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## SMALL INTESTINE ACETYLCHOLINESTERASE ACTIVITY IN EXPERIMENTAL ANIMALS EXPOSED TO LOW DOSES OF IONIZING RADIATION

A. S. Anschev

State Enterprise "Ukrainian Scientific Research Institute of Transport Medicine of the  
Ministry of Health of Ukraine", Odessa

### Abstract

Radioactive contamination of the environment has become one of the most important environmental factors and the problem of the possible consequences of the global and rapid change in the level of radiation load on the biosphere for all living things is of particular importance. **The objective:** to study the activity of AChE in the small intestine of rats - males and females of different ages - both under physiological conditions and under conditions of prolonged  $\gamma$ -irradiation in a total dose of 0.75 Gray. Experimental studies were performed on 510 rats of different ages and sexes of the Wistar line. All animals were kept under standard conditions and on the standard diet of the vivarium. Total  $\gamma$ -irradiation was performed with the use of gamma therapeutic unit "AGAT-R" №83 (isotope  $^{60}\text{Co}$ ). Analysis of the results showed that the most significant changes in AChE activity underwent in the small intestine of males and females 12 days after the end of the ionizing factor, and for all subsequent stages of the study was characterized by its gradual decrease. Previously unknown pathophysiological mechanisms of violation of the morphofunctional status of the small intestine of offspring obtained from predecessors after chronic  $\gamma$ -irradiation in low doses, the implementation of radiation effects of parental irradiation in their offspring have been obtained. The results of experimental research will provide an opportunity for theoretical justification and practical

development of ways of metabolic correction of negative manifestations of radiation-induced damage to the genome in the body of the offspring of irradiated parents. Given the dynamics of changes in acetylcholinesterase activity during the aging of animals, we concluded that prolonged exposure to ionizing radiation at a total dose of 0.75 Gray contributes to the strengthening of involuntional changes in the functional state of the small intestine.

**Key words: low doses of ionizing irradiation; experimental animals; small intestine; offspring of irradiated parents.**

**Introduction.** Currently, radioactive contamination of the environment has become one of the most important environmental factors. Almost 20% of the average annual effective dose a person receives is from man-made sources. In this regard, the problem of the possible consequences of the global and rapid change in the level of radiation load on the biosphere for all living things is of particular importance.

A deeper understanding of the regularities of the biological action of small doses of radiation will make it possible to assess their significance for the viability of organisms, the degree of risk, and possible ways of adaptation of living organisms to increased radioactive contamination of the environment. To predict possible biological effects, it is necessary to search for metabolically important parameters for a more complete assessment of the small doses chronic action. One of these promising areas is the study of lipid peroxidation processes, a process that occurs in all types of membranes and plays an important role in the regulation of cellular metabolism under normal conditions and under the action of damaging factors, including ionizing radiation.

In recent years, the concept of the reactions of various biological systems (from the cell to the population) to the effect of IR in small doses has fundamentally changed. Unlike IR high doses, which causes significant clinical disorders and can lead to the death of the body, low-intensity irradiation is not fatal, but is capable of modifying cellular and tissue processes, which ultimately leads to changes in many vital functions. At the same time, the risk of manifestation of negative consequences of the action of low-intensity IR in doses considered safe has not yet been subjected to special study, however, there are indications of the possibility of its significant increase in comparison with the expected results of extrapolation of biological effects from the region of high to the region of low doses.

Today we discuss the question of "critical" organs and tissues, "critical" cells and cellular structures, "critical" metabolic processes that form the primary response to any adverse factor, both external and internal environment. Such "critical" systems at the

metabolic level include the processes underlying endogenous radioresistance and nonspecific resistance, take an active part in the neutralization and disposal of excess oxygen-reactive intermediates formed after exposure to  $\gamma$ -irradiation.

At the molecular level, the above systems are provided by redox transformations of high- and low-molecular-weight thiol compounds, which form the so-called thiol-disulfide system.

Prolonged exposure to ionizing radiation in small doses gradually causes a decrease in the endogenous reserve of thiol-dependent systems and their complete depletion, which negatively affects the body's resistance to adverse environmental factors.

The digestive system is one of the body's radiosensitive systems. Even under the influence of small doses of  $\gamma$ -irradiation in the gastrointestinal tract there is a wide range of morpho-functional changes that lead to fluid loss, electrolytes, protein, digestive disorders, absorption of nutrients, excretion of endotoxins and exotoxins. The combination of these disorders determines the role of the gastrointestinal tract in the adaptive responses of the body as a whole.

