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EVIDENCE FOR FREE-RADICAL MEDIATED LIPID PEROXIDATION IN RATS AFTER COLD-IMMERSION STRESS

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ABSTRACT

The role of cold immersion stress for the free-radical mediated lipid peroxidation in rat plasma and erythrocytes was studied. A model of cold-induced stress in rats was created. A significant increase of the level of thiobarbituric acid-reactive substances (TBARS) in plasma was found after chilling. Activated catabolic processes and xanthine-oxidase reactions during hypoxia could cause the increase measured in plasma levels of uric acid one hour after cold stress. A decrease in the antioxidant defence measured by uric acid consumption in rat blood plasma took also place already at the 3rd hour after chilling. These effects were not accompanied neither by elevated levels of TBARS, nor by a decrease in the reduced glutathione or elevated ratio of oxidized/total glutathione in erythrocytes. The results indicated that lipid peroxidation was a component of the cold-induced stress in rats.

Key words: cold-induced stress, lipid peroxidation, uric acid, thiobarbituric acid-reactive substances, rats

INTRODUCTION

Every living organism is subjected to different types of stress during his life. One of the most common types of stress is the thermal stress: burns and cold exposures are common accidents and their pathogenesis is not completely studied yet.

Oxygen reactive species and peroxidation of biological substrates are known to be involved in a variety of pathologies. Evidences that peroxidation may play a major role in the thermal stress were found, too. Elevated levels of lipid peroxidation evaluated by the thiobarbituric acid reactive substances (TBARS) and decreased antioxidant defense were demonstrated for red blood cells and plasma in the early post-burn period, due to oxyradical burst from complement-activated polymorphonuclear neutrophil leukocytes (7). Few reports on the involvement of reactive oxygen species in the cold-induced stress in mammals (6), some related to cryopreservation of isolated tissues and organs (4), others to drug administration (8, 9). While the mechanisms of the burn-induced stress are already well-known, those of the cold-induced stress are not sufficiently clarified yet. It seemed interesting to investigate the chilling as a type of thermal stress and its effect on the free-radical mediated lipid peroxidation in rat plasma and erythrocytes.

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MATERIAL AND METHODS

Male Wistar rats weighting 220 ± 35 g were used. The experimental animals were divided into two groups as followed: the first group (n=10) was the cold restraint group in which the cold restraint stress was achieved by immersion of the back of the immobilized animals under anaesthesia with thiopentane sodium (30 mg/kg b.m.) into ice-cold water containing 10 % NaCl (-5°C), for 10 min. The control animals (n=10) were immersed into water (37°C) under the same conditions. Before immersion dorsal skin (20 % of the total body surface area) of all animals was shaved.

Venous blood was drawn at 1st and 3rd hour after cold exposure. After the removing the blood plasma, packed red blood cells were washed three times with saline (pH=7.4). The haemolysis of the washed erythrocytes was performed using distilled water (1:1v/v) and consequent freezing and thawing at -12°C. Plasma and fresh prepared haemolysate was employed immediately for determination of lipid peroxidation and content of antioxidants.

The content of TBA reactive substances was used as a marker for lipid peroxidation in plasma and erythrocyte haemolysates and was measured as described by Porter et al. (5). Malondialdehyde (MDA), an end product of lipid peroxidation, was measured spectrophotometrically at 532 nm and presented as nmol/g lipid. The 1,1,3,3-tetramethoxypropane from Aldrich Chemie (Steinheim, Germany) was used as a standard. Lipids were measured

spectrophotometrically at 530 nm with Biotest (Lachema, Brno, Czech Republic) based on the sulfophosphovanilin reaction.

The levels of glutathione in the reduced (GSH) and the oxidized (GSSG) forms in haemolysate of erythrocytes according to Hissin and Hilf (3), and of uric acid in plasma were determined as compounds of the antioxidant defence. The uric acid was assayed spectrophotometrically by its colour product with phosphowolframic acid (2). The content of glutathione was expressed as mol/g haemoglobin (Hb) of the haemolysate. The haemoglobin content of the haemolysate was measured according to the cyanhaemoglobin method.

Data were presented as the mean SEM and the statistical significance was established by means of the Student's *t* - test.

RESULTS AND DISCUSSION

The levels of TBARS in plasma and erythrocyte haemolysate were measured one and three hours after cold exposure. A significant increase in plasma MDA content ($p<0,05$) was found one and three hours after chilling. Simultaneous measurements of TBA-products in haemolysate did not show any significant changes both one and three hours after the cold exposure (Fig. 1).

