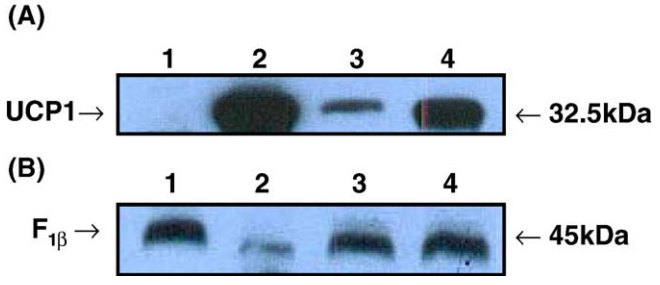

Fig. 1. UCP1 protein expression in thymus mitochondria isolated from rats acclimated to the cold for 5 weeks. Thymus mitochondria were isolated either from rats housed at room temperature or at 4 °C for 5 weeks as described in Materials and methods. Mitochondria were subjected to SDS-PAGE and Western blotted using in (A) an anti-UCP1 (Calbiochem) peptide antibody or in (B) an antibody to $F_1\beta$. Lane 1, liver mitochondria isolated from cold acclimated rats. Lane 2, BAT mitochondria isolated from cold acclimated rats. Lane 3 thymus mitochondria isolated from rats housed at 25 °C. Lane 4, thymus mitochondria isolated from cold acclimated rats.

Table 2
Summary of the effects of cold acclimation on thymus tissue

	Thymus from animals at room temperature	Thymus from cold-acclimated animals	Result of Students *t*-test
Tissue mass (g)	1.4±0.2(3)	1.3±0.1(3)	N.S.
Mitochondrial yield (mg/g tissue)	1.7±0.4(3)	1.4±0.1(3)	N.S.
B_{MAX} (pmol bound/mg mitochondrial protein)	131±20(3)	197±15(3)	$p\leq0.05$
UCP1/$F_{1\beta}$ (arbitrary units)	0.74±0.12(3)	1.25±0.07(3)	$p=0.0205$

Data is presented as mean±SEM for *n* determination performed in triplicate. B_{MAX}=maximal mitochondrial [^{3}H]-GDP binding capacity. UCP1/$F_{1\beta}$ represents UCP 1 protein expression, as assay by immunoblot, relative to that of the $F_{1\beta}$ subunit of the ATP synthase.

An independent method to confirm abundance of UCP 1 protein is to measure the degree of [^{3}H]GDP to UCP1 containing mitochondria. Fig. 2A shows saturable binding of [^{3}H]GDP to Dowex-treated thymus mitochondria isolated from room temperature and cold acclimated rats. The K_D values determined were 1.4±0.8 μM (3) and 0.9±0.3 μM (3) for mitochondria isolated from rats housed at room temperature and in the cold respectively. B_{MAX} values determined for Dowex-treated thymus mitochondria isolated from room temperature (131±20 pmol bound/mg protein (3)) and cold acclimated (197±15 pmol bound/mg protein (3)) were also calculated. No significant difference in K_D values for [^{3}H]GDP binding to thymus mitochondria was observed whether the source was rats at room temperature or cold acclimated rats. A significant ($p\leq0.05$) ~1.5-fold increase was observed in the B_{MAX} value as a result of cold acclimation. B_{MAX} values for thymus mitochondria from cold acclimated rats and rats kept at room temperature are summarized in Table 2.

Fig. 2B shows the [^{3}H]GDP binding kinetics of thymus mitochondria isolated from rats housed at room temperature to be non-saturable in the absence of Dowex treatment. Consequently, binding parameters B_{MAX} and K_D were

Table 3
Summary of the effects of cold acclimation on brown adipose tissue

	BAT from animals at room temperature	BAT from cold-acclimated animals	Result of Student's *t*-test
Tissue mass (g)	2.6±0.3(3)	2.2±0.1(3)	N.S.
Mitochondrial yield (mg/g tissue)	1.1±0.4(3)	6.9±0.8(3)	$p\leq0.01$
B_{MAX} (pmol bound/mg mitochondrial protein)	353±61(3)	736±128(3)	$p\leq0.05$
UCP1/$F_{1\beta}$ (arbitrary units)	0.97±0.07(3)	1.5±0.2(3)	$p\leq0.05$

Data is presented as mean±SEM for *n* determination performed in triplicate. B_{MAX}=maximal mitochondrial [^{3}H]-GDP binding capacity. UCP1/$F_{1\beta}$ represents UCP 1 protein expression, as assay by immunoblot, relative to that of the $F_{1\beta}$ subunit of the ATP synthase.

