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EARLY EFFECTS OF IONIZING IR-RADIATION ON THE ECTO-5'NUCLEOTIDASE ACTIVITY IN RAT BRAIN DURING POSTNATAL DEVELOPMENT

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Abstract

In the present study, early effects of low (50 cGy) and therapeutic dose (2 Gy) of ionizing γ-irradiation on ecto-5'nucleotidase activity in rat brain neuronal cells during postnatal development were studied. Ecto-5'-nucleotidase is the major enzyme that hydrolyzes extracellular AMP and is responsible for the formation of the P1 receptor agonist-adenosine. It was shown that the levels of AMP hydrolyses by the enzyme were not affected by irradiation in the rats during first 4 postnatal weeks. A both low- and therapeutic dose significantly decreased hydrolyses of extracellular AMP in pubertal and adult rats by 10-14%. These findings indicate that low dose exerts the same effects on ecto-5'nucleotidase activity as therapeutic one in first hour after irradiation. Another findings is that in early postnatal development, brain ecto-5'nucleotidase was resistant to irradiation damage.

Introduction

Adenine nucleotides represent an important class of extracellular molecules that are crucial for normal functioning of the nervous system having special functions during development. The events induced by extracellular adenine nucleotides are controlled by the action of ecto-nucleotidases (NTPDase 1, 2, 3), which play a central role in modulating the extracellular levels of these important signaling molecules. Ecto-5′nucleotidase (ecto-5′-NT/CD73) hydrolyses nucleoside monophosphates (AMP) to adenosine and is a key enzyme in the nucleotide degradation pathway. Adenosine, the final product of ATP hydrolysis, elicits important physiological responses related to neurotransmission modulation, neuroprotection and cell survival/death, activating specific P1 receptor subtypes. These effects are closely related to extracellular adenosine concentrations, cell surface expression of different adenosine receptor subtypes and signal transduction mechanisms activated following the binding of specific agonists [1].

Effects of ionizing radiation (IR) could not be explained simply by direct damage of the cell DNA molecules. The additional target is the plasma membrane, which is highly sensitive to IR, especially in early period after irradiation. Reactive oxygen species (ROS) formation and oxidative stress that follows are very important in injuries induced by ionizing radiation. 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. ROS, generated in the cell following IR, acts on polyunsaturated fatty

acids of cellular membranes producing lipid peroxides, which may alter functioning of plasma membrane proteins [2].

Thus, the aim of this work was to study the early (1h) effects of low and therapeutic doses of whole body irradiation with gamma-rays on ecto-5'nucleotidase activity during postnatal development by measuring the rate of AMP hydrolysis in young, prepubertal, pubertal and adult rat brain.

Experimental procedures

Female rats of the Wistar strain, 15, 30, 60 and 90 days old, were whole-body irradiated with 50 cGy or 2 Gy (10.7 cGy/min, ^{60}Co source). During irradiation, the 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 1 hour after irradiation. Nerve terminals (synaptic) plasma membranes (SPM) were isolated from whole brains. Activities of ecto-5' nucleotidases were determined under *in vitro* conditions: rate of AMP hydrolysis were measured by colorimetric determination of liberated phosphate in the presence of 80μg SPM proteins, 1mmol/l AMP, 5 mmol/l $MgCl_2$, 50 mmol/l Tris-HCl, pH 7,4 and incubations at 37ºC for 30 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 experimental results of age-dependent effects of low- (50 cGy) and therapeutic- (2 Gy) dose whole body irradiation on the brain ecto-5'nucleotidase hydrolyzing activity are presented in Figure 1. As can be seen, there is significant lower AMP hydrolyzing rate detected in young rats. During postnatal brain development synaptic contacts, dendrite elongation and myelinization, as well physiological apoptosis, are more potent events. Since we measured hydrolyzing activity of ecto-5'nucleotidase in isolated presynaptic plasma membranes in 15- and 30-day-old rats with lower number of synapses, the rates of hydrolyses were much lower in respect to the older age rat brains with formed synapses. Also, during early period of development, ecto-5'nucleotidase possess predominantly functions in brain cells communication. The analysis of the developmental profile of ecto-5'nucleotidase hydrolyzing activity revealed that this activity is minor in postnatal animals and reaches the maximum level at adult one [3]. Even though immature neurons are generally more sensitive to IR [4] we could not detect any changes in ecto-5'nucleotidase hydrolyzing activity. One hour after irradiation of 60- and 90-day-old rats with both doses, AMP hydrolysis decreased between 10% and 15% in respect to appropriate controls (Fig.1.). These results indicate that low-dose irradiation is sufficient to modulate ecto-5'nucleotidase activity as early as 1h. Several previous studies have reported that rat brain ecto-ATPase activity,

responsible for hydrolysis of extracellular ATP decreased under conditions that either promote or are associated with increased lipid peroxidation. It was found that 4-hydroxynonenal, the major product of membrane lipid peroxidation inhibits SPM ecto-ATPase activity [5]. Our results suggest that similar mechanisms may induce inhibition of ecto-5`nucleotidases. Inhibition of SPM ecto-5'nucleotidase activity would be expected to decrease formation of adenosine, generally accepted as neuroprotective compound.

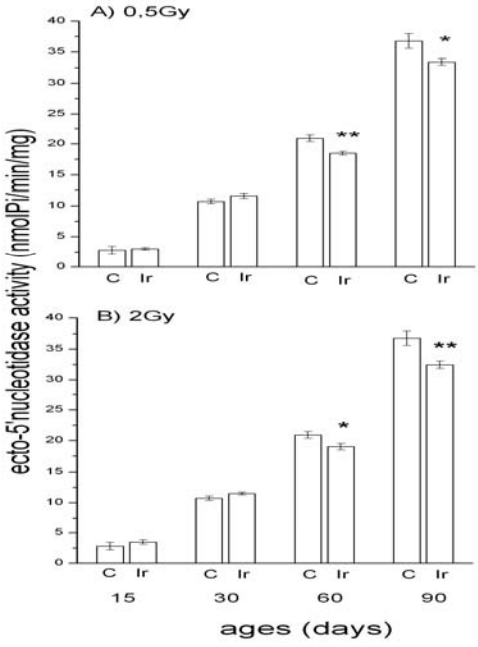

Fig. 1. Development-dependent ionizing radiation effects on pre-synaptic ecto-5'nucleotidase activity. The enzyme activity was measu- red 1h after (A) low-dose and (B) therapeutic-dose irradiation (Ir) and compared with control (C) activity. Results represent mean ±S.E.M from three experiments done in triplicate (*$p<0.05$, **$p<0.01$)

Conclusion

Whole body irradiation induces modulation of neuronal activity in pubertal and adult rat brain by decreasing extracellular AMP hydrolysis within 1h after irradiation, which may lead to increased cell death. The hydrolyzing activity of ecto-5'nucleotidase in young and prepubertal rats was not affected by irradiation.

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