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CORTICOSTERONE LEVEL ALTERS OPTIMAL HEAT SHOCK PROTEIN 90/GLUCOCORTICOID RECEPTOR RATIO IN HIPPOCAMPUS OF STRESSED RATS

A. Đorđević, M. Adžić, J. Đorđević and M.B. Radojčić

VINCA Institute of Nuclear Sciences
P.O. Box 522, 11001 Belgrade, Serbia

Abstract

Hippocampus (HIPPO) is one of the key brain structures, rich in glucocorticoid receptor (GR), a transcriptional factor involved in negative feedback of hypothalamic-pituitary-adrenal (HPA) axis in response to stress. Heat shock proteins accompany GR maintaining its optimal conformation, ligand binding ability and translocation to the nucleus. In order to evaluate the expression of GR, Hsp90 and their ratio we exploited three diverse types of stress (acute immobilization, chronic isolation and combination of the two). Our results indicated the same pattern of expression and compartmental distribution for both proteins, as well as for their ratio, under acute and combined stress when the level of corticosterone (CORT) was high. On the contrary, when CORT was low, such as in chronic stress, Hsp90/GR ratio exhibited opposite pattern of expression and GR was not translocated to the nucleus.

Introduction

The hypothalamic-pituitary-adrenal (HPA) axis plays a primary physiological role in response to stress. One of the key brain structures that regulates HPA axis is the hippocampus (HIPPO), a part of the brain "limbic system", abundant with glucocorticoid receptor (GR) [1]. GR is a hormone dependent transcriptional factor which mediates final effects of glucocorticoids. Under no stress conditions, GR is primarily located in the cytoplasm where it is associated with different heat shock proteins (HSPs) including Hsp90 [2]. Hsp90 predominantly resides in cytoplasm maintaining optimal GR conformation for ligand binding. After hormone binding, Hsp90 dissociates from the receptor, enabling its translocation to the nucleus. Nuclear GR regulates stress responsive genes and activity of HPA axis [3]. Moreover, Hsp90 is also found in the nucleus, alone, or in association with GR, where it may be involved in negative regulation of GR activity through removing receptor from its responsive elements [4]. Considering that fact, disrupted nuclear Hsp90/GR ratio or elevated nuclear Hsp90 level may be implicated in GR inactivation. Given the essential role of Hsp90 in GR translocation the aim of this work was to obtain information about GR and HSPs co-expression levels in the cytoplasm and nucleus of animals subjected to diverse types of stress.

Experimental

All experiments were performed on adult Wistar male rats kept according to the standards of the Ethical Committee for the Use of Laboratory Animals of the

VINCA Institute. The experimental groups were: (I) control group; (II) acute immobilization, 30 min; (III) chronic isolation, 21 day; (IV) combined stress-chronic isolation for 21 day followed by 30 min immobilization. After sacrifice, blood serum was prepared and used for corticosterone determination by OCTEIA Corticosterone kit. Cytoplasmic and nuclear extracts from HIPPO were obtained by differential centrifugation. Proteins were subjected to electrophoresis and detected by Western Blot. GR M-20 antibody was used to detect GR, Hsp90 (F-8) antibodies (Santa Cruz Biotechnology) were used for detection of Hsp90. β-actin was used as a loading control.

Results and Discussion

Glucocorticoid receptor: High corticosterone (CORT) levels found upon acute immobilization (626.94±107.08 ng/ml) or in combined stress (601.24±89.69 ng/ml) resulted in significantly reduced level of cytoplasmic and increased level of nuclear GR in hippocampus (HIPPO) ($p<0.001$, Figure 1a), implicating CORT dependent GR nuclear translocation. However, after chronic stress, both the cytoplasmic and nuclear levels of GR were reduced ($p<0.001$, Figure 1a), indicating decrease in GR expression and/or stability under low CORT conditions (64.73±28.29 ng/ml) found in prolonged isolation.

