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Adaptive changes in interscapular brown adipose tissue during reacclimation after cold: The role of redox regulation

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Abstract

1. The level of antioxidative defence, intensity of sympathetic innervation, expression of uncoupling protein 1 (UCP1), and ultrastructure of interscapular brown adipose tissue (IBAT) were studied in rats acclimated to room (22±1 °C), low temperature (4±1 °C, 45 days) and during reacclimation to 22 °C (days 1, 3, and 7).
2. The cold-induced increase in catalase, glutathione peroxidase, glutathione reductase, thioredoxin reductase, as well as a decrease in the activities of CuZn- and Mn-superoxide dismutases, shows specific dynamics of restitution during reacclimation to 22 °C.
3. The intensity of the cold-induced enhancement of the sympathetic innervation and UCP1 expression gradually decreases during reacclimation to 22 °C, but with the seventh day level still higher compared to the animals acclimated to room temperature.
4. Electron-microscopy study of IBAT shows that changes of the strongly activated tissue in cold undergo gradual restitution during reacclimation to 22 °C, but are observed already on the first day through a massive accumulation of glycogen, accompanied by a change in the lipid profile.

Keywords: Redox regulation; Brown adipose tissue; Uncoupling; Antioxidative defence; Cold; Reacclimation

1. Introduction

The redox equilibrium plays an important role in maintaining the total homeostasis of cells and tissues (Wiese et al., 1995; Kamata and Hirata, 1999). It has been shown to be tissue specific, and to change in various states: hyperplasia, cancer, cardiovascular and neurodegenerative diseases, etc. (Halliwell and Gutteridge, 1985; De Bono, 1994; Toyokuni et al., 1995; Davies, 1999). Maintenance of this equilibrium depends on the endogenous antioxidative defence (AD). The AD primarily includes the following enzymes: superoxide dismutase (CuZnSOD and MnSOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GR), as well as glutathione (GSH), necessary not only as an antioxidant, but also as a cofactor of numerous enzymes and the most significant molecule for maintaining the redox environment in the cells (McCord and Omar, 1993; Jacob, 1995; Aruoma, 1996). Also, AD shows plasticity in the response to intensity and metabolic tissue profile (Halliwell and Gutteridge, 1990; Spasić et al., 1993; Petrović et al., 2006).

On the other hand, interscapular brown adipose tissue (IBAT) shows extraordinary ability to remodel, given that its thermogenic capacity depends on the number of brown adipocytes, mitochondria, and the concentration of uncoupling protein 1 (UCP1) present (Lin and Klingenberg, 1982; Rial et al., 1983; Bukowiecki et al., 1986; Buzadžić et al., 1990; Nedergaard et al., 2001; Cannon and Nedergaard, 2004). These adaptive changes are directly mediated by sympathetic stimulation (Bukowiecki et al., 1978; Arch et al., 1984).

These processes in IBAT are induced by different stimuli. External temperature is among the most potent

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(Jansky, 1973; Rothwell and Stock, 1985). Exposure of animals to a low temperature triggers and activates (thermogenically inactive IBAT at room temperature) in a whole series of processes. The cold-induced changes in IBAT have been well studied (Bukowiecki et al., 1982, 1986; Darnley et al., 1988; Spasić et al., 1993; Petrović et al., 2005), in contrast to a smaller number of studies that describe tissue atrophy during reacclimation (Suter, 1969; Hori et al., 1998). For that reason the regulation of proliferation balance and normal cell death (hyperplasia/atrophy) is essential for maintaining the total IBAT homeostasis (Lindquist and Rehnmark, 1998). That homeostasis also includes structural, metabolic, and redox IBAT homeostasis (Petrović et al., 2006).

It has been established that adaptive IBAT changes induced by cold decrease during reacclimation (Hori et al., 1998). It has also been established that the restitution of thermogenically active tissue into inactive tissue depends on the duration of exposure to cold: it is rapid after a short exposure, and slower after a long exposure (Puigserver et al., 1992; Spasić et al., 1993). We have used here a long-term acclimation (45 days, $4\pm1\,^{\circ}C$). Also, 2–3 weeks are needed for a full restitution of the tissue (Suter, 1969). This indicates that molecular events leading to IBAT atrophy commence at an early stage, in the first week of reacclimation.

