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Free radical equilibrium in interscapular brown adipose tissue: Relationship between metabolic profile and antioxidative defense

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Abstract

Interscapular brown adipose tissue (IBAT) hyperplasia involves a new metabolic and structural profile, resulting from acclimation of animals to a cold environment. Cold-induced changes of several antioxidative defense (AD) components in IBAT and their interrelationship with uncoupling protein 1 (UCP1), sympathetic innervation and apoptosis were studied using cold-acclimated adult rat males (4 ± 1 °C, 45 days). Their age-matches were maintained at 22 ± 1 °C serving as the controls. In cold-adapted rats, activities of CuZn- and Mn-superoxide dismutase (SOD) and apoptosis were reduced, while catalase (CAT), glutathione peroxidase (GSH-Px), glutathione *S*-transferase (GST) activities and glutathione (GSH) content were increased compared to the control. IBAT mass, protein content, plasma free fatty acid (FFA) concentration, sympathetic innervation and UCP1 level were significantly increased in cold-acclimated group compared to the corresponding control. These results suggest that decreased CuZn and MnSOD activities in IBAT represent an adaptive response due to UCP1-induced mitochondrial uncoupling. Additionally, intensive fatty acid oxidation led to an increased H_2O_2 production which resulted in increased CAT, GSH-Px and GST activities and GSH level. Generally speaking, cold-induced changes of AD in the IBAT are closely connected with newly established metabolic profile in this tissue, thus making an important part of the entire tissue homeostasis including cell survival.

Keywords: Antioxidative defense; Apoptosis; Cold; Free radicals; IBAT; Superoxide; Uncoupling

1. Introduction

Endogenous antioxidative defense (AD) provides protection of tissues against reactive oxygen species (ROS) produced during both normal metabolic activity and in pathological conditions (Halliwell et al., 1992; Johnson, 2002). It primarily includes superoxide dismutases (SODs, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GSH-Px, EC 1.11.1.9), glutathione reductase (GR, EC 1.6.4.2), glutathione *S*-transferase (GST, EC 2.5.1.18) and thioredoxin reductase (TR, EC 1.6.4.5) and several low-molecular components such as vitamins E and C, glutathione (GSH), etc. (Aruoma, 1996). Organization of AD is tissue- and cell-specific (Halliwell and Gutteridge, 1990; Spasić et al., 1993). Specificities in maintaining redox equilibrium either in the tissues or in cells require plasticity of the response at the AD level when the changes in physiological state and intensity of metabolic activity take place (Buzadžić et al., 1997, 1999; Korać et al., 1998; Korać and Buzadžić, 2001).

Acclimation to low temperature acts by inducing a complex physiological response of an organism, particularly in interscapular brown adipose tissue (IBAT) (Jansky and Hart, 1968; Rothwell and Stock, 1983, 1985). Namely, cold-induced noradrenergic stimulation triggers a cascade of structural changes (proliferation and differentiation of brown adipocytes, angiogenesis, mitochondrial and peroxisomal biogenesis) playing the role in IBAT remodelling (Bukowiecki et al., 1978, 1986; Buzadžić et al., 1990; Spasić et al., 1993). Besides, new metabolic profile (intensive oxidation of fatty acids, increased oxygen consumption and basal metabolic level) gets

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established in IBAT (Suter, 1969). It is, first of all, characterized by the presence of a unique, uncoupling protein 1 (UCP1) within the inner mitochondrial membrane of brown adipocytes (Trayhurn and Milner, 1989; Martins et al., 1991; Nedergaard et al., 2001). Uncoupling respiration from phosphorylation provides a basic, thermogenic IBAT function.

Our previous studies demonstrated that the changes of AD in IBAT are specifically dependent on the duration of cold exposure (Spasić et al., 1993). So, acute (6 h) cold exposure was shown to increase MnSOD activity in IBAT (Petrović et al., 1989), while prolonged cold exposure (21 days) acted decreasing the activity of total SOD in rat brain, heart, kidney, liver and small intestine (Kaushik and Kaur, 2003). These data indicate that the changes of redox homeostasis depend on the phase of tissue remodelling. Also, investigations of AD changes in IBAT of ground squirrel (*Citellus citellus*) for the period of hibernation and circannual rhythm demonstrated that the maintenance of redox homeostasis is of utmost significance in the regulation of physiological processes (Buzadžić et al., 1990, 1992, 1998). Moreover, examinations of AD components in IBAT of rats showed that new homeostasis, at redox level, was established only after 40–45 days of cold exposure (Spasić et al., 1993; Korać et al., 2000), although, in numerous reports dealing with this topic, experimental animals were maintained at low temperature for 21 days.

