PHYSICAL CHEMISTRY 2010

10th International Conference on
Fundamental and Applied Aspects of
Physical Chemistry

Proceedings

The Conference is dedicated to the
100th Anniversary of the academician Pavle Savić birthday
and
20th Anniversary of the Society of Physical Chemists of Serbia

---

21-24 September 2010
B E L G R A D E

ISBN 978-86-82475-17-0
Title: Physical Chemistry 2010. (Proceedings)
Editors: S. Anić and Ž. Čupić
Published by: Society of Physical Chemists of Serbia, Studentski trg 12-16 P.O.Box 47, 11158 Beograd, 218, Srbija
Publisher: Society of Physical Chemists of Serbia
For Publisher: S. Anić, President of Society of Physical Chemists of Serbia
Printed by: "Jovan" Printing and Publishing Company; 200 Copies; Number of pages 16 + 388, **Format:** B5; Printing finished in September 2010.

Text and Layout: "Jovan"

200 - *Copy printing*

The Society of Physical Chemists of Serbia

in co-operation with

Institute of Catalysis, Bulgarian Academy of Sciences

Boreskov Institute of Catalysis, Siberian Branch of the Russian Academy of Sciences

Faculty of Physical Chemistry, University of Belgrade, Serbia

Institute of Chemistry Technology and Metallurgy, University of Belgrade, Serbia

Vinča Institute, University of Belgrade, Serbia

Institute of General and Physical Chemistry, Serbia

International Organizing Committee

Chairman: S. Anić (Serbia)
Vice-chairman: N. Stepanov (Russia)
B. Adnađević (Serbia)

Members:
M. Gabrovska (Bulgaria), N. Cvijetićanin (Serbia), T. Grozdić (Serbia), D. Jovanović (Serbia), M. Lalić (BiH), D. Marković (Serbia), B. Milosavljević (USA), N. Miljević (Serbia), M. Mojović (Serbia), N. Ostrovski (Serbia), C. Pona (Italy), B. Simonović (Serbia), D. Stanisavljev (Serbia), A. G. Stepanov (Russia), V. Vasić (Serbia), N. Vukelić (Serbia), V. Vukojević (Sweden)

International Scientific Committee

Chairman: Ž. Čupić (Serbia)
Vice-chairmans: V. N. Parmon (Russia)
M. Franko (Slovenia)
V. Vasić (Serbia)

Members:
A. Antić-Jovanović (Serbia), G. Bačic (Serbia), R. Cervellati (Italy), R. Compton (United Kingdom), V. Gaspar (Hungary), M. Jeremić (Serbia), A. L. Kawczyñski (Poland), Lj. Kolar-Anić (Serbia), S. Kuchanov (Russia), R. Leblanc (USA), S. Mentus (Serbia), S. Milonjić (Serbia), Lj. Morozova-Roche (Sweden), D. Moscone (Italy), J. Nedeljković (Serbia), Z. Noszticzius (Hungary), M. Perić (Serbia), V. Petruševski (Macedonia), M. Plavšić (Serbia), G. Smulevich (Italy), G. Schmitz (Belgium), I. Schreiber (Czech), P. Ševčik (Slovakia), N. Stepanov (Russia), M. Trtica (Serbia), D. Veselinović (Serbia)

Local Executive Committee

Chairman: B. Adnadjević
Vice-chairmans: S. Blagojević
A. Ivanović

Members:
A. Abu Rabi-Stanković, P. Banković, N. Begović, S. N. Blagojević, N. Cvjetičanin, M. Daković, A. Đerić, A. Ignjatović, Lj. Ignjatović, A. Jović, J. Krstić, S. Kuprešak, D. Lončarević, J. Maksimović, V. Marković, M. Milenković, M. Milojević, Z. Mojović, B. Nedić, I. Pašti, N. Pejić, A. Popović-Bjelić, M. Petković, N. Potkonjak, D. Ranković, R. Ranković, M. Stević, I. Stojković, B. Šljukić, M. Vujković

