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FLUOXETINE DECREASES GLUTATHIONE REDUCTASE IN ERYTHROCYTES OF CHRONICALLY ISOLATED WISTAR RATS

M. Adzic [a] *, A. Djordjevic [a], J. Djordjevic [a], I. Elakovic [b], I. Simic [a], M. Mitic [a], G. Rackov [a], G. Matic [b] and M. Radojcic [a]

[a] *Department of Molecular Biology and Endocrinology, VINCA Institute of Nuclear Sciences, Belgrade, Serbia,* [b] *Department of Biochemistry, Institute for Biological Research "Siniša Stanković", University of Belgrade, 142 Despot Stefan Blvd., 11060 Belgrade, Serbia*

Abstract

Alterations in the antioxidative defense parameters upon chronic stress are considered critical for pathophysiology of stress related psychiatric disorders and their status in blood serves as biomarker for effects of pharmacological treatments. We investigated the modulation of erythrocyte antioxidant enzymes (AOEs): superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GLR) protein expression and activity in Wistar male rats subjected to chronic psychosocial isolation and/or treated with fluoxetine. Chronically isolated animals exhibited decreased levels of plasma corticoserone (CORT). AOEs status was not altered either by chronic social isolation or by fluoxetine. The only exception was GLR, whose level and activity were both markedly reduced by fluoxetine. Our study indicates that fluoxetine treatment of chronically isolated male Wistar rats, leads to significant reduction in the level and activity of GLR in the erythrocytes.

Introduction

Oxidative stress is defined as the imbalance between production of reactive oxygen species (ROS) and the activity of antioxidant defense system [1]. During chronic exposure to neuroendocrine stress, alterations in antioxidative defense are considered to be one of critical conditions promoting pathophysiology of stress induced psychiatric disorders. Treatment with fluoxetine (selective serotonin reuptake inhibitor, SSRI) can reverse and prevent oxidative damage induced by stress, due to elevation of antioxidative defense [2]. Thus, alterations of antioxidant parameters, particularly in blood, can serve as valuable biomarker to follow effects of chronic stress and/or pharmacological treatment of stress induced psychiatric disorders [3]. Therefore, the present study was designed to examine the pharmacological modulation of antioxidant enzymes by fluoxetine in the erythrocytes of Wistar male rats stressed by chronic psychosocial isolation.

Experimental

Animals, adult male Wistar rats, were divided into four experimental groups: control group; group II animals were subjected to chronic psychosocial isolation for 21 days; group III was subjected to chronic isolation stress for 21 days and treated with distilled water for another 21 days (vehicle), group IV was subjected to chronic isolation for 21 days and treated with fluoxetine for another 21 days.

For Western blot, erythrocyte lysates were prepared according to Laemmli [4], and 30 µg of proteins were subjected to electrophoresis (SDS-PAGE). Western blot technique was performed using following antibodies: anti-β-actin anti-CuZnSOD, anti-catalase (CAT), anti-GPx, anti-glutathione reductase (GLR), to detect β-actin, CuZnSOD, CAT, GPx and GLR, respectively.

Total SOD activity in the erythrocytes lysates was determined using a commercial kit. Catalase activity was determined according to Claiborne [5]. Activity of glutathione peroxidase was determined according to Maral *et al.* [6] and activity of glutathione reductase was measured according to Glatzle *et al.* [7]. Activities of AOEs were expressed as unit per mg of proteins.

Results and Discussion

The corticosterone (CORT) levels were only significantly decreased in animals subjected to chronic social isolation for 3 weeks (Table 1, *p<0.05). In all other treatments, the CORT levels remained unaltered.

Table 1. Serum corticosterone concentration (ng/ml) in the experimental groups. Data are presented as mean ± SD. *p<0.05 *vs* control.

	Control	Chronic stress	Chronic stress/Vehicle	Chronic stress/Fluoxetine
Corticosterone (ng/ml)	84.6±16.8	45.8±8.8 *	78.1±22.4	66.7±20.1

Antioxidant enzymes activities

The only significant change was observed in decreased activity of glutathione reductase (GLR) in chronically stressed animals treated with fluoxetine, both in respect to the control and chronically stressed animals that received distilled water (Table 1, *p<0.05, *vs* control, $^{\#}$p<0.05 *vs* chr+veh).

Table 2. Antioxidant enzyme activities in the control and treated animals.

Enzyme	Control	Chronic stress	Chronic stress/Vehicle	Chronic stress/Fluoxetine
CuZnSOD	15.7±3.1	18.0±3.8	15.4±3.1	16.5±3.0
Catalase	233.8±32.8	239.8±34.5	215.1±20.8	225.2±46.8
Glutathione peroxidase	149.2±16.3	172.4±29.2	176.7±32.5	166.0±30.5
Glutathione reductase	56.5±7.1	46.6±2.9	52.1±6.22	29.3±3.7 * #

Level of antioxidant enzymes in the rat erythrocytes

The only change was observed in the protein level of GLR in chronically stressed animals treated with fluoxetine (Fig. 2, *p<0.05, *vs* control, #p<0.05 *vs* chr+veh) where its level was decreased. The consequences of reduced levels of GLR under fluoxetine treatment may be multiple. Namely, GLR is required for the stability and integrity of the red cells, while its decrease was connected induced haemolytic anaemia, eye cataracts, and its reduction may compromise haemoglobin function and consequently affect oxygen transport [8].

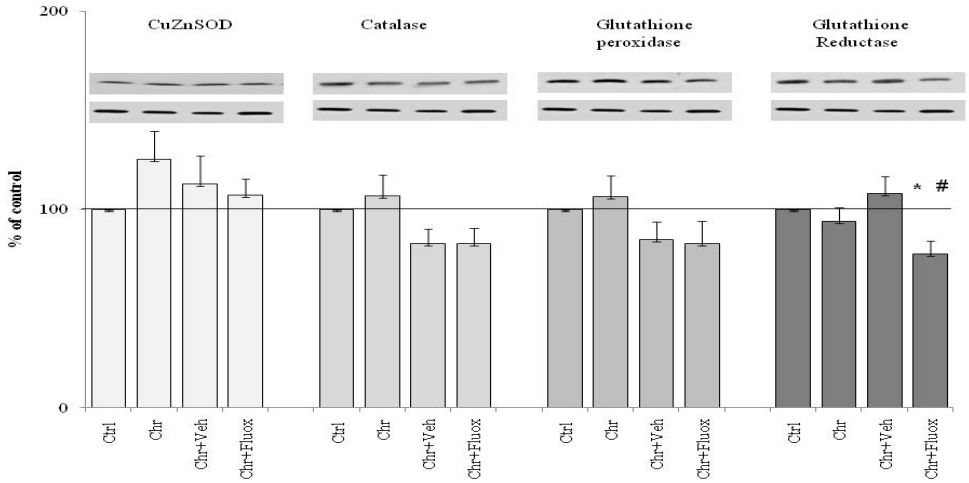

Fig.2. Representative Western blot of CuZnSOD, Catalase, Glutathion peroxidase and Glutathion reductase and their relative levels in the erythrocytes of control (Ctrl), chronically stressed (Chr), chronically stressed and vehicle treated (Chr+Veh) and chronically stressed and fluoxetine treated (Chr+Fluox) Wistar rats Data are presented as mean ± SD of 10-11 animals per group.

Conclusion

Fluoxetine treatment significantly reduced the level and activity of glutathione reductase in the erythrocyte of chronically isolated male Wistar rats, potentially disturbing overall oxido-reductive balance in those cells and organism as whole.

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