This is why our aim is to investigate the changes of AD (CuZnSOD, MnSOD, CAT, GSH-Px, GR, and thioredoxin reductase (TR) activity), UCP1 expression, innervation and structural changes of IBAT in various functional states. To investigate further the latter possibility the animals were acclimated to room temperature ($22\pm1\,^{\circ}C$; thermogenically inactive tissue) and to a low temperature (45 days, $4\pm1\,^{\circ}C$; thermogenically active tissue). Also, in order to investigate the mechanisms that lead to the opposite changes (IBAT atrophy) we used the model of reacclimation to $22\,^{\circ}C$ (1, 3, and 7 days) of animals previously acclimated to low temperature.

2. Materials and methods

2.1. Animals

The male Mill Hill hybrid hooded, 4-months-old rats were divided into five groups (six animals in each group). The control group was maintained at room temperature ($22\pm1\,^{\circ}C$) for the whole term of the experiment. Four other groups were kept in a cold chamber at a temperature of $4\pm1\,^{\circ}C$ for 45 days. One of the groups was sacrificed after that, the three remaining groups were transferred from the cold chamber to the control room and then sacrificed after 1, 3, and 7 days of reacclimation to $22\,^{\circ}C$. The protocol was approved by the Institute's Ethical Committee on Animal Experiments.

The animals were sacrificed by decapitation, the IBAT dissected out within 3 min after death, and thoroughly rinsed with physiological saline to remove traces of blood. The tissue was homogenized (a Janke and Kunkel Ka-Werke Ultra-Turrax homogenizer, $0–4\,^{\circ}C$) in 0.25 M sucrose, 0.1 mM EDTA and 50 mM Tris–HCl buffer, pH 7.4. The homogenates were sonicated as suggested by Takada et al. (1982).

2.2. Activity of antioxidative enzymes

Total SOD activity was determined by a modified epinephrine method of Misra and Fridovich (1972). This method is based on the capacity of SOD to inhibit autoxidation of adrenaline to adrenochrome. One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the autoxidation of adrenaline at $26\,^{\circ}C$ in the volume of 3.2 ml. Reaction mixture contained 3×10^{-4} M adrenaline, 1×10^{-4} M EDTA and 0.05 M Na_2CO_3, pH 10.2. For determination of MnSOD activity, the assay was performed after preincubation with 4 mM KCN. The CuZnSOD activity was calculated as the difference between total SOD and MnSOD activities. The activity was expressed in U mg^{-1} protein. Catalase was assayed as suggested by the supplier (SIGMA Chemicals, St. Louis, MO, USA) and the activity expressed as μmol H_2O_2 min^{-1} mg^{-1} protein. The method is based on the rate of H_2O_2 degradation by the action of catalase contained in the examined sample followed spectrophotometrically at 230 nm in 5 mM EDTA, Tris–HCl solution, pH 8.0. The GSH-Px was determined using *t*-butylhydroperoxide as a substrate (Paglia and Valentine, 1967) and the activity expressed in nmol NADPH min^{-1} mg^{-1} protein. The activity of GR was assayed as suggested by Glatzle et al. (1974) and expressed in nmol NADPH min^{-1} mg^{-1} protein. The activity of TR was determined by the method of Luthman and Holmgren (1982) and expressed as nmol NADPH min^{-1} mg^{-1} protein.

2.3. SDS–PAGE and Western blotting

Proteins were resolved according to Laemmli (1970). The amount of 10 μg of proteins was boiled and electrophoresed in 15% SDS–polyacrylamide gel (PAGE), transferred onto a nitrocellulose membrane and incubated with rabbit polyclonal antibody against UCP1 (Sigma-Aldrich, Inc.). Incubation with primary antibody was performed in TBS-T with 5% bovine serum albumin (BSA) at 1:1000 dilutions, overnight in the cold room. After multiple washes in TBS-T the membrane was incubated with horseradish peroxidase-conjugated IgG secondary antibody (Santa Cruz Biotechnology) at 1:2000 dilution. Peroxidase activity was detected using 4-chloro-1-naphthol and hydrogen peroxide as substrate.

2.4. Electron-microscopic examination

Immediately after removal, the samples of IBAT were cut into small pieces, fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2), and post-fixed in 2%