CHRONIC PSYCHOSOCIAL ISOLATION ALTERS HSP70/GR AND HSP90/GR RATIOS IN RESPONSE TO NOVEL ACUTE STRESS IN RAT HYPOTHALAMUS

I. Simić, A .Đordjević, J. Đorđević, M. Mitić, M. Radojčić and M. Adžić

Vinča Institute of Nuclear Science, P.O. Box 522, 11001 Belgrade, Serbia

Abstract

It is known that chronic psychosocial isolation (CPSI) exerts maladaptive effect on the hypothalamic-pituitary-adrenal (HPA) axis activity. Since the hypothalamus (HT) is a major driver of the HPA axis activity and since glucocorticoid receptor protein (GR) mediates HPA axis negative feedback particularly in this structure, we studied the effect of CPSI by following the expression of GR and its chaperones hsp70 and hsp90 in HT. Our results showed that the ratios of HSPs/GR set by the CPSI were altered in response to a novel acute stress, which indicated negative CPSI influence on GR functions in HT.

Introduction

Stress response involves the activation of the hypothalamic-pituitary-adrenal (HPA) axis and the key driver of this activity is hypothalamus (HT). The final products of the HPA axis activity are glucocorticoids (GCs) that act through glucocorticoid receptor (GR) and mediate feedback inhibition of the HPA axis particularly *via* the HT [1]. The GR is a ligand-dependent transcription factor that in absence of GCs resides in the cytoplasm as a multiprotein heterocomplex with heat shock proteins (HSPs) hsp70 and hsp90 [2]. While hsp70 protein is essential for maturation of GR, hsp90 helps GR to achieve a hormone-binding conformation [2]. Upon hormone binding, GR dissociates from hsp90 and translocates to the nucleus where both HSPs continue to influence its further functions. Namely, hsp90 is required for nuclear GR to regain hormone-binding competence [2], but it may also reduce GR activity when hsp90/GR ratio is increased [3]. Hsp70 plays a general role in sequestering denaturated proteins in nucleus including GR [2]. Considering the essential role of HT in regulating HPA activity under stress conditions, as well as, the role of hsp70 and hsp90 in GR signaling, we investigated a pattern of expression of these proteins and their cellular redistribution in the HT of Wister rat under different types of stress. We were mostly interested in the effect of CPSI on mentioned parameters and in their reversibility in response to subsequent acute stress [4].

Experimental

The animals (adult Wistar male rats) were divided into four groups: (I) controls (untreated); (II) acute stress (30 min of immobilization); (III) chronic psychosocial isolation (CPSI) (for 21 days) and (IV) combined stress (chronic isolation followed by 30 min immobilization). After sacrifice, blood was collected and corticosterone

(CORT) concentration was determined by OCTEIA Corticosterone kit. The cytosolic and nucleosolic samples of HT were prepared by differential centrifugation. The proteins were detected using Western blot technique and antibodies M-20, hsp70 (N27F3-4) and hsp90 (F-8) for GR, hsp70 and hsp90, respectively. β-actin was used as a loading control.

Results and discussion

All results are represented in Figure 1.

