PHYSICAL CHEMISTRY 2006

Proceedings

of the 8th International Conference on Fundamental and Applied Aspects of Physical Chemistry

September 26-29,
Belgrade, Serbia

ISBN	86-82139-26-X
Title:	Physical Chemistry 2006. (Proceedings)
Editors	Prof. dr A. Antić-Jovanović
Published by:	The Society of Physical Chemists of Serbia, Studentski trg 12-16, P.O.Box 137, 11001 Belgrade, 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 + 442; Format B5; Printing finished in September 2006.
Text and Layout:	Aleksandar Nikolić

250 – copy printing

LOW-DOSE IONIZING IRRADIATION AFFECTS NTPDase ACTIVITY IN NEUORONAL CELLS OF YOUNG FEMALE RATS

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Abstract

In the present study, time-dependent effects of low-dose ionizing irradiation on membrane-bound enzyme activity in neuronal cell endings of young female rat brains were sudied. The ecto-adenosine triphospho diphosphohydrolases (NTPDases) hydrolyse the extracellular nucleotide di- and tri- phosphates (ADP and ATP) in the presence of divalent cations (Ca^{2+} and Mg^{2+}). The influence of whole-body irradiation on membrane enzymes ATP and ADP hydrolysing activity were monitored 1, 24 and 72 h after irradiation. Animals were divided into three groups: non-treated, under physiological conditions (C), immobilized and whole body irradiated with 50 cGy by γ-rays (R) and immobilized non-irradiated (I) animals. It was shown that the levels of ATP and ADP hydrolyses were not affected within 72h after immobilisation. Low-dose irradiation significantly decreased hydrolyses of extracellular ATP as early as 1h after irradiation. ADP hydrolyses within 72 h and ATP hydrolysis after 24 h were not altered.

Introduction

Low-dose ionizing irradiation (IR) effects cannot be explained only by direct damage to the DNA. The alternative target for low-dose effects (LDE) initiation in a cell are the plasma membranes which are highly sensitive to IR [1]. IR affects plasma membrane functions mediated through transmembrane proteins by altering their expression or changing the interaction(s) that normally take place between membrane lipids and proteins. Reactive oxygen species (ROS), generated following IR in the cell, act on polyunsaturated fatty acids of cellular membranes producing lipid peroxides which may alter functioning of plasma membrane proteins.

The ecto-enzymes are a family of transmembrane enzymes with a substrate-active site located in extra-cellular spaces. These enzymes hydrolyse nucleotide mono- di- and triphospates. Adenosine triphosphate (ATP) functions as a fast excitatory neurotransmitter and neuromodulator in the central nervous system. The extracellular (synaptic) level of ATP is rapidly regulated by the conjugated action of synaptic plasma membrane (SPM)-bound, surface-located enzyme chain of ectonucleotidases. Ecto-ATPase (NTPDase2) are enzymes that hydrolyse preferentially ATP, whereas the ecto-ATPdiphosphohydrolase (NTPDase1) hydrolyses ATP equally well as ADP to AMP. The AMP formed is metabolized to adenosine by ecto-5'-nucleotidase [2]. As a result, an increased adenosine level, a main inhibitory neuromodulator, exerts neuroprotective function preserving the brain from excitoxcity damage and has

various trophic roles during developement. Inhibition of SPM ecto-ATPase activity would be expected to potentiate excitatory synaptic transmission by supporting synaptic efficacy of ATP and inhibiting the formation of adenosine.

Thus, the aim of this work is to study the LDE (50 cGy) of NTPDase activity through the rate of ATP and ADP hydrolysis, from the young rat brain SPM; 1h, 24h and 72h after whole body irradiation with gamma-rays.