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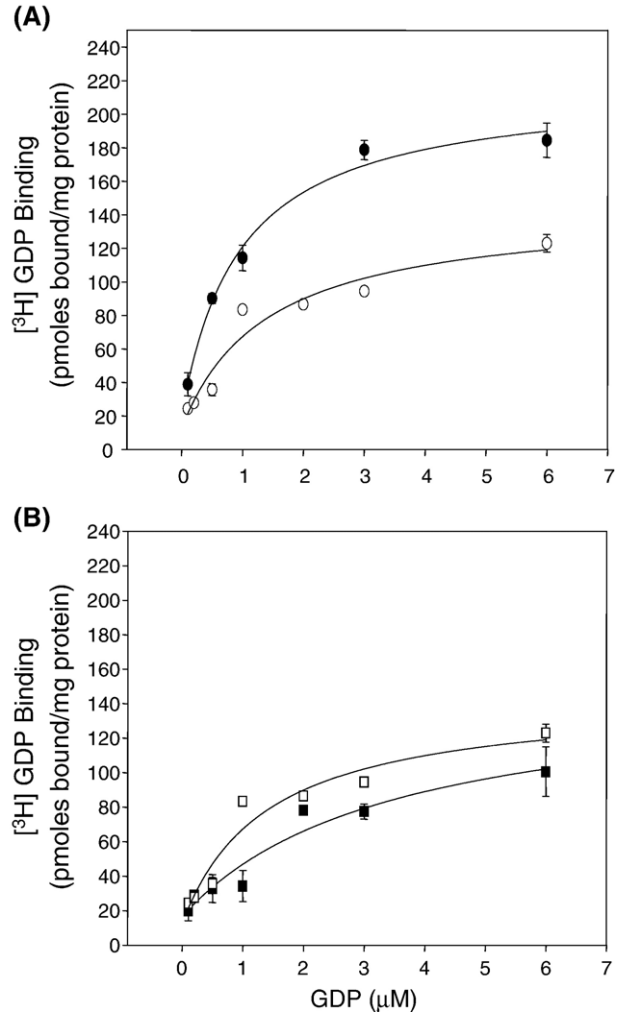

Fig. 2. Comparison of [^{3}H] GDP binding kinetics in thymus mitochondria. [^{3}H] GDP binding kinetics were determined for (A) Dowex-treated thymus mitochondria from rats kept at room temperature (○) and cold-acclimated (●) rats and (B) Dowex-treated (□) and non-Dowex-treated (■) mitochondria from thymus of rats kept at room temperature. Experiments were performed to determine B_{MAX} and K_D values for the GDP binding parameters. Dowex-treated mitochondria were incubated with [^{3}H] GDP (11.0 Ci/mmol; 0.1–6 μM) and [^{14}C] sucrose for 15 min at 37 °C in the absence (total binding) and presence (non-specific binding) of unlabelled GDP (1.5 mM) as described in Materials and methods. Specific binding was calculated from the difference between total and non-specific binding. Data are expressed as the mean±SEM of at least 3 experiments, each experiment performed in triplicate. Statistical differences were assessed using an unpaired Student's *t*-test.

unattainable. In contrast, Dowex-treated thymus mitochondria bind [^{3}H]GDP in a saturable manner up to and including 6 μM GDP.

