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IONIZING IRRADIATION AFFECT EXTRACELLULAR NUCLEOTIDE HYDROLYSIS IN BRAIN OF RATS IN DIFFERENT STAGES OF DEVELOPMENT: I 15-DAY-OLD RATS

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Abstract

Ionizing radiation affects plasma membrane functions mediated through transmembrane proteins including enzymes. Plasma membrane surface-located enzyme chain of ecto-nucleotide triphospho diphosphohydrolases (NTPDases) are involved in termination of cell purinergic signalization by hydrolysing extracellular adenosine tri- and di-phosphate (ATP and ADP). In the present study, effects of low (50 cGy) and therapeutic (2 Gy) dose of ionizing γ-irradiation on NTPDase activity in early postnatal rat brain neuronal cells were studied. Both low- and therapeutic doses significantly decreased hydrolyze of extracellular ATP (by 11% and 30%) and ADP (18% and 46%) in postnatal rats. These findings indicate that gamma-radiation inhibits the enzyme activity in dose-dependent manner. This decreasing NTPDase activity 24h after whole body irradiation may lead to neuronal cell function disturbance, even cell death.

Introduction

The high sensitivity of cellular membranes to the action of great variety of chemical and physical agents including ionizing radiation (IR) is well known. Reactive oxygen species (ROS), generated following IR in the cell, act on polyunsaturated fatty acids of cellular membranes producing lipid peroxides, which may alter plasma membrane proteins function. Although, ionizing radiation may affects the expression of membrane proteins or change the interaction(s) that normally take place between membrane lipids and proteins [1].

Adenosine triphosphate (ATP) functions as a fast excitatory neurotransmitter and neuromodulator, activating various purino-receptors in the central nervous system. ATP released in the synaptic cleft can be hydrolyzed to adenosine by the conjugated action of synaptic plasma membrane (SPM)-bound, surface-located enzyme chain of NTPDases and ecto-5'nucleotidase. Thus, ectonucleotidase pathway has a double function of removing the one signal (ATP) and generating second one (adenosine), consequently controlling the levels of adenine nucleotides in the extracellular environment and the duration and extent of their receptor activation. 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 [2].

The aim of this work was to study the effects of low and therapeutic doses one day after whole body gamma-ray irradiating on ATP and ADP hydrolysis by

NTPDase of postnatal rats brain SPM.

Experimental procedures

Female rats of the Wistar strain, 15 and 90 days old, were whole-body irradiated with 50 cGy or 2 Gy (10.7cGy/min, ^{60}Co source). During irradiation animals were confined in plywood boxes and the second group of animals were treated as the irradiated group but not subjected to irradiation (control group). All groups were sacrificed 24 hour after irradiation. Nerve terminals (synaptic) plasma membranes (SPM) were isolated from whole brains. Activities of NTPDases were determined under *in vitro* conditions: rate of ATP and ADP hydrolysis were measured by colorimetric determination of liberated 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 as mean nmolPi/min/mg SPM protein ± S.E.M. from three independent examinations performed in triplicate. Statistical analyses were performed by one-way analysis of variance (ANOVA), followed by a Tukey's test as post-hoc, considering $p < 0.05$ as significant.

Results and Discussion

The results of low- (50 cGy) and therapeutic- (2 Gy) dose radiation on the brain NTPDase activity 24h after irradiation are presented in Figure 1. In 15-day-old rats both doses decrease ATP hydrolysis in dose-dependent manner by 11% and 30% compared to non-irradiated control (Fig. 1.A). Changes in ADP hydrolysis go along ATP hydrolysis, and are decreased by 18% and 46% in respect to control (Fig. 1.B). The results indicate that even a low dose is enough for induce inhibition of NTPDase activity 24h after irradiation. In the nerve terminals of adult rat brain, 24h after irradiation, ATP hydrolysis is increased by 30% compared to non-irradiated control only with low dose (Fig. 1.A), while no change was observed with therapeutic dose. The ADP hydrolysis was unchanged irrespective of radiation dose (Fig. 1.B). It was assumed that low doses initiate activation of enzymatic reaction because of disturbed plasma membrane permeability [1]. Several previous studies have reported that rat brain ecto-ATPase activity decreased under conditions that either promote or are associated with increased lipid peroxidation. The results demonstrate that low-pathologically relevance of 4-hydroxynonenal, the major product of membrane lipid peroxidation, selectively inhibits SPM NTPDase [3]. Lack of changes in ADP hydrolyses in adult rats indicate presence of two NTPDases with different sensitivity to membrane disturbance. Increased ATP hydrolysis indicates increased adenosine formation, possessing neuroprotective effects. Another finding from the results was that infant brain NTPDase(s) was differently vulnerable on ionizing radiation or cellular events induced by radiation. It is well known that immature neurons are more sensitive to ionizing irradiation. By decreased NTPDase activity in infant rats and consequently decreased adenosine production, neuronal cells in that stage of development were susceptible to apop-

tosis. Certain cells of the CNS have been reported to undergo p53-dependent apoptosis after 2 Gy irradiation and its inhibition of NTPDase activity may have implication in cell death of immature brain. Whole-brain irradiation with large-scale doses (2-10 Gy) also caused a decrease in number of cells and their progenitor cells in young rodent brain in the dose-dependent fashion a few hours after irradiation [4].

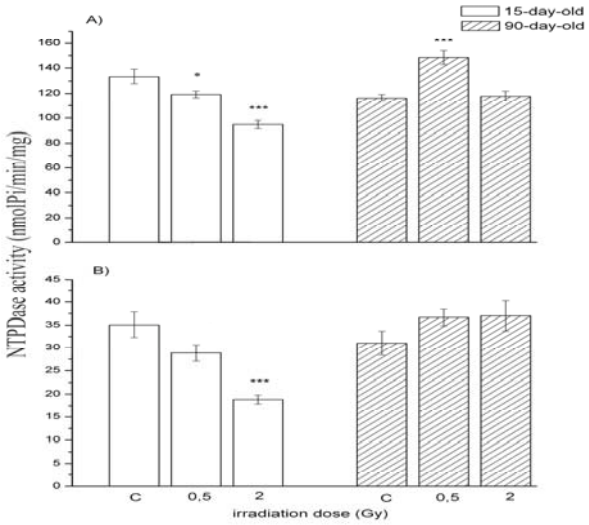

Fig. 1. The ATP (A) and ADP (B) hydrolysis in 15- and 90-old-rats 24h after 50 cGy and 2 Gy irradiation. Results represent mean ±S.E.M from three experiments done in triplicate. Significance in respect to non-irradiated controls (C) (*$p<0.05$, ***$p<0.001$).

Conclusion

In young rat brain, 24h after low and therapeutic doses of gamma-irradiation induce decreasing in NTPDase activity that may lead to disturbance in neuronal cells function and increased cell death.

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