As a result of the action of  $\gamma$ -irradiation in organs and tissues, the generation of free radicals increases uncontrollably, and not only lipid peroxidation but also proteins intensify. The latter is of particular importance, given the participation of proteins in the structure of receptors, functioning of enzyme systems, regulating metabolism at the body, organ and tissue levels.

Because acetylcholinesterase (AChE) contains SH groups, the possibility of inactivation of the enzyme is not excluded with the possible free radical oxidation of sulfhydryl groups. The latter can in turn lead to changes in the transmission of nerve impulses and, accordingly, to disruption of the small intestine. The aim of the study was to study the activity of AChE in the small intestine of rats - males and females of different ages - both under physiological conditions and under conditions of prolonged  $\gamma$ -irradiation in a total dose of 0.75.

**The objective:** to study the activity of AChE in the small intestine of rats - males and females of different ages - both under physiological conditions and under conditions of prolonged  $\gamma$ -irradiation in a total dose of 0.75 Gray.

**Object** of the study. Experimental studies were performed on 510 rats of different ages and sexes of the Wistar line. All animals were kept under standard conditions and on the standard diet of the vivarium of Odessa State Medical University. Groups of animals subjected to  $\gamma$ -irradiation were formed from healthy mature males and females aged three

months and a body weight of 180-200 g. The selection of animals was performed per 1 male 4-5 females. For the experiment, females were selected so that they were at the same stage of the estrous cycle, which was determined using vaginal swabs.

Total  $\gamma$ -irradiation was performed on the basis of the X-ray therapeutic department of the Odessa Regional Oncology Center, using the gamma therapeutic unit "AGAT-R" №83 (isotope  $^{60}\text{Co}$ ). To irradiate the animals, they were placed in specially made cages made of organic glass. Irradiation was performed on the vanity at 9 o'clock in the morning. To obtain a total dose of 0.75 Gray irradiation was performed under the following technical conditions: dose rate 107 rad / min, the distance from the source to the field was 75 cm, field size 20 • 20 cm, single dose 0.15 Gray, exposure 8 seconds, amount repetitions-5, every 72 hours. The dosimetric control was carried out by the dosimetric service of the Odessa regional oncological dispensary. Animals were removed from the experiment under ether anesthesia by rapid decapitation. The activity of AChE was determined by potentiometric method according to the formula:

$$A_{A.E} = \frac{pH \cdot 40}{t \cdot B} = \mu\text{mol of acetylcholine per 1 min per 1 g of tissue.}$$

When working with experimental animals, bioethics was observed in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the provisions of the Rules of Conduct for Experimental Animals of August 12, 1997, as well as the "Common Ethical Principles of Animal Experiments" approved by the First National Congress on Bioethics (Kyiv, 2001) and the Law of Ukraine "On Protection of Animals from Cruelty" (Law of February 21, 2006 № 3447 -IV, edition of December 9, 2015, grounds 766-19).

**Results.** The activity of AChE in the small intestine in 18-day-old embryos was  $367.8 \pm 18.4 \mu\text{mol} / \text{g}$ .

Studies of AChE activity in the small intestine in 2-day-old rats showed that it increased compared to similar values in 18-day-old embryos. This increase in AChE activity is obviously aimed at adapting the small intestine to new living conditions and increasing its motility due to the supply of exogenous products (milk). The activity of AChE in the small intestine of 14-day-old embryos by 23.4% and did not differ from similar values in two-day-old rats (Table 1).

In the small intestine of 14-day-old intact females, AChE activity outperformed males by 10.8%. AChE activity in the small intestine of 1-month-old males also increased relative to all previous indicators and at the same time prevailed the level of 18-day-old embryos by

3.4% (Table 1). AChE activity in the small intestine of 3-month-old males also did not differ from 14-day-old and 1-month-old rats and at the same time was significantly lower than in 2-day-old animals and was 25.3% higher than in 18-day-old embryos.

Table 1.

