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Physical Chemistry

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FLUOXETINE FAILED TO PREVENT ISOLATION-INDUCED CHANGES OF GLUTATHIONE-DEPENDENT DEFENSE IN RAT HIPPOCAMPUS

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ABSTRACT

Chronic exposure to psychosocial stress is implicated in the pathophysiology of depression. We investigated the effect of 21d of chronic social isolation (CSIS) stress (an animal model of depression) and/or chronic administration of fluoxetine (15 mg/kg/day), an antidepressant, on GSH content, protein expression and activity of glutathione peroxidase (GPx) and glutathione reductase (GLR) in the cytosolic fraction of rat hippocampus. CSIS stress caused reduced GPx and GLR protein expression which was not prevented with fluoxetine treatment. Moreover, fluoxetine administration intensified reduction of these proteins expression. Decreased GSH content, GPx and GLR activity was also found in chronically-isolated animals (vehicle- or fluoxetine treated). Data indicate that fluoxetine not only failed to prevent CSIS-induced changes but itself compromised GSH-dependent defense system in control animals.

INTRODUCTION

Psychosocial stress leads to oxidative stress in the brain that contributes to the development of mental disorders [1]. Chronic social isolation (CSIS) represents a naturalistic type of stress in rodents that has been shown to produce behavioral and structural changes, similar to human depression [2]. Fluoxetine is an antidepressant which belongs to the selective serotonin reuptake inhibitor, a class of drugs used for the treatment of depression [3]. Literature data have shown that fluoxetine protects the hippocampus against the adverse effects of stress-related mental illnesses such as maternal separation followed by social isolation [4]. We have previously published that CSIS causes oxidative stress in rat hippocampus compromising antioxidative activity of MnSOD [5]. Major component of the first line antioxidative defense system is glutathione (GSH) which plays central role in maintaining physiological redox status. GSH is a substrate for glutathione peroxidase (GPx), enzyme which reduces peroxides and converts GSH to its oxidized form (GSSG). Reduction of GSSG back to GSH is catalyzed by

glutathione reductase (GLR). The aim of this study was to examine the influence of CSIS stress on GSH-dependent defense system functioning and possible ability of antidepressant fluoxetine to prevent eventual CSIS-induced detrimental changes.

EXPERIMENTAL

Adult male Wistar rats, 2.5 months old, were divided on control (unstressed) group that was comprised of four animals per cage and chronically-isolated rats that were housed individually for 21d, deprived of any visual or tactile contacts with other animals. Fluoxetine-hydrochloride was administered daily by intraperitoneal (i.p.) injections of 15 mg/kg during the 21d in both control (Cont+Fluox) and chronically-isolated (CSIS+Fluox) rats. This dose of fluoxetine produced serum drug concentrations that correspond to those reported in human patients treated with therapeutically effective doses [6]. Vehicle-treated (Cont+NaCl and CSIS+NaCl) groups received daily i.p. injections of normal saline (0.9% NaCl). Cytosolic fractions of hippocampus were used for determination of biochemical parameters. GSH content was measured according to Ellman's method modified by Hissin and Hilf [7]. GPx and GLR protein expression was monitored by Western blot, while activity of these enzymes was determined by spectrophotometric assay [8, 9]. Data are expressed as mean ± S.E.M. of 5-6 animals per group and analyzed by two-way ANOVA followed by Duncan's post-hoc test.

RESULTS AND DISCUSSION

The relative changes in GPx and GLR protein expression are presented in Figure 1.

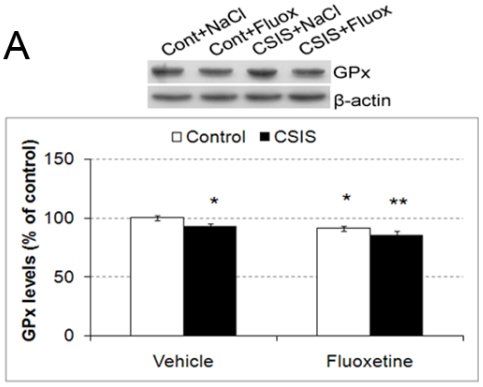
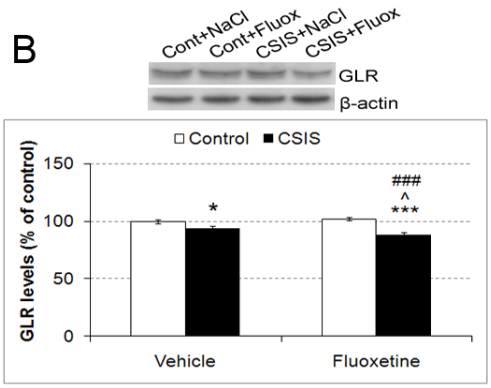


