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ANTIOXIDANT ENZYMES EXPRESSION IN LIVER OF STRESSED WISTAR RAT

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Abstract

The antioxidant enzymes (AOEs) expression was studied in Wistar rat liver under two types of stress: acute (immobilization) and chronic (isolation). The acute stress induced increase in blood corticosterone (CORT) and glucose (GLU), but decreased AOEs expression, and such conditions may result in oxidative stress. In contrast to acute stress, in chronic stress conditions, when both CORT and GLU were low, the AOEs expression was markedly induced. This increase in MnSOD, CuZnSOD, and catalase exhibited similar trend implying efficient detoxification of $O_2^{\cdot -}$ and H_2O_2.

Introduction

Adaptation to neuroendocrine stress involves action of glucocorticoids (GCs), the hormones of hypothalamo-pituitary-adrenal (HPA) axis, which mediate central and peripheral recovery of organism's homeostasis. The main peripheral GCs target is liver, in which GCs stimulate metabolic processes serving to increase and/or maintain normal concentrations of blood glucose, thus providing other organs with energy necessary for successful adaptation [1]. Although beneficial, the increased energy requirements are also followed by the enhanced production of potentially toxic reactive oxygen species (ROS) [2]. The cellular regulatory pathways activated by ROS involve action of antioxidant defence enzymes (AOE) that may efficiently detoxify cells from transiently elevated ROS. However, prolonged stress and constantly high (mM) concentration of ROS may compromise AOE detoxifying capacity of cells, and thus influence energy/glucose production necessary for successful cell/organism adaptation. The aim of the presented study was to characterize AOEs protein expression in Wistar rat liver under different types of stress: acute (immobilization) and chronic (social isolation). Liver enzymes: CuZn-superoxide dismutase (CuZnSOD), Mn-superoxide dismutase (MnSOD), and catalase (CAT), were measured and their expression was correlated with the input signal of blood GCs and the resulting glucose level.

Experimental

Wistar male rats were kept according to the standards of the Ethical Committee for the Use of Laboratory Animals of the VINCA Institute. The experimental groups were: (a) control; (b) acute immobilization, 30 min; (c) chronic isolation, 21 day. After sacrifice, blood serum was prepared by 15 min centrifugation at 3000 rpm and used for corticosterone determination by OCTEIA Corticosterone EIA kit, and

glucose determination by Accutrend strips. Livers were perfused, homogenized, and lysed by ice-cold 1% Triton X-100 in 10mM TrisHCl pH 7.4, containing 0.32M sucrose and 5mM $MgCl_2$, centrifuged at 12,000rpm for 15 min and protein concentration was determined by method of Lowry. Cell extracts were denatured in sodium dodecyl-sulphate (SDS) buffer, separated by 7.5% SDS-polyacrylamide gel electrophoresis, transferred to PVDF membranes, and probed with specific primary antibodies: anti-MnSOD, anti-CuZnSOD, anti-catalase or anti-β-actin, and secondary goat anti-rabbit IgG-HRP conjugate. Quantification of protein bands on X-ray film was performed by Image J analysis PC software. Statistical significance was determined by one-way ANOVA and Tukey's posthoc test.

Results and Discussion

Since stress-induced gluconeogenesis in liver is controlled by the level of corticosterone (CORT) and since it is reflected in the serum level of glucose (GLU), we determined these parameters in Wistar rats subjected to different stress

Table 1 Stress effects on serum corticosterone and glucose level

*Results are presented as mean± SD (n=4), *p<0.05, **p<0.01, ***p<0.001.*

Stress / ***Parameters***	**Control**	**Acute**	**Chronic**
Corticosterone (ng/ml)	136,80±44,51	626,94±107,08***	64,73±28,29**
Glucose (mmol/l)	5,67±0,80	8,08±0,66 ***	3,40±0,71***