[^{3}H]GDP binding to BAT mitochondria from cold acclimated rats and rats kept at room temperature were also determined and B_{MAX} values are summarized in Table 3. No significant difference in K_D values for [^{3}H]GDP binding to BAT mitochondria was observed whether the source was rats at room temperature (0.5±0.4 μM (3)) or cold acclimated rats (0.8±0.6 μM (3)). However, as expected, there was a significant ($p \leq 0.05$) ~2.1-fold increase in the B_{MAX} for GDP-binding to BAT mitochondria from cold -acclimated rats (736±128 pmol bound/mg protein (3)) when compared to rats kept at room temperature (353±61 pmol bound/mg protein (3)) (Table 3).

Data is also presented which shows the effect of cold-acclimation on tissue abundance and mitochondrial yield. Data in Table 2 shows that there is no significant change in average thymus mass from cold acclimation rats (1.3±0.1 g(3)) when compared to rats kept at room temperature (1.4±0.2 g(3)), nor is there a significant change in mitochondria yield from the thymus of cold-acclimated rats (1.4±0.2 mg/g tissue(3)) compared with the yield from rats kept at room temperature (1.7±0.4 mg/g tissue(3)). By contrast data in Table 3 show that although cold acclimation does not increase interscapular BAT mass (2.2±0.1 g(3) from cold-acclimated rats; 2.6±0.3 g(3) from rats kept at room temperature), mitochondrial yield per unit mass of BAT is increased 6.3-fold (from 1.1±0.4 mg/g tissue (3) to 6.9±0.8 mg/g tissue(3); $p \leq 0.01$). Extrapolating UCP 1 expression and binding data to the whole tissue, shows that cold-acclimation results in a ~1.2- to 1.3-fold increase in UCP 1 in the thymus of rats, whereas cold-acclimation results in an ~8.2- to 11-fold increase in UCP 1 in interscapular BAT of rats.

The present study also investigated the effect of cold-acclimation on in vitro activity of UCP 1 in non-Dowex treated mitochondria. Tables 4 and 5 show that state 4 (non-phosphorylating) oxygen consumption rates, the oxygen consumption rates following addition of saturating amounts of GDP, and the oxygen consumption rates following addition of 40 nM free palmitate for mitochondria isolated from BAT (Table 4) and thymus (Table 5) from cold-acclimated rats and rats kept at room temperature. 2,4-DNP was added to determine the maximal electron transport chain rate and to demonstrate that the mitochondria were coupled. As expected, Table 4 clearly demonstrates that the state 4 oxygen consumption rate for BAT mitochondria from rats kept at room temperature (167.8±14.2 nmol O/min/mg protein(3)) can be inhibited to a significant degree ($p \leq 0.01$) by addition of GDP (99.5±7.5 nmol O/min/mg protein(3)). The GDP-inhibitable oxygen consumption rate can in turn be overcome by addition of palmitate (40 nM) (139.4±7.5 nmol O/min/mg protein(3)) ($p \leq 0.05$). Similarly, the data demonstrate that the state 4 oxygen consumption rate for BAT mitochondria from cold-acclimated (384.2±88 nmol O/min/mg protein(3)) can be inhibited to a significant degree ($p \leq 0.001$) by addition of

Table 4
Summary of the effects of cold acclimation on brown adipose tissue mitochondrial oxygen consumption rates

	Mitochondrial oxygen consumption rates (nmol O/min/mg protein)		Result of Student's *t*-test
	BAT mitochondria from rats kept at room temperature	BAT mitochondria from cold-acclimated rats	*p* value
State 4	167±14.2(3)	384.2±8.8(3)	$p=0.0002$
GDP-inhibited	99.5±7.5(3)	169.4±18.2(3)	$p=0.0238$
Palmitate-activated	139.4±7.5(3)	333±4(3)	$p=0.0001$

Data is presented as mean±SEM for *n* determination performed in triplicate.

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Table 5
Summary of the effects of cold acclimation on thymus mitochondrial oxygen consumption rates

	Mitochondrial oxygen consumption rates (nmol O/min/mg protein)		Result of Student's *t*-test
	Thymus mitochondria from rats kept at room temperature	Thymus mitochondria from cold-acclimated rats	*p* value
State 4	28±2.8(3)	41±6.2(3)	$p=0.002$
GDP-inhibited	9.6±2.5(3)	12.6±1.4(3)	N.S.
Palmitate-activated	33.6±4.3(3)	55.3±7.3(3)	$p=0.012$

Data is presented as mean±SEM for *n* determination performed in triplicate.