Figure 1. Hippocampal GPx (A) and GLR (B) protein of controls and chronically-isolated rats treated with saline or fluoxetine. Symbols indicate differences between: treated group and Cont+NaCl $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; CSIS+Fluox and CSIS+NaCl $^{\wedge}p<0.05$; CSIS+Fluox and Cont+Fluox $^{\#\#\#}p<0.001$.



GPx protein expressions were reduced in fluoxetine-treated rats (both controls and chronically-isolated) ($^{*}p<0.05$, $^{**}p<0.01$) and CSIS group alone ($^{*}p<0.05$) compared to vehicle-controls (Figure 1A). The levels of GLR were decreased in chronically-isolated rats (vehicle- and fluoxetine-treated) ($^{*}p<0.05$, $^{***}p<0.001$) (Figure 1B). Although fluoxetine alone didn't cause change in GLR level in controls, its intensified reduction of GLR expression following CSIS, as compared to CSIS stress alone ($^{\wedge}p<0.05$). Fluoxetine-induced depletion of GSH content in controls ($^{**}p<0.01$) may be the result of reduced GLR activity ($^{***}p<0.001$) (Table 1). Moreover, decreased GPx activity noted in these animals ($^{*}p<0.05$) may suggest either enzyme inactivation by reactive oxygen species or already decreased GSH availability. Significantly decreased GSH content, as well as activity of GPx and GLR, which was in accordance with their reduced protein expression in chronically-isolated rats ($^{**}p<0.01$), suggest that CSIS compromised GSH-dependent defense system in the hippocampus promoting prooxidative state. We already reported that rats exposed to the CSIS used here (21d) (vehicle- or fluoxetine treated) showed a decrease of GSH in the liver whereby fluoxetine caused more pronounced changes in chronically-isolated animals than in controls [6]. These data lead to the conclusion that, in addition to the liver, fluoxetine also compromises GSH regulation in the hippocampus.

Table 1. Hippocampal GSH, GPx and GLR activity in controls and CSIS treated with saline or fluoxetine. Symbols indicate differences between: treated group and Cont+NaCl $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$; CSIS+Fluox and CSIS+NaCl $^{\wedge\wedge\wedge}p<0.001$; CSIS+Fluox and Cont+Fluox $^{\#\#\#}p<0.001$.

Groups	GSH levels (nmol/mg protein)	GPx activity (mU/mg protein)	GLR activity (mU/mg protein)
Cont+NaCl	67,24 ± 0,95	47,01 ± 1,60	51,96 ± 0,84
Cont+Fluox	58,32 ± 3,93 **	40,71 ± 3,02 *	45,21 ± 0,85 ***
CSIS+NaCl	57,45 ± 0,77 **	38,89 ± 1,89 **	47,28 ± 1,58 **
CSIS+Fluox	56,72 ± 1,13 **	36,36 ± 1,54 **	38,41 ± 0,91 *** ^^^ ###

Moreover, chronic fluoxetine treatment failed to prevent CSIS-induced reduction of abovementioned parameters. In addition, fluoxetine treatment of chronically-isolated rats resulted in even more decreased GLR activity comparing to CSIS alone ($^{\wedge\wedge\wedge}p<0.001$), indicating that this antidepressant failed to prevent CSIS-induced compromise of GSH-dependent antioxidative defense but itself has harmful effects on rat hippocampus.

CONCLUSION

CSIS stress increased susceptibility of rat hippocampus to oxidative stress by compromising GSH-dependent defense system. Treatment with antidepressant fluoxetine didn't prevent this detrimental effect, moreover, its administration in controls disturbed functioning of this system as well.

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