Based on these considerations, the present study was designed to investigate the contribution of several enzymatic and non-enzymatic AD components (CuZn- and Mn-SOD, CAT, GSH-Px, GST and GSH) in establishing redox equilibrium in relation to new metabolic and structural profile in IBAT during prolonged 45-day exposure of adult rat males to cold.

2. Materials and methods

2.1. Animals

Adult (4-month-old) Mill Hill hybrid hooded rat males (*Rattus norvegicus,* Berkenhout 1769) were divided into two groups. One group was maintained at 22±1 °C and the second one in a cold room at 4±1 °C for 45 days. The animals were housed in individual transparent plastic cages with food and water ad libitum. Each experimental group consisted of six rats.

The animals were sacrificed by decapitation, the IBAT dissected out within 3 min after death and thoroughly rinsed with physiological saline to wash out traces of blood. After removal of muscular and white adipose tissues, the IBAT mass was weighed (a Mettler analytical scale at a precision level of 10^{-6} g). The tissue was homogenized (Ultra/Turrax homogenizer, Janke and Kunkel Ka/Werke, Staufen, Germany, 0–4 °C) in 0.25 M sucrose, 0.1 mM EDTA and 50 mM Tris–HCl buffer, pH 7.4, and the homogenates were sonicated (Takada et al., 1982).

2.2. Activity of antioxidative enzymes

Superoxide dismutase activity was determined by the method of Misra and Fridovich (1972), but at 26 °C and expressed in U mg^{-1} protein. Total specific SOD activity and MnSOD activity after the inhibition with 4 mM KCN were measured and then CuZnSOD activity calculated. One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the autooxidation of adrenaline at 26 °C in the volume of 3.2 mL. CAT was assayed as suggested by the supplier (Sigma-Aldrich, St. Louis, MO, USA) and the activity is expressed in μM H_2O_2 min^{-1} mg^{-1} protein. GSH-Px was determined with *t*-butylhydroperoxide as a substrate (Paglia and Valentine, 1967) and the activity is expressed in nM NADPH min^{-1} mg^{-1} protein. GST was measured by the method of Habig et al. (1974) and the activity is expressed in nM GSH used min^{-1} mg^{-1} protein.

2.3. Determination of GSH

Samples of IBAT were deproteinized with 10% sulfosalicylic acid, and total GSH content was measured by an enzyme-recycling assay (Griffith, 1980) and expressed in nmol g^{-1} tissue.

2.4. SDS-PAGE and Western blotting

Proteins were resolved according to Laemmli (1970). Ten micrograms of protein aliquots were boiled and electrophoresed in 15% SDS–polyacrylamide gel, transferred onto a nitrocellulose membrane and incubated with rabbit polyclonal antibody against UCP1 (Sigma-Aldrich Inc.). The incubation with the primary antibody was performed in TBS-T supplemented with 5% BSA at 1:1000 dilution overnight in a 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 revealed using 4-chloro-1-naphthol and hydrogen peroxide as a substrate.

2.5. Detection of apoptosis

Immediately after dissection, the samples of IBAT were fixed in 10% formaldehyde at 4 °C overnight and processed routinely for embedding in paraffin. For immunohistochemical detection of apoptosis by TUNEL labeling of the nuclei showing specific oligonucleotide sequences resulting from DNA strand breaks 5 μm thick IBAT sections were used. Staining was performed with the In Situ Cell Death Detection Kit POD (Roche, Germany) according to the manufacturer's instructions.

2.6. Other assays and statistics

For Bodian's nerve staining (Bodian, 1936), 5-μm-thick IBAT sections were used. Plasma level of free fatty acids (FFA) was determined after Duncombe (1964). Protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin as reference protein. Student's *t*-test was used for data comparison between different groups according to Hoel (1966). The $p<0.05$ level was chosen as the point of minimal acceptable statistical significance.