GDP (169.4±18.2 nmol O/min/mg protein(3)). The GDP-inhibitable oxygen consumption rate can in turn be overcome by addition of palmitate (40 nM) (333±4 nmol O/min/mg protein (3)) ($p \leq 0.01$).

UCP 1 dependent (GDP-inhibitable/fatty acid activatable) can also be observed in thymus mitochondria although the rates of oxygen consumption are approximately 8 times less per mg mitochondrial protein in mitochondria that in mitochondria from BAT. Table 5 clearly demonstrates that the state 4 oxygen consumption rate for thymus mitochondria from rats kept at room temperature (28±2.8 nmol O/min/mg protein(3)) can be inhibited to a significant degree ($p \leq 0.01$) by addition of GDP (9.6±2.5 nmol O/min/mg protein(3)). The GDP-inhibitable oxygen consumption rate can in turn be overcome by addition of palmitate (40 nM) (33.6±4.3 nmol O/min/mg protein(3)) ($p \leq 0.05$). Similarly, Table 5 shows that the state 4 oxygen consumption rate for BAT mitochondria from cold-acclimated rats (41±6.2 nmol O/min/mg protein(3)) can be inhibited to a significant degree ($p \leq 0.01$) by addition of GDP (12.6±1.4 nmol O/min/mg protein(3)). The GDP-inhibitable oxygen consumption rate can in turn be overcome by addition of palmitate (40 nM) (55.3±7.3 nmol O/min/mg protein(3)) ($p \leq 0.01$).

There was no GDP-inhibitable/palmitate activatable oxygen consumption rates in mitochondria isolated from liver (which contain no constitutively expressed UCPs) [19], kidney (which contains UCP2) [20] or spleen (which contain both UCP2 and UCP3) [21] from cold-acclimated rats or rats kept at room temperature [results not shown].

Tables 4 and 5 also show a comparison of mitochondrial oxygen consumption rates for mitochondria isolated from cold-acclimated rats and rats kept at room temperature for BAT and thymus, respectively. Cold-acclimation resulted in a significant ($p=0.0002$) ~2.3-fold increase in state 4/ non-phosphorylating oxygen consumption rate, a significant ($p=0.0238$) ~1.7-fold increase in GDP-inhibitable oxygen consumption rate and a significant ($p=0.0001$) ~2.4-fold increase in palmitate activatable oxygen consumption rate, per unit mass of BAT mitochondria (Table 4). Cold-acclimation resulted in a significant ($p=0.002$) ~1.5-fold increase in state 4/ non-phosphorylating oxygen consumption rate, a non-significant ~1.3-fold increase in GDP-inhibitable oxygen consumption rate and a significant ($p=0.0121$) ~1.6-fold increase in palmitate activatable oxygen consumption rate, per unit mass of thymus mitochondria (Table 5).

In contrast, cold-acclimation had no effect on state 4 oxygen consumption rates of liver mitochondria (which contain no constitutively expressed UCPs) [19], kidney mitochondria (which contains UCP2) [20] or spleen mitochondria (which contain both UCP2 and UCP3) [21] (results not shown).

4. Discussion

Cold-acclimation clearly has a dramatic effect on metabolism in thymus. Our thymocyte oxygen consumption data show that cold acclimation of rats resulted in a significant 8-fold decrease in oxygen consumption of isolated thymocytes (Table 1). In addition, we observed that the oxygen consumption rates of thymocytes from cold acclimated rats were noradrenaline insensitive. It is known that BAT cells from cold acclimated hamsters have increased oxygen consumption rates and rates which are further stimulated (~10-fold) by in vitro addition of noradrenaline [1]. The lack of response of the thymus is in contrast to the response of BAT following cold acclimation as reported in the literature for [1]. Thus the stress response in rats due to the cold, presumably manifest through the action of thyroid hormone and noradrenaline, has opposing effects on metabolism in BAT and thymus. However, it is reassuring to know that our thymocyte oxygen consumption data are consistent with the process of involution known to occur in thymus following cold exposure [22].

