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COMPROMISED GLUTATHIONE-DEPENDENT REDOX SYSTEM OF CHRONICALLY-ISOLATED RATS: A HARMFULL SIDE EFFECT OF CLOZAPINE

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ABSTRACT

Changes in glutathione (GSH)-related systems are implicated in depressive disorders. Since chronic psychosocial stress contributes to depression, we investigated the effects of 21d of chronic social isolation (CSIS) stress, an animal model of depression, as well as chronic administration of clozapine, an atypical antipsychotic, on GSH content, glutathione peroxidase (GPx) and glutathione reductase (GLR) in the prefrontal cortex of rats. Increased GPx protein expression and its activity in clozapine-treated (controls or chronically-isolated) rats as well as in CSIS group were found. Nonetheless, clozapine administration caused decrease in GSH content but no effects on GLR in controls and CSIS group. Data indicate that CSIS compromises GSH-dependent redox system promoting oxidative stress in rat prefrontal cortex which can't be protected by clozapine. Moreover, clozapine administration in controls has a harmful side effect on this redox system.

INTRODUCTION

Chronic psychosocial stress, as a risk factor of depression, may disturb redox-status in the cell, causing oxidative stress. To maintain redox-homeostasis and counteract to oxidative stress, cells use antioxidant defense system such as glutathione (GSH)-dependent redox system. This system includes GSH, GPx that eliminates peroxides with a concomitant oxidation of GSH to GSSG, and GLR which catalyses reduction of GSSG to GSH. Compromised GSH associated with oxidative stress in the brain has been shown as feature of depression [1]. Based on our previous finding that 21d of chronic social isolation (CSIS) stress on male Wistar rats induces depressive- and anxiolytic-like behaviours [2], we treated chronically-isolated animals with antipsychotic clozapine because recent study has suggested that some atypical antipsychotic drugs may have protective properties against oxidative stress [3]. Clozapine is an atypical antipsychotic effective in treating depressive patients who are resistant to typical antipsychotic drugs [4]. Hence, we investigated the effects of social CSIS,

on GSH content, GPx and GLR protein expression and its activity, in the rat prefrontal cortex and role of clozapine (21d) in rectifying possible changes. The prefrontal cortex was chosen for investigation as it is included in stress response and susceptible to a neurochemical and structural changes under the chronic psychosocial stress as those observed in mental illness.

EXPERIMENTAL

Adult male Wistar rats, 2.5 months old at the onset of the experiment, served as subjects. Control groups consisted of four animals per cage, while rats underwent CSIS were housed individually for 21d. Clozapine was administered daily by intraperitoneal (i.p.) injections of 20 mg/kg during the 21d in both control (Con+Cloz) and chronically-isolated (CSIS+Cloz) groups. We already reported that chronically administration of 20 mg/kg/day of clozapine in rats produces a serum drug level therapeutically relevant in humans [5]. Vehicle-treated rats received daily i.p. injections of 0.9% NaCl (Con+NaCl and CSIS+NaCl groups). Isolated prefrontal cortexes were subcellular fractionated to prepare cytosolic tissue protein extracts. GPx and GLR protein expression was investigated by Western blot, while GSH content was determined by spectrophotometric method [6]. GPx activity was determined using Randox commercial kit [7], while GLR activity was measured by method of Halliwell and Foyer [8]. Results were analyzed by two-way ANOVA followed by Duncan's post-hoc test. The data are expressed as mean ± S.E.M. of 5-6 animals per group.

RESULTS AND DISCUSSION

The relative changes in GPx and GLR protein expression are presented in Figure 1, while GSH content as well as GPx and GLR activity are presented in Table 1.

