PHYSICAL CHEMISTRY 2012

11th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

Under the auspices of the
University of Belgrade

Proceedings

The Conference is dedicated to
Professor Ivan Draganić

September 24-28, 2012
Belgrade, Serbia

ISBN 978-86-82475-27-9 ***Volume*** **1**
ISBN 978-86-82475-28-6 ***Volume*** **II**

Title: PHYSICAL CHEMISTRY 2012 (Proceedings)
Editors: S. Anić and Ž. Čupić
Published by: Society of Physical Chemists of Serbia, Studenski trg 12-16, 11158, Belgrade, Serbia
Publisher: Society of Physical Chemists of Serbia
For Publisher: S. Anić, President of Society of Physical Chemists of Serbia
Printed by: "Jovan" Priting and Publishing Company; 200 Copies;
Number of pages: 6+ 497; **Format**: B5; Printing finished in September 2012.

Text and Layout: "Jovan"

200- *Coppy printing*

CONTENTS

F-05-P

DIFFERENTIAL EFFECTS OF CHRONIC ISOLATION STRESS ON NNOS AND INOS PROTEIN EXPRESSION IN RAT PREFRONTAL CORTEX AND HIPPOCAMPUS

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Abstract

Stress-related glucocorticoids and glutamate release have been implicated in mood disorders such as depression. Since glutamatergic mechanisms activate nitric oxide synthase (NOS), we examined protein expression of neuronal NOS (nNOS) and inducible NOS (iNOS) in the cytosolic fraction of prefrontal cortex and hippocampus of rats exposed to acute, chronic and combined stresses (chronic stress followed by acute stress). While nNOS protein expression was high in all animal groups in both brain regions, iNOS protein expression was increased only in prefrontal cortex following chronic isolation indicating state of oxidative/nitrosative stress. Moreover, serum corticosterone level, as a marker of neuroendocrine stress response, revealed deregulated HPA axis after chronic isolation stress. Data have shown the ability of hippocampus to maintain homeostasis (redox balance) after exposure of animals to chronic isolation stress, while iNOS may be implicated in the progression of neurotoxicity in the prefrontal cortex.

Introduction

Acute and chronic stress, as well as stress-induced release of glucocorticoids, induces changes in glutamate neurotransmission in the prefrontal cortex and the hippocampus. Numerous studies have indicated the role of glutamate and its receptors in depression. Glutamatergic activation of N-methyl-D-aspartate (NMDA) receptor stimulates nitric oxide synthase (NOS), the enzyme that catalyzes the production of nitric oxide (NO) which possess both neuroprotective and neurodestructive properties. Brain NO is mainly generated either by neuronal (nNOS) which play physiological and protective role or by inducible (iNOS) associated with pathological processes. Therefore, we examined the protein expression of cytosolic nNOS and iNOS in the prefrontal cortex and hippocampus of rats exposed to acute stresses, chronic isolation stress as an animal model for depression or their combination to assess the maladaptive effect of chronic isolation.

Experimental

Adult male Wistar rats were divided into four groups: (I) control group; (II) groups exposed to 2h of acute stress immobilization (IM) or cold (C, 4°C); (III) chronic isolation stress where animals were individual housed for 21 days; IV groups of chronically stressed animals subsequently exposed to 2h IM or C (4°C) stress (IS+IM, IS+C). After series of tissue centrifugations, in obtained cytosolic fractions, iNOS, nNOS protein expression was measured using Western blot technique. Serum

corticosterone (CORT) level was monitored by enzyme-linked immunosorbent assay (ELISA). Analysis of variance (Two-way ANOVA) followed by Duncan post hoc test was used for statistical data analysis.

Results and discussion

Decreased CORT level to a novel IM or C stress of chronically-isolated animals compared to acute stresses alone indicate compromised hypothalamic-pituitary-adrenal (HPA) axis activity by previous experience of chronic IS stress which could not be resumed after subsequent acute stressors (Table 1).

Table 1. Serum CORT level (ng/ml). Symbols depict significant difference between, stress *vs.* control $^{***}p<0.001$; acute *vs.* combined stress $^{\#\#}p<0.01$; chronic *vs.* combined stress $^{\wedge\wedge\wedge}p<0.001$.