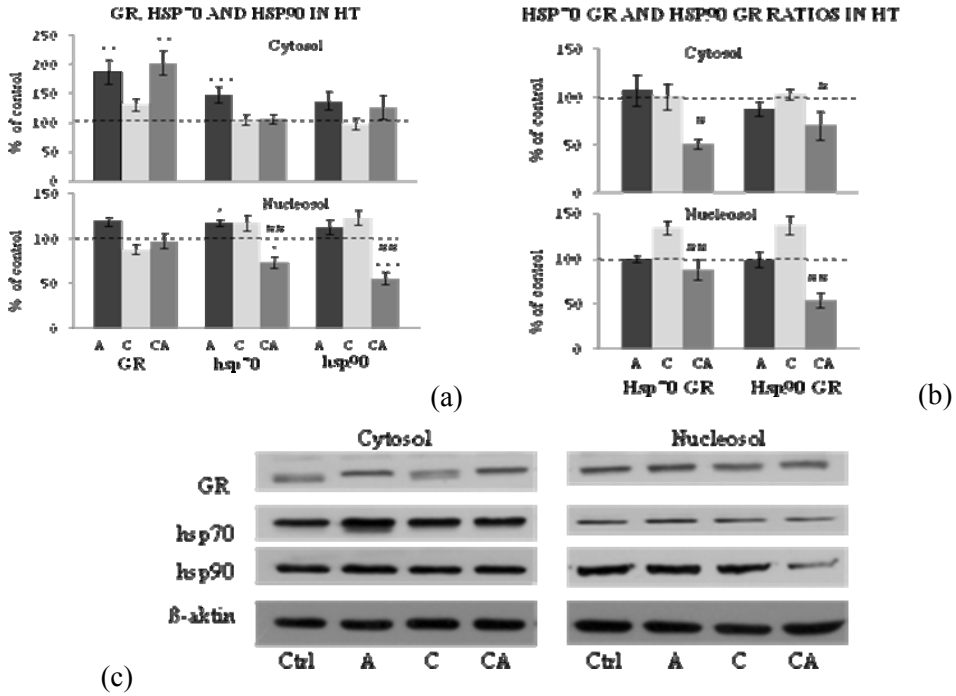

Fig.1. Hypothalamic GR, hsp70 and hsp90 in the cytosol and nucleosol of animals exposed to acute (A), chronic (C) and combined (CA) stresses: expression levels (a); hsp70/GR and hsp90/GR ratios (b) and representative Western blots (c). Data are presented as mean±SEM (n=10) and analyzed by one-way ANOVA (*p<0.05, ** p<0.01, *** p<0.001 stress vs control; #p<0.05, ##p<0.01 chronic vs combined stress).

In response to acute stress, when CORT levels were elevated (612.75±20.12 ng/ml) in respect to control (98.35±20.76 ng/ml), significantly increased levels of GR in the cytosol and hsp70 in both compartments were detected. This elevation of GR in the cytosol could be result of increased synthesis and/or stability and could represent enhanced sensitivity to GCs of this brain structure. The augmentation of hsp70 could indicate activation of cellular stress response [5].

Chronically stressed animals showed decreased CORT levels (54.26±9.21 ng/ml) which were consistent with our previous experiments [4]. The levels of either studied proteins were not statistically changed.

When chronically stressed animals were subjected to novel acute stress, CORT levels (604.31±21.05 ng/ml) as well as GR levels in the cytosol were elevated again. In contrast to acute stress, HSPs levels were decreased in the nucleosol. That could represent inadequate cellular stress response [5] and thus maladaptive effect of CPSI regarding this parameter.

Examining the hsp70/GR and hsp90/GR ratios we found that response of chronically stressed animals to novel acute stress was different from response of control animals to acute stress. Particularly, novel acute stress turned both HSPs/GR ratios in favor of GR, in both compartments. Considering the fact that for appropriate GR function optimal levels of its HSPs in both cellular compartments are required, these results indicated maladaptive influence of CPSI on GR function in the HT.

Conclusion

Chronic stress (CPSI) altered hypothalamic HSPs/GR ratios and compromised response of this structure to a subsequent acute stress. This finding suggested potentialy maladaptive influence of CPSI with regard to the HPA axis feedback activity in hypothalamus.

References

[1] E. R. de Kloet et al., Endocr. Rev., 1998, **19**, 269-301.
[2] D. B. DeFranco, Kidney International, 2000, **57**, 1241-1249.
[3] K. I. Kang et al., Proc. Natl. Acad. Sci., 1999, **96**, 1439-1444.
[4] A. Djordjevic et al., Neuropsychobiology, 2009, **59**, 213–221.
[5] R. I. Morimoto, M. G. Santoro, Nature Biotechnology, 1998, **16**, 833-838.