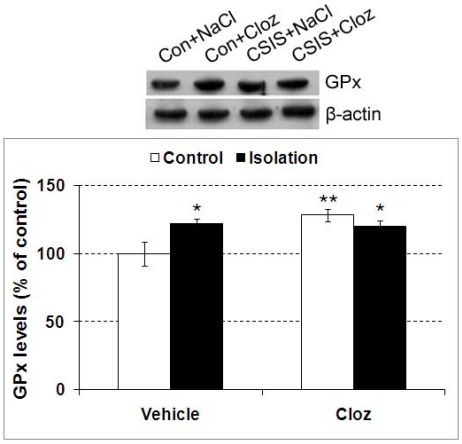
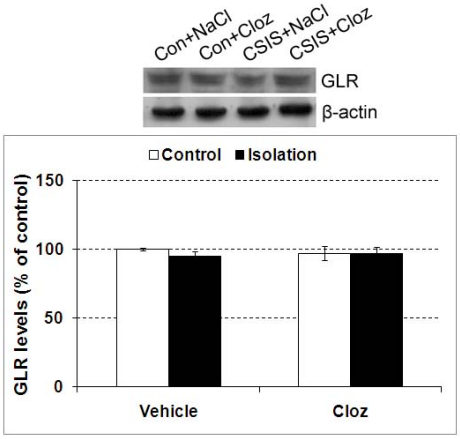


Figure 1. Cytosolic GPx and GLR protein expression in the prefrontal cortex of controls and chronically-isolated male rats treated either with vehicle (0.9% NaCl) or 20 mg/kg/day of clozapine, respectively.



Significant differences are indicated as follows: *p<0.05, **p<0.01 clozapine treatment or/and isolation *vs* vehicle-treated controls.

Table 1. Prefrontal cytosolic GSH content, GPx and GLR activity in controls and chronically-isolated rats treated with either 0.9% NaCl or 20 mg/kg/day of clozapine. Asterisks indicate comparison between: clozapine treatment or/and isolation *vs* vehicle-treated controls, *p<0.05, **p<0.01

Groups	GSH levels (nmol/mg protein)	GPx activity (mU/mg protein)	GLR activity (mU/mg protein)
Con + NaCl	68.78 ± 3.90	43.48 ± 0.57	24.70 ± 0.45
Con + Cloz	54.95 ± 2.35 **	52.30 ± 2.49 **	24.23 ± 1.35
CSIS + NaCl	60.25 ± 2.89 *	51.42 ± 2.05 **	24.61 ± 1.14
CSIS + Cloz	54.03 ± 0.58 **	51.55 ± 1.54 **	22.59 ± 1.57

Significant decrease of GSH content in CSIS group as compared to controls (*p < 0.05) may be consequence of increased oxidative stress as well as increased GPx activity that uses the GSH for the catalytic reduction of hydrogen peroxide. Increased GPx protein expression (*p<0.05) and its activity (**p<0.01) in CSIS rats might contribute to protection of the prefrontal cortex from CSIS-induced oxidative stress. Despite GSH consumption during CSIS, unchanged protein expression and activity of GLR may diminish GSH recycling due to inability of GLR to compensate increased GSH consumption by GPx. Clozapine treatment alone, as well as in combination with CSIS, caused oxidative stress judged by increased GPx protein expression (**p < 0.01, *p < 0.05) and its activity (**p < 0.01) associated with decreased GSH content (**p < 0.01) and unaltered GLR protein expression and activity. The present findings concur with previous

obtained results in the liver where clozapine administration used here (21d) reinforce hepatic oxidative stress in chronically-isolated rats, judged by increased nitric oxide and lipid peroxidation, GSH depletion and caused hepatotoxicity [5]. This implies that chronic administration of clozapine, in addition to the liver, also compromises GSH redox system in the prefrontal cortex and failed to protect this structure from oxidative stress following CSIS, moreover it caused the same.

CONCLUSION

Results demonstrate presence of oxidative stress following CSIS in the rat prefrontal cortex, as evidented by GSH depletion, increased GPx and unchanged GLR protein expression and its activity. Chronic administration (21d) of 20 mg/kg/day of clozapine failed to prevent oxidative stress in chronically-isolated rats and compromised GSH-dependent redox system in controls, as well. Although in terms of efficacy versus typical antipsychotic, clozapine remains an unparalleled choice, our finding together with clozapine study, which demonstrated oxidative cell injury in the brain via increased levels of membrane lipid peroxidation and total protein oxidation [9], confirms that clozapine should be considered as a second choice drug in depressive therapy.

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