Acetylcholinesterase activity in the small intestine of males and females obtained from intact rats ( $M \pm m$ ,  $\mu\text{mol} / \text{g}$  of small intestine tissue;  $n = 10$ )

Experiment conditions		AChE activity
14-days-old	M	453,86±22,69*123,4
	F	502,0±25,09**110,6
1 -mnth-old	M	494,3±24,7*134,4
	F	610,4±30,5***121,6
3-mnth-old	M	492,0±24,6108,4
	F	554,03±27,7***110,3
6-mnth-old	M	479,3±23,96105,6
	F	565,3±28,3***112,6
12-mnth-old	M	352,2±17,6**77,6
	F	447,8±22,4***89,2

AChE activity in the small intestine of 3-month-old females decreased significantly compared to the previous indicators, but at the same time was 10.3% higher than 14-day-old females and 12.6% higher than the level of age-old males.

The activity of AChE in the small intestine of 6-month-old intact males did not differ from similar indicators of 14-day-old and 3-month-old animals of this sex and was at the same time significantly lower than the value of 1-month-old. AChE activity in 12-month-old males was 26.5% lower than in 6-month-old animals.

Thus, in aging there were changes in AChE activity, i. e. there was a decrease in nonspecific resistance, increased involucional changes in the small intestine, changes in its functional state, resulting in decreased nerve impulses and the intensity of peristalsis. In addition, in the process of aging the body observed an increase in gender differences.

Prolonged  $\gamma$ -irradiation in a total dose of 0.75 Gray leads to ambiguous in depth and direction changes in the content of various compounds in the small intestine of males and females.

AChE activity in the small intestine of 3-month-old rats 12 days after prolonged exposure to total  $\gamma$ -irradiation at a total dose of 0.75 Gray was increased compared to the predecessors of the same-age control by 68.8%. In the small intestine of one-year-old

irradiated females, AChE activity increased relative to that of intact animals, exceeding the latter by 31.4%. Despite the fact that under physiological conditions the activity of AChE is much higher in females than in males, after  $\gamma$ -irradiation for 12 days it in the latter prevailed by 14.1% the indexes of the first. Three months after total exposure to  $\gamma$ -irradiation in a total dose of 0,75 Gray AChE activity in the small intestine of males exceeded the same indicators of one-year control by 50.6%.

Studies of AChE activity in the small intestine of 6-month-old females three months after radiation exposure showed that it increased by 19.6% relative to one-year control.

Table 2

Acetylcholinesterase activity in the small intestine of males and females irradiated at a total dose of 0.75 Gray ( $M \pm m$ ,  $\mu\text{mol} / \text{g}$  of small intestine tissue;  $n = 10$ )

Experiment conditions			AChE activity
Age of animal	Term after influence	gender	
3 mnth	control	F	492,0 $\pm$ 24,6
		M	554,03 $\pm$ 27,7
	12 day	F	830,5 $\pm$ 41,5*168,8
		M	728,0 $\pm$ 36,4*131,4
6 mnth	Control	F	479,3 $\pm$ 23,96
		M	565,3 $\pm$ 28,3
	3 mnth	M	721,8 $\pm$ 36,1*150,6
		F	676,1 $\pm$ 33,8*119,6
12 mnth	Control	M	352,2 $\pm$ 17,6
		F	447,8 $\pm$ 22,4
	6 mnth	M	481,5 $\pm$ 24,1*136,7
		F	494,8 $\pm$ 24,7*110,5

\*  $P < 0.05$  relative to the same age control

In this case, the intensity of increased AChE activity in the small intestine of irradiated males was slightly higher than similar values of age-old females. At the sixth month after the end of long-term  $\gamma$ -irradiation at a total dose of 0.75 Gray, its activity in the small intestine of 12-month-old males increased by 36.7% compared with one-year-old control. At this time, in 12-month-old irradiated females, AChE activity in the small intestine exceeded the level of age-related control by 10.5%. This stage of research was characterized by the fact that the indicators of AChE activity in the small intestine of males and females in absolute terms at this stage did not differ. Analysis of the results showed that the most significant changes in

AChE activity underwent in the small intestine of males and females 12 days after the end of the ionizing factor, and for all subsequent stages of the study was characterized by its gradual decrease. But it should be emphasized that in all cases it significantly outweighed the control.

**Conclusion.** Given the dynamics of changes in acetylcholinesterase activity during the aging of animals, we concluded that prolonged exposure to ionizing radiation at a total dose of 0.75 Gray contributes to the strengthening of involuntional changes in the functional state of the small intestine.

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