	CONTROL	**ISOLATION**
	142.905±14.16	123.39±10.89
IMMOBILIZATION	$648.89\pm166.78^{***}$	$477.98\pm74.03^{***\ \#\#\ \wedge\wedge\wedge}$
COLD	$342.112\pm112.53^{**}$	$86.41\pm37.68^{\#\#}$

While nNOS protein expression was high in all animal groups in both brain regions, iNOS protein expression was increased by chronic isolation and remained upregulated after both combined stresses only in the prefrontal cortex (Fig. 1.). Unlike hippocampus, in which activities of MnSOD and CuZnSOD were unchanged, their reduced activities in prefrontal cortex following chronic isolation could be due to oxidative/nitrosative stress caused by increase of iNOS levels [1]. Since cortical iNOS induction is associated with extensive and prolonged release of NO (and further to generation of peroxynitrite) this could be in part responsible for stress-induced neurodegenerative changes [2]. Given that iNOS expression was decreased in hippocampus, increased nNOS expresession following chronic isolation suggests its physiological and protective role. Protective role in hippocampus could be also achieved over increased level of inducible heat shock protein that prevents NF-kB activation and therefore inhibition of iNOS protein expression (manuscript in preparation).

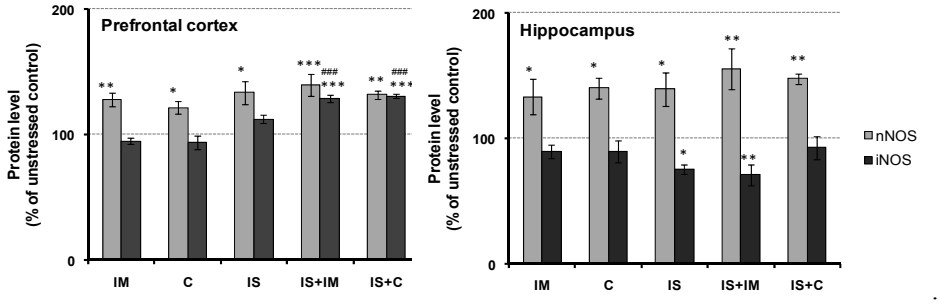

Figure 1. nNOS and iNOS protein level in cytosolic fraction of rat prefrontal cortex and hippocampus, expressed as % of control. Symbols indicate significant difference between stressed groups and control (*p<0.05, **p<0.01, **p<0.001); acutely stressed animals and those previously exposed to IS (p<0.001)

Conclusions

Obtained results indicate the ability of hippocampus to maintain homeostasis (redox balance) after exposure of animals to chronic isolation stress, while iNOS expression may be implicated in the progression of neurotoxicity in the prefrontal cortex. In contrast to iNOS, increased nNOS could be involved in synaptic plastic, contributing to learning and memory. Given that antidepressant-like activity can be produced by agents that affect subcellular signaling systems linked to excitatory amino acid receptor of nitric oxide synthase [3], molecular mechanisms involved in the antidepressant-like effect induced by iNOS inhibition will be further investigated.

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CIP Volume I

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

544(082)
621.35(082)
66.017/.018(082)

MEĐUNARODNA konferencija iz fundamentalne i primenjene fizičke hemije (11 ; 2012 ; Beograd)
Physical Chemistry 2012 : proceedings. #Vol. #1 / 11th International Conference on Fundamental and Applied Aspects of Physical Chemistry, September 24-28, 2012, Belgrade ; [editors S.[Slobodan] Anić and Ž.[Željko] Čupić ; organized by Society of Physical Chemists of Serbia ... et al.]. - Belgrade : Society of Physical Chemists of Serbia, 2012 (Belgrade : Jovan). - VI, 498 str. : ilustr. ; 24 cm

"The Conference is dedicated to Professor Ivan Draganić" --> nasl. str. - Tiraž 200. - Bibliografija uz svaki rad.

ISBN 978-86-82475-27-9
1. Društvo fizikohemičara Srbije (Beograd)
a) Физичка хемија - Зборници b) Електрохемијско инжењерство - Зборници c) Наука о материјалима - Зборници
COBISS.SR-ID 193432332

CIP Volime II

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

544(082)
621.35(082)
66.017/.018(082)

MEĐUNARODNA konferencija iz fundamentalne i primenjene fizičke hemije (11 ; 2012 ; Beograd)
Physical Chemistry 2012 : proceedings. #Vol. #2 / 11th International Conference on Fundamental and Applied Aspects of Physical Chemistry, September 24-28, 2012, Belgrade ; [editors S.[Slobodan] Anić and Ž.[Željko] Čupić ; organized by Society of Physical Chemists of Serbia ... et al.]. - Belgrade : Society of Physical Chemists of Serbia, 2012 (Belgrade : Jovan). - VI str., 499-782 str. : ilustr. ; 24 cm

"The Conference is dedicated to Professor Ivan Draganić" --> nasl. str. - Tiraž 200. - Bibliografija uz svaki rad. - Registar.

ISBN 978-86-82475-28-6
1. Društvo fizikohemičara Srbije (Beograd)
a) Физичка хемија - Зборници b) Електрохемијско инжењерство - Зборници c) Наука о материјалима - Зборници
COBISS.SR-ID 193433356