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THE LEVEL OF CuZn SUPEROXIDE DISMUTASE IN LIVER OF RATS EXPOSED TO ACUTE, CHRONIC OR COMBINED STRESS

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Abstract

The CuZn superoxide dismutase (CuZnSOD) protein expression in liver of Wistar male rats exposed to acute (immobilization or cold), chronic (isolation, crowding or daily swimming) or combined stressors was followed by Western immunoblotting. Relatively increase levels of CuZnSOD in acute stress conditions are required to remove high level of ROS in order to protect against ROS damage in liver. The moderate SOD up-regulation under chronic and combined stress may lead to inefficient ROS defense and increased oxidative damage of liver under the stress conditions In that view, special care should be played to development of antioxidant therapeutics for antagonizing stress induced redox disbalances in cells.

Introduction

It is well known that intensive stress response results in creation of reactive oxygen species (ROS), e.g. hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and superoxide anion radical ($O_2^{\cdot-}$) that can play an important role in tissue injury[1]. It has been suggested that chronic stress and high level of glucocorticoids (GCs), the adrenal steroids secreted during stress, affect diverse processes involve ROS and increase ROS by approximately 10% basally [2]. Also, GCs have been implicated as a regular factor for antioxidant enzymes in peripheral tissues that express corticosteroid receptor. In order to neutralize ROS, living cells possess three protective enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) to help the cells against a variety of oxidative stress. CuZnSOD (EC1.15.1.1) is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2 [3]. However the changes of endogenous defense enzymes in the liver of stressed rats remained obscure. Because liver is a prime target tissue for glucocorticoid action, in the present study, we were investigated the effect of 21 day isolation, crowding or daily swimming as chronic stressors, sole or in combination with 2h acute stress of immobilization or cold (4^{o}C) on the level CuZnSOD protein expression in liver cytosol of Wistar male rats.

Experimental

Adult Wistar rat males, aged three months, weighing 330-400 g, were housed in groups of four individuals *per* cage and offered water and food *ad libitum*. For experimental purposes the animals were randomly divided into five groups. Group I con-

sisted of the unstressed animals (control); Group II contained animals exposed to acute stress by immobilization or cold; Group III was exposed to chronic isolation *i.e.* the rats were individually housed for 21 days; Group IV was exposed to chronic social crowding for 21 days, with eight animals *per* cage; Group V comprising four animals *per* cage was exposed to forced 15 min swimming for 21 day. Chronically stressed animals were subsequently exposed to immobilization or cold (4°C) for 2h. Unstressed controls or stressed animals were sacrificed 2 hrs following the end of the stress procedure. Separation of proteins in liver cytosol was examined by SDS-PAGE and quantification of level CuZnSOD by Western immunoblotting**.** The obtained data were analyzed by two-way ANOVA by Tukey post-hoc test. Statistical significance was accepted at $p<0.05$.

Results and Discussion

Figure 1 (left panel) shows that both acute stressor, immobilization (IM) and cold (C), induced a significant up-regulation of CuZnSOD levels in the liver cytosol ($p<0.05$), in relation to the unstressed controls. Interestingly enough, in spite of the differences in both type and intensity of acute stressors, a similar level of CuZnSOD changes was observed, as judged by the Tukey post-hoc test. The elevated CuZnSOD expression could indicate that the studied stress conditions, either directly or indirectly, act on the liver cell redox-equilibrium by shifting it towards prooxidant state. At least in part, the prooxidant state could result from 3-6 fold elevation of plasma corticosterone (CORT) under different stress conditions, as reported in our previous work [4]. Also, oxidative metabolic pathway of increase concentration of catecholamines may be the reason of elevated protein expression of CuZnSOD under acute stress conditions. Compared to acute stress effects, chronic stress conditions led to a moderate increase in the level of CuZnSOD (middle panels of Fig.1). The Tukey post-hoc test indicated that, within chronic stressors swimming (Sw) led to a significant increase ($p<0.05$) of the level CuZnSOD, relative to the unstressed control. Evaluation of combined stress data indicated that only isolation followed by cold induced significant up-regulation of level CuZnSOD ($p<0.05$; Fig.1, right panel). A moderate up-regulation the CuZnSOD level in chronic and combined stress may be inactivation of SOD by interaction with oxygen radical. Pigeolet et al., [5] reported the inactivation SOD by hydroxyl radical and hydrogen peroxide. The depression of SOD level may result in cellular injury by superoxide radical which could the propagate the chain reaction, producing one hydrogen peroxide and one GSSG for each turn in cycle [6]. As CuZnSOD expression is regulated by GR, moderate protein level of this enzyme may lead to inefficient ROS defense and more pronounced oxidative damage of liver.

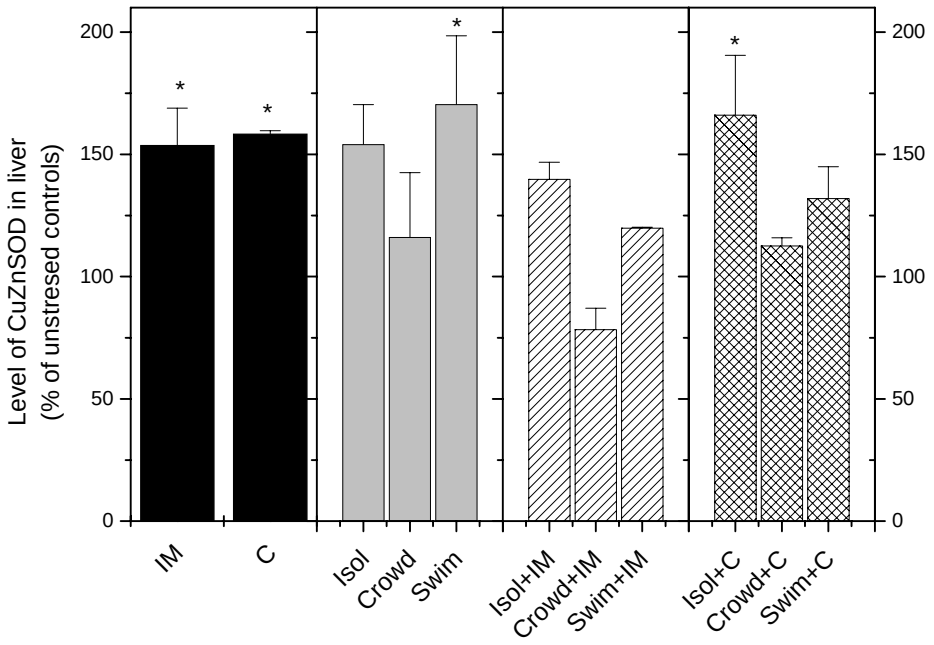

Fig. 1. Relative changes in the level of CuZnSOD in liver cytosol of animals exposed to acute stressors: immobilization (IM) or cold (C); chronic stressors: isolation (Isol), crowding (Crowd) or daily swimming (Swim), or combined stressors (mean±S.E.M, significant differences by Tukey post-hoc test is $^{*}p<0.05$).

Conclusions

Our study indicated that both acute and chronic stressors most probably generate intracellular imbalance between production and elimination of ROS, as judged by differential CuZnSOD protein induction. The presumed stress induced changes in redox equilibrium in liver may be prerequisite in generation and propagation of variety of pathological processes.

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