The elements involved in the mechanism by which there is an 8-fold reduction in oxygen consumption rate by thymocytes from cold-acclimated animals when compared to thymocytes from animals kept at room temperature must include mitochondria as they are the major oxygen consumers in cells. How the mitochondria in situ are regulated directly or indirectly to reduce their oxygen consuming rates will now be a matter for investigation. Possible lines of investigation include (a) a decrease in ATP turnover in the cells, (b) a decrease in the contribution of in situ proton leak and (c) a decrease in supply of substrates to the mitochondria. The potential for noradrenaline to stimulate UCP 1 containing BAT cells led us to investigate whether the reduced oxygen consumption rate in rat thymocytes from cold-acclimated rats, was due to UCP 1, a protein recently shown to be present in mitochondria from rat (and mouse) thymus [8,9]. Although it is known that proton leak contributes to ~20% of the oxygen consumption of thymocytes from rats kept at room temperature [14], we were unable to estimate the contribution of proton leak (or ATP turnover) in thymocytes from the cold-acclimated animals due to the low resolution of the methodology at such low oxygen consumption rates. On the other hand, UCP 1 protein expression in thymus mitochondria does appear to be sensitive to cold acclimation. Our combined data, for protein expression and GDP-binding, demonstrate that rat thymus UCP1 expression increases 1.5- to 1.7-fold as a result of cold acclimation (Figs. 1and 2; Table 2). The extent of the increase in UCP 1 protein expression and GDP-binding per unit mass of thymus mitochondria, parallels the observed increase (1.5- to 2.1-fold) by us (Table 3) and others [1] for UCP

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1 expression per unit mass of BAT mitochondria following cold-acclimation.

It should also be noted that we attribute the GDP-binding in thymus mitochondria to UCP 1, although we know that thymus mitochondria also contain UCP 2 [23] and UCP 3 [21]. The reason we do so is that we know that kidney and spleen, both of which contain UCP 2, show no significant GDP-binding, either because UCP 2 can't bind GDP or because there is very little UCP 2 present [9]. Similarly, skeletal muscle mitochondria which contain UCP 3 also show no significant GDP-binding [9]. In addition, our mass spectrometry analysis of the purified fraction for native UCP 1 from thymus, detected no UCP 2 or UCP 3, suggesting low expression levels of these proteins compared with UCP 1 [9].

Consistent with our observations for UCP 1 abundance per unit mass of mitochondria from thymus, is our further observations that oxygen consumption rates by UCP 1 containing mitochondria are increased 1.5-fold in state 4 and 1.6-fold in palmitate activation of GDP-inhibited mitochondria (Table 5) as a result of cold-acclimation. The observation that there is masking of UCP 1 in thymus mitochondria from animals kept at room temperature (Fig. 2B), parallels masking data for BAT mitochondria from animals kept at room temperature reported in the literature [9,16]. Clearly because abundance and activity of UCP 1, as determined in isolated mitochondria, doesn't decrease as a result of cold-acclimation, we can conclude that the increased abundance and activity of UCP 1 is not a factor in determining the reduced oxygen consumption rates in thymocytes. The corollary, however, being that because the abundance and activity of UCP 1 in the whole thymus only increases ~1.2- to 1.4-fold (Table 2 and 5) compared with 10- to 13-fold for whole BAT (Table 3 and 4), we therefore conclude that the role of UCP 1 in thymus is not a thermogenic role.

This suggests an as yet unknown function for UCP 1 in thymus. One possible role for UCP 1 in thymus is regulation of reactive oxygen species (ROS) production by mitochondria which may be linked to involution. Support for such a role is consistent with the observation by Nègre-Salvayre et al. [24] that reactive oxygen containing species (ROS) production by rat thymus mitochondria is GDP sensitive. Future work will focus on whether UCP 1 has a role ROS production by thymus mitochondria and whether that role that may be linked to thymus function.

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