Experimental Procedures

Female rats of the Wistar strain, 30 days old, were divided into three groups: the control group were under physiological conditions, the second was whole-body irradiated (50 cGy, 10.7cGy/min, ^{60}Co source). During irradiation, the animals were confined in plywood boxes. Because of the immobilisation stress as a positive control, the third group of animals were treated as the irradiated group but not subjected to irradiation (I). All groups were sacrificed 1h, 24h, and 72h after irradiation. Synaptic plasma membranes (SPM) were isolated from whole brains. Activities of 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 37 °C for 15 min. The specific enzyme activity was expressed as mean nmolPi/min/mg SPM protein ± S.E.M. of the I animals in the respect to control animals or as a mean percentage ± S.E.M. of irradiated animals in the respect to I, from 3 independent examinations performed in triplicate. The data were analyzed using Student's t-test and $p<0.05$ values were considered significant.

Results and Discussion

To clarify if immobilization stress affects ATP and/or ADP hydrolyses, hydrolizing activity of NTPDase from rats exposed to immobilising stress and non-treated (control) group were examined. Results show that hydrolysis of ATP and ADP were not affected by acute immobilisation (Fig.1).

Figure 2. presents the experimental result of time-dependent LDE (50 cGy) on the enzyme hydrolyzing activity. One hour after irradiation, ATP hydrolysis decreased by 20% ($p<0,01$) when compared to I, but after 24 h as well as ADP hydrolyses within 72 h were not affected.

Analysis of the developmental profile of ATP hydrolyzing activity revealed that the activity reaches the maximum level at day 30. Thus, both NTPDase1 and 2 have a role in brain dveleopement at day 30 [3]. Several previous studies have reported that rat brain ecto-ATPase activity decreased under conditions that either promote or are associated with increased lipide peroxidation. The results demonstrate that low-pathologically relevance of 4-hydroxynonenal, the major product of membrane lipide peroxidation, selectively inhibits SPM NTPDase activity [4]. The low-dose (50 cGy) produces an inhibition of cell metabolism employed acutely.

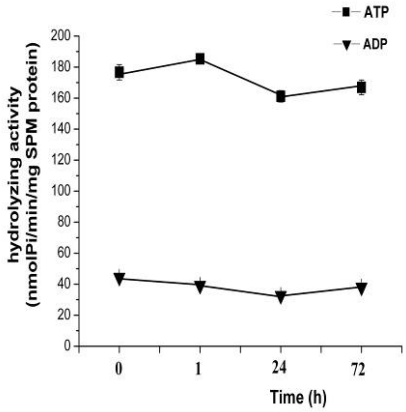

Fig. 1. Time-dependent effects of the immobilization on ATP and ADP hydrolysis activity of NTPDase. Enzyme specific activitiy of nucleotide hydrolyses presented as mean ±S.E.M from three experiments done in triplicate.

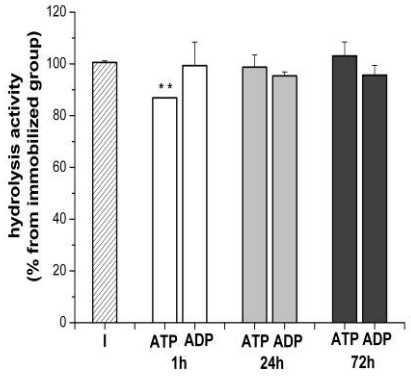

Fig. 2. Time-dependent LDE (50 cGy) on NTPDase activity represented in relative units, as percentage (%) of ATP and ADP hydrolysis in irradiated group compared with immobilized control I, (** $p<0.01$). Results represent mean ±S.E.M from thre experiments done in triplicate.

Eidus [1] hypothetised that the inhibitory effect declines when the post-irradiation time period is sufficiently prolonged to enable the adaptive response to appear. Inhibition of ATP hydrolysing activity 1h after LD irradiation as shown in our study is in accordance with this hypothesis. Twentyfour hours is more than enough for the adaptive response to appear. On the other hand, only ATP hydrolysis is decreased but not ADP hydrolysis. This indicates that the enzyme structure of the NTPDase2 is more sensitive to LD of irradiation.

Conclusion

Whole body irradiation induces modulation of neuronal activity in young rat brain by decreasing extracellular ATP hydrolysis 1h after irradiation. After 24h, due to the adaptive response of neuronal cells, the effect is diminished.

Acknowledgements

This sudy was supported by the Serbian Ministry of Sciences and Environmental Protection, Project No. 143044.

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