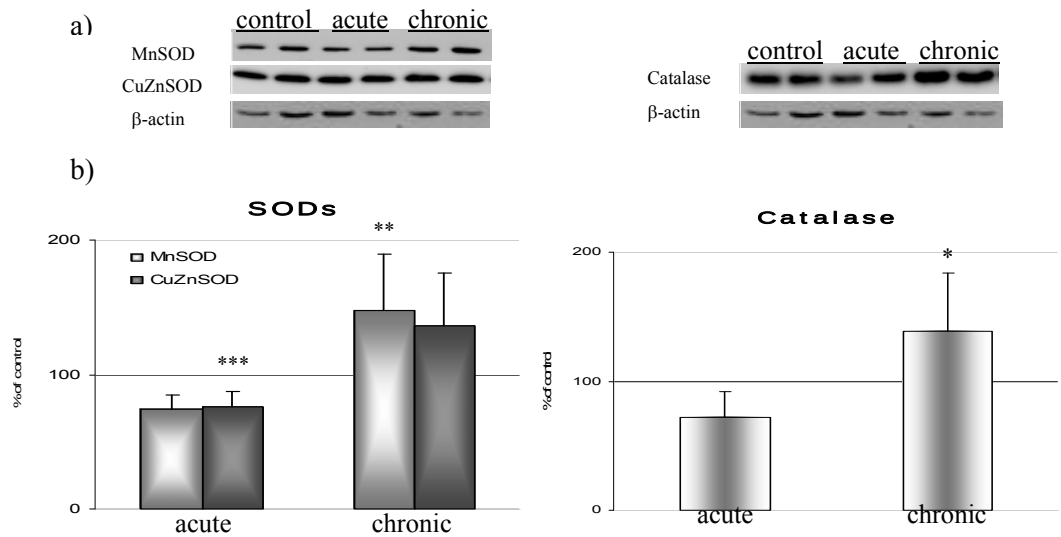

Fig. 1 Stress effects on antioxidant enzymes expression measured by Western blot (a) and quantified by Image J analysis PC software (b). Results are presented as mean± SEM (n=4), *p<0.05, **p<0.01, ***p<0.001.

conditions. The results indicated that in acute stress when the serum CORT was high the concentration of blood GLU was also increased (Table 1). Under the chronic stress both CORT and GLU concentration were below the control level (Table 1). Quantification of protein expression of the antioxidant enzymes (AOEs): Mn-and CuZn-superoxide dismutases (MnSOD and CuZnSOD), and catalase (CAT) indicated that all three enzymes were increased in chronic stress, but unaltered or lowered in acute stress (Figure 1). It is known that CORT triggeres increase in the metabolic rate (respiration, syntesis of ATP) required for gluconeogenesis which would result in the equal increase in ROS. Discrepancy between ROS generation due to increased metabolism and decreased antioxidant capacities may result in the state of oxidative stress. Since the increased expression of AOEs is well known indicator of elevated ROS our data suggest that state of oxidative stress may also exist under low CORT conditions. The observed increase in MnSOD, CuZnSOD, and CAT in chronic stress exhibited similar trend which may imply efficient detoxification of $O_2^{\cdot -}$ and H_2O_2 under these conditions. The apparent discrepancy between the stress intensity defined by CORT level, and oxidative stress, defined by AOEs expression, may be due to downregulation of AOEs by glucocorticoid receptor [3] or to altered activity of AOEs due to inhibitors, such as NO [4].

Conclusion

The increase in blood glucose (GLU) due to stress-induced elevation of CORT (*i.e.* increased rate of gluconeogenesis) in rat liver is not followed by the respective increase in antioxidant enzymes (AOEs) expression. AOEs expression is markedly elevated in chronic stress, when both CORT and GLU are low. This increase in MnSOD, CuZnSOD, and catalase exhibited similar trend implying efficient detoxification of $O_2^{\cdot -}$ and H_2O_2.

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References

[1] H. DeWulf, H.G. Hers, European J. Biochem., 1967, **2**, 57-60.
[2] N.Lane, J.Theoretical Biol., 2003, **225**, 531-540.
[3] H.T. Kim, Y.H. Kim, J.W. Nam, H.J. Lee, H.M. Rho, G. Jung, Biochem. Biophys. Res. Commun. 1994, **201,** 1526–1533.
[4] Y.S.Kim, S.Han, Biol.Chem., 2000, **381**, 1269-1271.