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*

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F-12-P

RADIATION-MEDIATED MODULATIONS OF EXTRACELLULAR NUCLEOTIDE HYDROLYSIS IN ADULT FEMALE RAT BRAIN

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Abstract

The present study was performed to investigate whether acute whole-body exposure of female adult rats to low dose (0.5 Gy) of ionizing irradiation (IR) is sufficient to alter ectonucleotidase enzyme activities in the brain. All measurements were done at time points 1, 24 and 72h after irradiation. Neuronal synaptic plasma membranes (SPMs) were isolated from whole brains and enzyme activities were determined by monitoring ATP, ADP and AMP hydrolysis *in vitro*. Our results indicate that whole-body IR is able to modulate investigated brain enzyme activities in a time-dependent manner.

Introduction

All over the world each living being is everyday exposed to ionizing irradiation (IR), almost all from natural sources in the environment or for medical reasons. Acute low dose IR is able to cause current and irreversible damages in the brain by forming reactive oxygen species (ROS) that directly or indirectly modulate protein-protein and protein-lipid interactions and thus, inhibit several metabolic processes, disrupt structure of DNA molecule as well structure, permeability and fluidity of plasma membrane [1].

Ectonucleotidases (NTPDase1,2,3 (nucleoside triphosphate diphospho-hydrolases 1,2,3) and 5'-NT (ecto-5'-nucleotidase)) are surface-sited synaptic plasma membrane (SPM) enzymes that hydrolyze adenine nucleotides with different affinities [2]. NTPDase1 equally hydrolyzes ATP and ADP; NTPDase2 and NTPDase3 prefer ATP while 5'-NT is essential for AMP degradation to neuroprotective adenosine [2]. The modulation of ectonucleotidase activities caused by different stimuli provokes disruption in synaptic transmission and adenosine formation that leads to cell dysfunction, permanent neuronal injury, apoptosis or necrosis and finally to cognitive and other permanent disorders [2].

Therefore, this study tested the hypothesis that whole-body exposure of adult female rats to low (0.5 Gy) IR dose could alter NTPDase1,2,3 and 5'-NT enzyme activities in the whole brain. The possible enzyme activities modulations were examined through the rate of ATP, ADP and AMP hydrolysis at different time points (1h, 24h and 72h).

Materials and Methods

Animals were kept according to the standards of Ethical Committee for the use of laboratory animals of VINCA Institute of Nuclear Sciences (INN VINCA). Adult (60 days old) female rats of Wistar strain were divided at random into two groups:

I) control non-irradiated animals (C) and II) animals irradiated with 0.5 Gy (IR). The whole-bodies of IR rats were exposed to a single 0.5 Gy dose of γ-rays using 10.7 cGy/min, ^{60}Co source at INN VINCA. During the irradiation procedure animals were confined in plywood boxes while rats from control group were treated as the IR group but not subjected to irradiation. Immobilization for all groups lasted approximately 90 min. Animals were decapitated at different time points (1h, 24h and 72h after treatments) and synaptosomal plasma membranes (SPMs) from whole brains were isolated according to Stanojevic et al. [1]. The rate of ATP, ADP and AMP hydrolysis as indicators of enzyme activities, were measured by colorimetric determination of liberated inorganic phosphate as previously described [1]. The data were obtained from three independent SPMs 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

The experimental results of synaptosomal ATP, ADP and AMP hydrolysis as indicators of NTPDase1,2,3 and 5'-NT activities in each investigated time-point following acute immobilization are shown in Table 1.

control	1h	24h	72h
ATP	144.5 ± 5.5	128.2 ± 6.3	147.8 ± 9.1
ADP	23. 7 ± 2.2	29. 8 ± 2. 7	33.6 ± 2.0
AMP	18.4 ± 1.3	16.9 ± 1.8	27.1 ± 0.5

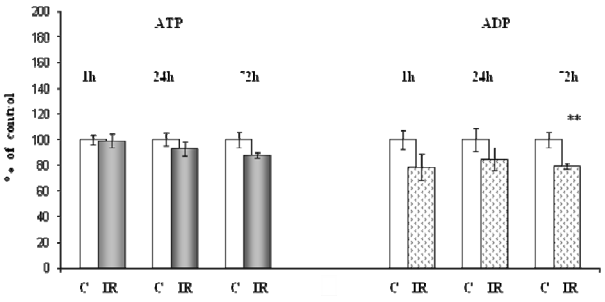
Table 1. Specific enzyme activities for control groups presented as a mean nmolPi/min/mg ± SEM, from 3 independent experiments done in triplicate

In the nerve terminals of 60-days old female Wistar rats, whole-body irradiation with 0.5 Gy was not sufficient to alter ATP hydrolysis in any investigated time point (Fig 1a, left). Although IR provoked decrease of ADP hydrolysis at all time points, the greatest impact appeared after 72h when ADP hydrolysis was reduced by 21% (Fig 1a, right). Thus, in this experimental model system, the ATPase component of investigated enzymes was less sensitive to low dose IR then the ADPase part.

Furthermore, the significant decrease in AMP hydrolysis by 20% was observed 72h after irradiation while after 1h and 24h the investigated hydrolyses were reduced but not considerably (Fig 1b). Knowing that adenosine is an important neuroprotective agent, the observed diminished AMP hydrolysis as an indicator of 5'-NT activity may contribute to lesser extracellular adenosine formation and be harmful for neurons.

Conclusions

In the present study, detected hydrolyzing activities point out that more than one ectonucleotidase is present on the neural cell surface and all of them are differently sensitive to whole-body irradiation. Furthermore, our findings indicate that acute whole-body IR exposure via modulation of the neuronal ectonucleotidases activity in adult female rats might be capable to reduce the formation of adenosine, impair the function of neuronal cells and increase the possibility of cell death occurrence in time-depended manner.



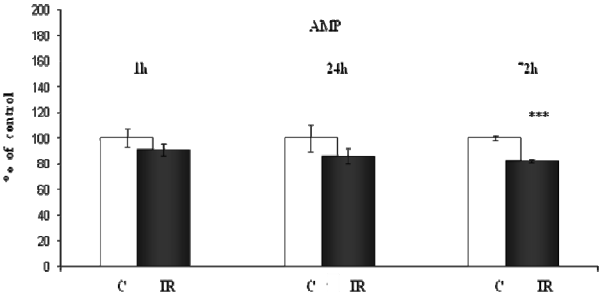


Figure 1. a) ATP and ADP hydrolyses as indicators of NTPDase1,2,3 activities in synaptic plasma membranes 1h, 24h and 72h following acute whole-body irradiation with 0.5 Gy dose. Results are presented as a percentage of control, mean ± SEM from 3 independent experiments done in triplicate (*p<0.05)

b) AMP hydrolysis as a marker of 5'-NT activity in synaptic plasma membranes 1h, 24h and 72h following acute whole-body irradiation with 0.5 Gy dose. Results are presented as a percentage of control, mean ± SEM from 3 independent experiments done in triplicate (*p<0.05)

Acknowledgment

This study is supported by Ministry of Education and Science, Project No. 173044.

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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