PHYSICAL CHEMISTRY 2008

Proceedings

of the 9th International Conference on Fundamental and Applied Aspects of Physical Chemistry

Volume I

The Conference is dedicated to the 200th Anniversary of the University in Belgrade

UNIVERSITY OF BELGRADE
1808 • 2008

September 24-26, 2008,
Belgrade, Serbia

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ISBN	978-86-82475-16-3
Title:	Physical Chemistry 2008. (Proceedings)
Editor:	Prof. dr A. Antić-Jovanović
Published by:	The Society of Physical Chemists of Serbia, Studentski trg 12-16, P.O.Box 47, 11158 Belgrade, 218, Serbia
Publisher:	Society of Physical Chemists of Serbia
For publisher:	Prof. dr S. Anić, president of the Society of Physical Chemists of Serbia
Printed by:	"Jovan" Printing and Published Comp; 250 Copies; Number of Pages: x + 468; Format B5; Printing finished in September 2008.
Text and Layout:	Aleksandar Nikolić

250 – copy printing

The Conference is organized by
the Society of Physical Chemists of Serbia

in cooperation 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

Institute of Chemistry, Technology and Metallurgy, Belgrade

Institute of General and Physical Chemistry, Belgrade

IONIZING IRRADIATION AFFECT EXTRACELLULAR NUCLEOTIDE HYDROLYSIS IN BRAIN OF RATS IN DIFFERENT STAGES OF DEVELOPMENT: II 30-DAY-OLD RATS

D. Drakulić, I. Stanojević, M. Milošević and N. Veličković

Laboratory for Molecular Biology and Endocrinology,
"Vinča" Institute of Nuclear Science, P.O.Box 522, 11001 Belgrade, Serbia

Abstract

The effect of acute gamma irradiation (IR) on enzyme activity of rat brain Ecto-Nucleotide Diphosphohydrolase (E-NTPDase), in presence of adenosine tri- and diphophashates (ATP and ADP) and divalent cations (Ca^{2+} and Mg^{2+}), has been investigated. The aim of research was to study the influence of low (50 cGy) and therapeutic (2Gy) doses of whole-body irradiation on rat brain E-NTPDase enzyme activity 24h after treatment in prepubertal and adult rats. Our results suggest that whole-body irradiation could induce modulation of neural activity in rat brain, especially in young rats.

Introduction

E-NTPDase1, 2, 3 are glycoproteins, expressed on of brain cell′s plasma membranes. These proteins contain transmembrane domain; N- and C-terminus, located in intracellular and large highly conserved active domain, located in extracellular space. Substrate-active site enables hydrolysis of extracellular nucleotides to nucleosides and induces purinergic signalling modulation and termination. E-NTPDases1, 2, 3 hydrolyze ATP and ADP with different affinities. E-NTPDase1 hydrolysis ATP equally as ADP, other two E-NTPDases (E-NTPDase2 and E-NTPDase3) prefer ATP [1]. In central nervous system, ATP acts as universal signal and fast excitatory neurotransmitter and co-transmitter, as well as neuromodulator. Binding of extracellular ATP and products of its hydrolysis (ADP, AMP and adenosine) to specific surface located purinergic receptors, triggers signalling cascade and regulates many physiological processes. Changes in E-NTPDase activity causes disruption in synaptic transmission and adenosine formation, leading to cell dysfunction, apoptosis and cognitive disorders [2].

Ionizing radiation, that is commonly used in every day life, for instance in diagnostic protocols and therapeutic purposes, is able to induce plasma membrane structure alterations, metabolic process inhibitions, ion transport changes as well as DNA damages [3]. Through arising of reactive oxygen species (ROS), IR modulates permeability and fluidity of plasma membrane by altering expression and interaction of transmembrane proteins and protein-lipid connections.

It is supposed that low and therapeutic doses alter brain′s E-NTPDase enzyme activity through the rate of ATP and ADP hydrolysis 24h after whole-body

irradiation and this hypothesis was tested in the present study. Also, susceptibility of young brain to IR was studied.

