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-12-P

TIME-DEPENDENT EXPRESSION OF BCL-2 AND BAX PROTEINS IN CORTICAL BRAIN AREA OF ADULT WISTAR RATS AFTER PERMANENT BILATERAL OCCLUSIONS

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Abstract

Model of permanent bilateral occlusion of common carotid arteries (2VO) is generally used to investigate mechanisms of chronic cerebral hypoperfusion that occurs in aging and other neurodegenerative processes. The aim of this study was to determine time-dependent modulation of mitochondrial apoptotic signaling in cortical brain area following chronic cerebral hypoperfusion. Using Western blot technique we monitored the changes in the expression of proteins of Bcl-2 family (Bcl-2, Bax) 3, 7 and 90 days following the insult. According to our results the greatest impact of chronic cerebral hipoperfusion occurred on 7th day.

Introduction

Chronic cerebral hypoperfusion may be the cause or at least a partial cause of various neurodegenerative impairments including vascular dementia, stroke, Alzheimer's disease etc. Since these disorders affect large part of population, it is essential to determine mechanisms in which chronic cerebral hypoperfusion modifies function of neuronal cells. Furthermore it is important to understand the time course of these changes.

Bcl-2 protein family governs mitochondrial outer membrane permeabilization. It includes either pro-apoptotic (Bax, BAD, Bak and Bok) or anti-apoptotic (Bcl-xL, and Bcl-w) proteins. It is established that Bcl-2-related anti- and pro-apoptotic proteins are important in the decision step of the intracellular death program initiated by caspase proteasis. Bcl-2 is a prominent anti-apoptotic protein, but its mechanism of action is currently not completely understood [1]. This suppressor of programmed cell death, homodimerizes with itself or forms heterodimers with a homologous protein Bax, a promoter of cell death. Bax can form an oligomeric pore in the outer mitochondrial membrane. Moreover, Bax is believed to interact with the mitochondrial voltage-dependent anion channel and induce its opening. This results in the release of cytochromeC and other pro-apoptotic factors from the mitochondria [2].

The aim of this study was to determine the time course of possible apoptotic changes and peak of neurodegeneration processes in order to define optimal time point for analyzing beneficial effects of certain substances.

Materials and Methods

Animals used in this experiment were kept according to the standards of Ethical Committee for the Use of Laboratory Animals of INN VINCA. Animals were maintained under standard conditions *ad libitum* access to food and water.

Experiments were performed on adult (3 months old) male Wistar albino rats randomly assigned to different experimental groups: I) animals subjected to permanent bilateral occlusions of common carotid artery (2VO), and II) control, sham operated animals. Procedure was performed on chloral hydrate (400mg/kg) anesthesized animals according to [3]. After given time (3, 7 or 90 days) animals were sacrificed. Crude synaptosomal fraction (P2) was isolated from cortex with differential centrifugation as previously described [4]. Western blot was performed using following antibodies: Bcl-2, Bax and β-actin as loading control (Santa Cruz Biotechnology). The data were obtained from three independent P2 isolations and all measurements were done in triplicate. Statistical significance was determined by one-way ANOVA followed by Tuckey's posthoc test.

Results and Discussion

Knowing that the ratio of pro- and anti-apoptotic proteins of Bcl-2 family might define the cell's destiny, we examined the time-dependent protein expression of anti-survival Bax and pro-survival Bcl-2 in cortical brain area of both sham and 2VO rats.

Chronic cerebral hypoperfusion after 3 days caused no relevant alternation in the quantity of Bax. on 7 day after 2VO, in comparison to controls, a significant increase in expression of Bax was detected. The expression of Bax reached control level on 90 day following the 2VO suggesting that neuronal cells begin to recover (Fig 1a). Further, the expression of Bcl-2 was not significantly altered after 3 and 7 days post operation, while 90 days after 2VO insult a significant increase in quantity of Bcl-2 was observed (Fig 1b).

In addition, to correlate the changes in the protein levels of two members of Bcl–2 protein family as indicators of apoptosis, Bax as pro–apoptotic and Bcl–2 as anti–apoptotic molecule, we calculated their relative ratio. The ratio value above one indicates the dominance of Bax and possible pro-apoptotic changes in the cells, whereas value below one points to prevalence of Bcl-2 protein and anti-apoptotic modulations. The calculated protein ratio showed that Bax predominated in cortical brain area after 7 days while Bcl–2 was predominant 90 days following 2VO insult (Fig 1c).

Findings of current study might indicate that 3 days following the 2VO insult might not be sufficient period for pro-apoptotic changes to occur. On the other hand, according to the expression of Bax and Bcl-2, as well their relative protein ratio, the greatest impact of chronic cerebral hypoperfusion might happen after 7 days. 90 days following the insult, anti-apoptotic processes might start as well as the recovery of neuronal cells.

Conclusions

The relevance of this study was to determine at what investigated time point the chronic cerebral hypoperfusion insult was the greatest in order to test neuroprotective effects of certain substances, which is planned for our further experiments.

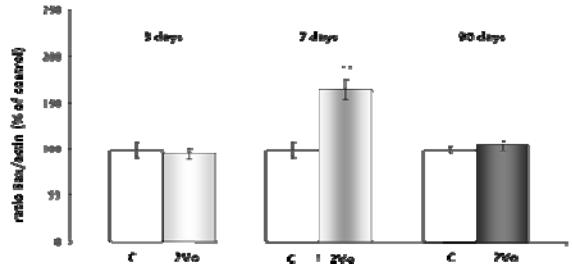
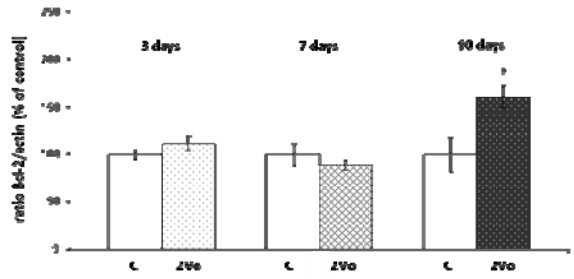
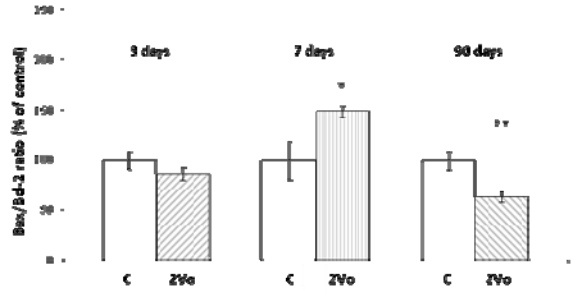


Figure 1. a) Expression of Bax in the cortical P2 fraction on 3, 7, 90 day following permanent 2VO procedure. Results are presented as a percentage of control, mean ± SEM (*p<0.05).



b) Expression of Bcl-2 in the cortical P2 fraction on 3, 7, 90 day following permanent 2VO procedure. Results are presented as a percentage of control, mean ± SEM (*p<0.05).



c) Bax/Bcl-2 protein ratio in the cortical P2 fraction on 3, 7, 90 day following permanent 2VO procedure. Results are presented as a percentage of control, mean ± SEM (*p<0.05).

Acknoledgemnt

This study is supported by the Ministry of Education and Science, Projects No: 173044 and 41014.

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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