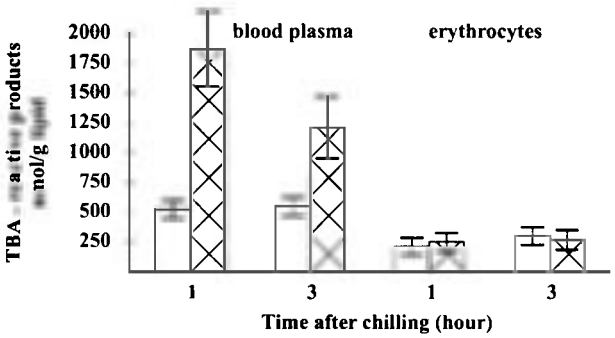

Fig 1. Changes of TBA-reactive products in plasma and haemolysates of erythrocytes in controls (□) and in cold-exposed rats (⊠). = - p<0,05 vs. Controls

Plasma uric acid and glutathione content in haemolysate of erythrocytes (GSH and GSSG/GSH+GSSG) were used to estimate the antioxidant capacity after chilling. One hour after the cold stress no significant changes in the plasma levels of the uric acid were found in the animals exposed to chilling compared to the controls.

Significantly lower content ($p<0.05$) of uric acid in the plasma of the animals exposed to cold was measured at the third hour (Fig. 2). The abovementioned effects were not accompanied by the decrease of the GSH (controls – 11,65 0,83 (mol/gHb); cold stressed animals – 11,92 0,66 (mol/gHb) or elevated ratio of GSSG/GSH+GSSG (controls – 0,365 0,018; cold stressed animals – 0,329 0,032) in haemolysate after cold exposure.

Activated lipid peroxidation and reduced antioxidant capacity after cold stress were more demonstrative in blood plasma than in erythrocytes, opposite to burns, where these changes were as significant in plasma as in erythrocytes (1,8,9). Cold-induced stress causes changes in the microcirculation resulting in ischemia in the early post-cold period. Ischemia is known to be accompanied by activation of xanthine oxidase and enhanced production of uric acid that is in agreement with our results (1st hour after cold exposure). On the other hand, during reperfusion following ischemia reactive oxygen species are actively produced resulting in membrane lipid damage estimated as elevated TBA-reactive substances.

Besides a decrease in the antioxidant defence measured by uric acid consumption in rat blood plasma took place already at the 3rd hour after chilling. Probably, the leading mechanisms of the two types of thermal stress (burning and chilling) are different.

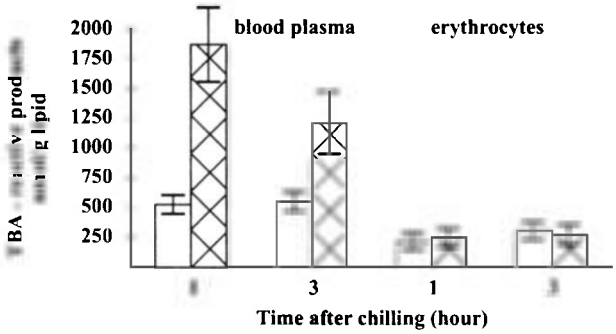

*Fig. 2. Effect of chilling on plasma levels of uric acid in rats. Controls (□) and cold-exposed rats (■). * - p<0,05 vs. Controls*

Oxyradical burst from complement-activated polymorphonuclear leukocytes could be the cause for oxidative alterations in erythrocyte membranes after thermal stress. Damaged erythrocytes, on the other hand, could activate leukocytes. That could explain why changes in the antioxidant defence after burning were measured in plasma and in erythrocytes in the early postburn period (1) while no changes in the content of glutathione in erythrocytes were found after chilling.

It seems likely that the burn stress is the more drastic type of thermal stress and, probably, the more harmful one under conditions of activated lipid peroxidation.

In conclusion, activated free-radical mediated lipid peroxidation after cold stress is demonstrated by increased levels of TBARS in rat blood plasma at the first and third hour after cold exposure. There is a significant decrease of uric acid content in plasma at the third hour after cold exposure. Neither changes in the lipid peroxidation, nor changes of GSSG and GSSG/GSH+GSSG levels are established in erythrocytes after chilling.

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