Experimental procedures

Experiments were performed on 30 (prepubertal) and 90 (adult) days old female Wistar albino rats obtained from the local colony. Animals were divided into three groups: the control group non-irradiated (C); second and third group whole-body irradiated with 50 cGy or 2 Gy (10.7 cGy/min, ^{60}Co source INN Vinča). Animals from all three groups were confined in plywood boxes, and sacrificed 24h after treatments. Synaptic plasma membranes (SPM) were isolated from whole brains. Activities of E-NTPDase were determined under *in vitro* conditions: rate of ATP and ADP hydrolysis were measured by colorimetric determination of liberated inorganic phosphate in the presence of 40μg SPM proteins, 1mmol/l ATP or ADP, 5 mmol/l $MgCl_2$, 50 mmol/l Tris-HCl pH 7.8, and incubations at 37ºC for 15 min. The specific enzyme activity was expressed from 3 independent experiments performed in triplicate as mean nmolPi/min/mg SPM protein ± S.E.M. The data were analyzed using one-way ANOVA followed by post-hoc Tukey's test and $p<0.05$ values were considered significant.

Results and Discussion

In attempt to reveal if whole-body irradiation alters the E-NTPDase enzyme activity, the production of inorganic phosphate, as result of ATP and ADP hydrolysis, was monitored. Analyses showed that in prepubertal rat brain, compared to control (C), 24h after irradiation with 50 cGy, ADP hydrolysis was decreased by 20% and irradiation with therapeutic dose caused decrease of ATP hydrolysis by 10%. Low dosage whole-body irradiation of adult (90-day-old) animals leaded to ATP hydrolysis augmentation by 28%.

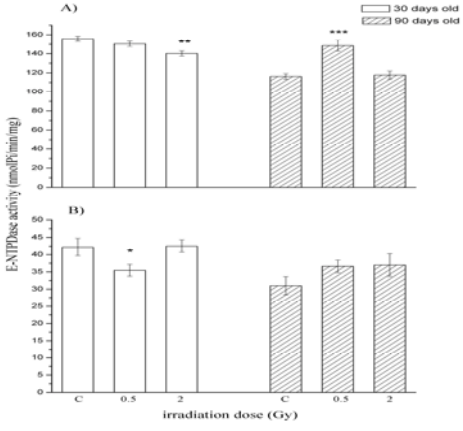

Fig. 1. E-NTPDase activity presented as a mean ± S.E.M. from 3 experiments done in triplicate. A) ATP and B) ADP hydrolysis in imobilized control (C) group and groups irradiated with 50 cGy (0,5) and 2 Gy (2). *** p< 0,001, ***p<0,01, * p<0,05 *vs*. control.

Results suggest that more than one E-NTPDases is present on the neural cell surface and all of them are differently sensitive to whole-body irradiation. In 90 days old rat's brain, ATPase part of these enzymes was more sensitive to low dose

irradiation then ADPase component, while in young rat ADPase component was more sensitive.

In developing central nervous system cells are highly sensitive to certain chemical and physical agents such as IR [4]. Few previous studies demonstrated that all three E-NTPDase enzymes play an important role in neural development. Decreased hydrolitic activity of these enzymes in prepubertal rats could be a consequence of reactive oxygen and nitrogen species formation and plasma membrane disturbance, lipide peroxidation, as well as inhibition of enzyme activity [3]. Futher, inhibition of the enzyme activity could cause augmentation of excitatory nucleotides amount in synaptic cleft and apoptosis inducement by activation of P2 receptor, that results in appearence of motoric and cognitive disfunctions.

Ionizing irradiation could alter signal transduction and gene expression. Cells are supplied with defense mechanisms and 24 h after irradiation is enough time for the adaptive response. According to our results, 24h after whole-body irradiation with 2 Gy, defence cells mechanisms in adult rats, were induced. Apparantly, increased ATP hydrolysis after 50 cGy treatment is protective because IR *per se* could induce augmentation of extracellular ATP and cause cytotoxity. Observed increased enzyme activities may reduce the amount of these nucleotides in synaptic cleft. In prepubertal rat brain ADPase part of the enzymes was more sensitive to low dose irradiation, while ATPase component was sensitive to 2 Gy. Perhaps young rats were not able to induce defence mechanisms. Futher, ADPase part of the E-NTPDases was more senzitive to IR with 50 cGy then ATPase component.

Conclusion

Our results showed that whole-body irradiation with low- and therapeutic doses affects neuronal activity in prepubertal rat brain by decreasing extracellular ATP and ADP hydrolysis 24h after irradiation. In this way, concentrations of excitatory ATP and ADP were increased, while concentration of neuroprotective adenosine was decreased. Low dose irradiation modulates neuronal activity in adult brain by increasing ATP hydrolysis and it enables neuroprotective effect.

Acknowledgements

The Serbian Ministry of Sciences, Project No. 143044 supported this study.

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