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FLUOXETINE DECREASES THE LEVEL OF NUCLEAR GLUCOCORTICOID RECEPTOR IN WISTAR RAT HIPPOCAMPUS UNDER CHRONIC STRESS

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Abstract

Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis has been implicated in the pathophysiology of depression and stress disorders. Glucocorticoids, key regulators of stress response, have diverse effects on cellular processes in the hippocampus. Beside non genomic pathways, glucocorticoids effects are mediated through activation of the glucocorticoid receptor (GR), a ligand activated transcriptional factor that belongs to the nuclear hormone receptor superfamily. We analysed the GR protein level both, in the cytoplasmic and nuclear compartments in Wistar rat hippocampus, exposed to 3 week social isolation stress upon chronic fluoxetine treatment. Under chronic stress, corticosterone level was decreased compared to the control and treatment with fluoxetine did not change its level significantly in stressed animals. At the molecular level, fluoxetine significantly decreased the level of nuclear GR protein in the brain hippocampus of the chronically stressed rats. Fluoxetine reversed the nuclear level of GR disrupted by chronic psychosocial isolation (CPSI), but it failed to normalize HPA axis activity.

Introduction

Stress disorders are characterized by a reduced ability of the brain to cope and adapt under challenging conditions [1]. Activation of the hypothalamic-pituitary-adrenocortical (HPA) axis mediates physiological responses that enable an organism to maintain or return to homeostasis. The end product of HPA axis activation is synthesis and secretion of glucocorticoids from the adrenal cortex thus enablening coping with stress that is essential for survival [2]. The magnitude and duration of stress responses are controlled, in large part, by glucocorticoid receptor (GR), which provides feedback signals that inhibit the activity of HPA axis [3]. The GR is a member of nuclear hormone receptor superfamily of the ligand-activated transcription factors. Along this line, the aim of this study has been to investigate the ability of chronic SSRI antidepressant fluoxetine treatment to modulate the responsiveness of GR under challenging conditions. Accordingly, we have examined its changes in response to the chronic 3 week CPSI. We focused our analysis on the effects of fluoxetine treatment on the cytoplasmic and nuclear distribution of GR in the rat hippocampus, a brain structure that is affected in animals exposed to chronic psychosocial isolation [4].

Experimental

The experiments were performed on adult (3-months old) male albino Wistar rats that were divided into four groups: control group consisted of unstressed animals treated with vehicle for 3 weeks VEH;water); group II consisted of unstressed animals treated with fluoxetine 3 weeks (FLU); Two other groups (groups III and IV) consisted of 3 weeks chronically isolated animals that were further treated with either vehicle or fluoxetine for another 3 weeks. Blood from each animal was collected at the time of sacrifice. Corticosterone (CORT) concentration was determined from blood serum by using the OSTEIA Corticosterone EIA kit according to manufacturers instructions (American Laboratory Products Co.). The cytoplasm and nucleus samples were prepared by differential centrifugation and western blot technique was performed using following antibodies: M20 (Affinity Bioreagents) and rabbit polyclonal anti-β-actin as a loading control.

Results and discussion

Effect of stress and fluoxetine on CORT level: To asses the effectiveness of the stress paradigm adopted, the serum CORT was first measured. Even tough, two-way ANOVA showed a significant relevance of chronic psychosocial isolation stress on CORT concentration, post-hoc tukey analysis did not reveal any differences between experimental groups due to high variations in individual values of corticosterone. These results showed that the responsiveness of the HPA axis was not affected either in control or in stressed groups by fluoxetine.

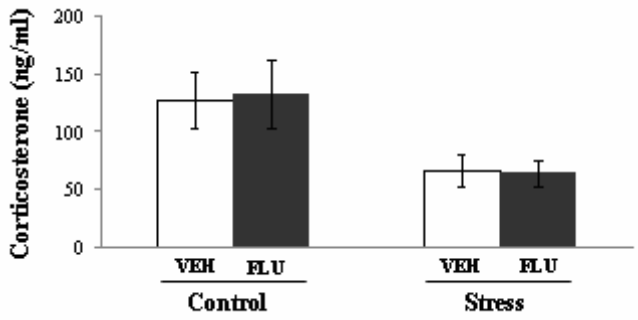

Fig.1. Serum corticosterone concentration (ng/ml) in rats treated for 3 weeks with vehicle (VEH) or fluoxetine (FLU) either in basal condition (control) or exposed to 3 weeks chronic psychosocial isolation (stress). Values are presented as mean ± SEM and measured individually.

*Effect of stress and fluoxetine on GR level***:** Furthermore, as shown in Figure 2a, GR levels in the cytosolic compartment were not affected either by stress ($F=0.005$, $p>0.05$) or by FLU treatment ($F=0.795$, $p>0.05$). In contrast, the GR level in the nucleus was significantly increased by CPSI in respect to the control ($F=21.691$, $p<0.05$) and its level was reversed by additional fluoxetine treatment ($F=26.031$, $p<0.05$).

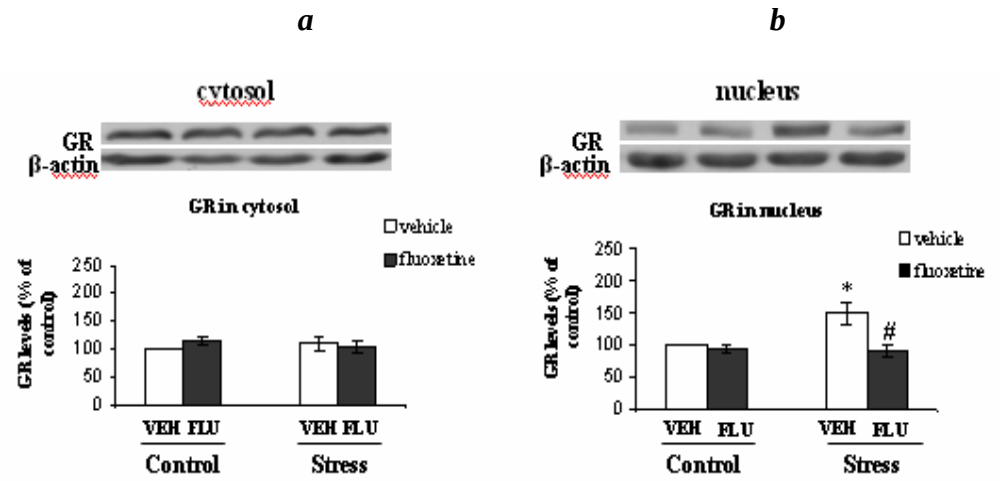

Fig.2. Western blot (WB) experiment demonstrating the effect of chronic social isolation stress on glucocorticoid receptor protein levels in the cytosolic (a) and nuclear (b) compartments obtained from the hippocampus of rats chronically treated with fluoxetine (FLU;5 mg/kg) or vehicle (VEH). Data are analysed with two-way ANOVA followed by post-hoc Tukey test and presented as a percentage of control values (unstressed animal treated with VEH, set as 100%). Results are presented as mean ± S.E.M, *$p<0.05$ Stress vs Control,# $p<0.05$ VEH stress vs FLU stress.

Conclusion

Fluoxetine reversed the nuclear level of GR disrupted by chronic psychosocial isolation, but it failed to normalize HPA axis activity. The changes in nuclear GR trafficking observed in fluoxetine treatment of chronically isolated animals might have impact on the regulation of several hippocampal genes and therefore on the coping responses to a challenging conditions.

Acknowledgements

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