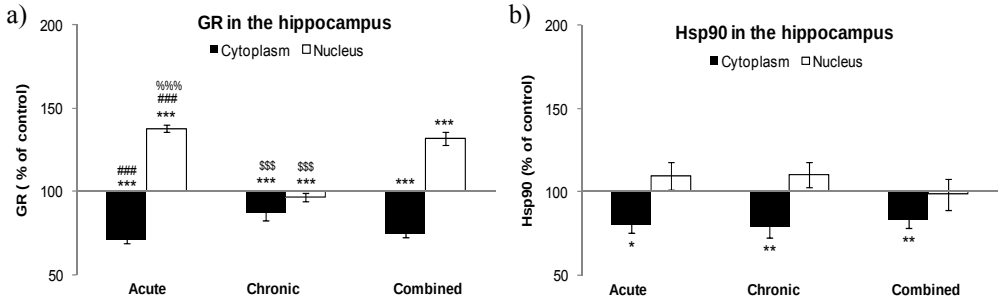

Fig. 1 Hippocampal GR (a) and Hsp90 (b) expression in cytoplasm and nucleus of rats subjected to acute, chronic and combined stress (data presented as mean± SEM, n=6)

Heat shock protein 90: The analysis of Hsp90 protein indicated that its pattern of expression in the cytoplasmic compartment was similar to the expression level of GR. Namely, in all three types of stress the cytoplasmic Hsp90 protein levels were decreased ($p<0.05$, $p<0.01$, respectively, Figure 1b). As it may be observed in Figure 1b, upon acute and chronic stress, Hsp90 was translocated to the nuclear compartment, judged by its nuclear increase. In case of combined stress nuclear level of Hsp90 remained unchanged.

Hsp90/GR ratio: Under the high serum CORT conditions Hsp90/GR ratio was elevated in cytoplasm and decreased in the nuclear compartment. Although both GR and Hsp90 were translocated to the nucleus under these conditions, it seemed that GR translocation exceeded the one of Hsp90 (Figure 2b). On the contrary, when the CORT level was low, i.e. under the chronic stress, the Hsp90/GR ratio

was changed in the opposite direction, with significant elevation in the nuclear compartment, and decrease in the cytoplasm.

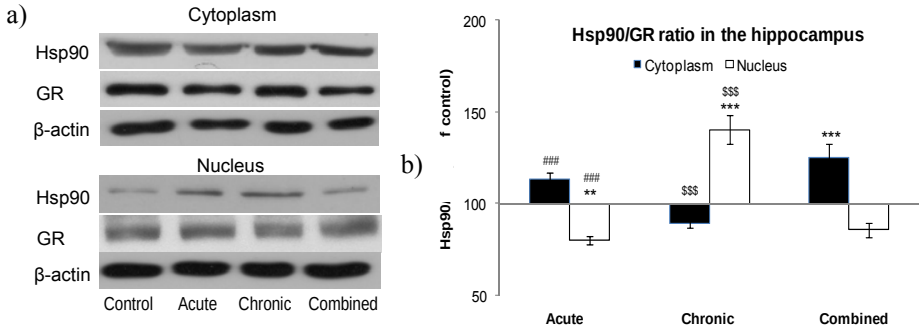

Fig. 2 Hippocampal Hsp90/GR ratio in cytoplasm and nucleus of stressed rats; (a) representative Western blots; (b) densitometric scans (data presented as mean± SEM, n=6)

Conclusion

The results indicated that acute and combined stress induced hippocampal GR translocation to the nucleus, whereas chronic stress resulted in altered localization of GR in this brain structure. In case of Hsp90, acute and chronic stress induced nuclear translocation and decreased its level in cytoplasmic compartment. As the optimal Hsp90/GR ratio is necessary for modulation of steroid dependent response, in case when this ratio is altered (either enhanced or diminished) steroid dependent responses may be disturbed [4]. Therefore, the pattern of changes in Hsp90/GR ratio seemed to be related to CORT level. Namely, when CORT was high the ratio of Hsp90/GR was elevated in the cytoplasm, and decreased in nucleus, while when CORT was low, the opposite pattern was found.

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