POSTRADIATION DISTURBANCES IN THE CYCLIC AMP SYSTEM OF LYMPHOCYTES OF THE MOUSE SPLEEN AND THYMUS

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In the early periods after irradiation of muscles in a dose of 800 rad changes in the activity of the enzymes of cyclic AMP metabolism are found in the lymphocytes of the spleen and thymus. The postradiation disturbance of the relative activities of these enzymes leads to changes in the steady-state intracellular cyclic AMP concentration. It is also shown that irradiation reduces the ability of lymphocytes to respond to isoproterenol by accumulating cyclic AMP.

KEY WORDS: irradiation; adenylate cyclase; phosphodiesterase; cyclic AMP level; lymphocytes.

Disturbance of the regulatory mechanisms of the cell is one of the most important aspects of the harmful action of ionizing radiation on the living organism [3]. The cyclic AMP system regulates a wide range of vitally important processes and phenomena in the cell [11]. Accordingly, the study of the state and functioning of this regulatory system after irradiation is of great importance. The writers showed previously [6] that ionizing radiation changes the activity of enzymes of cyclic AMP metabolism (adenylate cyclase and phosphodiesterase) in the liver, with a consequent disturbance of the function of the cyclic AMP system.

The object of this investigation was to study the effect of ionizing radiation of the cyclic AMP system and, in particular, on the enzymes of metabolism of cyclic AMP and its content in lymphocytes of the thymus and spleen, which are among the most radiosensitive cells in the body [1].

EXPERIMENTAL METHOD

Male SHK mice weighing 18-20 g were used. The animals were irradiated with the RUM-13 apparatus in a single dose of 800 rad, at the rate of 100 rad/min (180 kV, 15 mA, Cu 0.5, Al 1.0). The mice were decapitated 1, 3, and 24 h after irradiation and their spleen and thymus were removed. The organs were homogenized carefully in a Potter's homogenizer in buffer consisting of 5 mM KH_2PO_4, pH 7.5, containing 0.9% NaCl. The cells were filtered through four layers of Kapron tissue. The suspension of thymus cells thus prepared was a homogeneous suspension of lymphocytes. Lymphocytes from the suspension of spleen cells were separated on a Ficoll–Na amidotrizoate gradient. By means of a Pasteur pipet a solution containing 0.56 g Ficoll (from Pharmacia Fine Chemicals, Sweden), 1.8 ml 76% Na amidotrizoate (Verografin, Czechoslovakia), and 8.2 ml water was introduced beneath this suspension. After centrifugation (at 1800g, 20 min) the splenic lymphocytes were harvested from the interphase boundary. The isolated spleen and thymus cells were washed in buffer and lysed in a small volume of 50 mM Tris-HCl, pH 7.5, containing 5 mM $MgSO_4$. The lysate was centrifuged at 6000g for 15 min. Phosphodiesterase activity was determined in the supernatant and adenylate cyclase activity in the residue. Isolation of the cells and of the enzyme preparation was carried out at 0-4°C. Adenylate cyclase and phosphodiesterase activity was determined by methods described previously [4, 5]. To determine the cyclic AMP content in the lymphocytes the cells were washed in Hanks' solution, incubated for 15 min at 37°C in the same solution (10^7-$2 \cdot 10^7$ cells/ml), and then centrifuged for 2 min at 500g. The residue was lysed with 4 mM EDTA solution, heated on a boiling waterbath for 3 min, after which the denatured particles were removed by centrifugation (500g, 15 min), and the cyclic AMP level in the supernatant was determined by means of the kit from the Radiochemical Centre, Amersham (England).

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TABLE 1. Adenylate Cyclase and Phosphodiesterase Activity of Thymus and Spleen Lymphocytes of SHK Mice Irradiated in a Dose of 800 rad (M ± m)

Test object	Experimental conditions	Normal	Time after irradiation (in h)		
			1	3	24
	Adenylate cyclase, pmoles cyclic AMP/mg protein/min				
Spleen	b.a	16,9±2,7	14,2±2,5	11,0±2,2	3,9±0,9†
	I -10^{-5}M	30,6±4,8	24,4±3,5	18,5±2,8	4,4±0,5
	I -10^{-2}M	104,1±13,6	76,6±8,4	66,7±11,0	29,6±6,5
Thymus	b.a	27,8±2,0	21,3±2,4	14,8±2,0†	7,3±1,4†
	I -10^{-5}M	51,5±4,8	35,4±3,8	26,7±2,6	9,9±1,6
	I -10^{-2}M	96,7±12,0	81,2±11,3	62,7±7,1	42,7±15,2
	Phosphodiesterase, pmoles cyclic AMP/mg protein/min				
Spleen	$2\cdot10^{-7}$M*	53,8±1,5	39,7±4,3†	31,9±1,7†	8,1±0,7†
	$1\cdot10^{-4}$M*	2617±207	2084±325	1794±242†	392±65†
Thymus	$2\cdot10^{-7}$M*	21,2±0,5	30,8±1,1†	27,5±0,9†	2,6±0,3†
	$1\cdot10^{-4}$M*	997±66	1528±157†	1199±79	198±54†

Legend. b.a) Basal activity, I) isoproterenol. Here and in Table 2: *) cyclic AMP concentration for determination of enzyme activity; †) $P < 0.05$ (compared with control).

TABLE 2. Cyclic AMP Concentration in Lymphocytes of Spleen and Thymus of SHK Mice Irradiated in a Dose of 800 rad (M ± m)

Test object	Experimental conditions	Normal	Cyclic AMP level (in pmoles/10^7 cells) after irradiation		
			1 h	3 h	24 h
Spleen	s.c.	29,5±3,0	25,4±4,5	27,6±2,9	16,1±0,6†
	I $-5\cdot10^{-3}$M	42,8±3,0	32,3±6,6	32,1±1,3†	27,4±3,6†
Thymus	s.c.	46,4±7,7	35,5±3,6	14,1±1,5†	24,0±3,6
	I $-5\cdot10^{-3}$M	108,3±32,8	42,5±4,9	19,5±3,0†	35,5±7,1

Legend. s.c.) Steady-state concentration. Remainder of legend as in Table 1. Cyclic AMP level determined 5 min after addition of isoproterenol.

EXPERIMENTAL RESULTS

Basal (unstimulated) adenylate cyclase activity fell after irradiation in the thymus and spleen cells (Table 1). After irradiation, besides the basal adenylate cyclase activity, activity of that enzyme stimulated by isoproterenol and NaF also fell; initially (after 1 and 3 h) the isoproterenol-stimulated adenylate cyclase activity fell parallel with the decrease in basal activity, but 24 h after irradiation the degree of stimulation of adenylate cyclase activity by isoproterenol was only one third to one half of the normal value. The ability of F^- to stimulate adenylate cyclase activity was tested at all times after irradiation. Since NaF and the hormones stimulate adenylate cyclase in different ways [9], it can be suggested that 24 h after irradiation the mechanism of hormonal stimulation was disturbed.

A decrease in the ability of adenylate cyclase of spleen and thymus lymphocytes to be stimulated by catecholamines (adrenalins) 24 h after irradiation has been reported [10]. The workers cited did not determine activity of the enzyme in the earlier periods after irradiation.

Phosphodiesterase activity of the splenic lymphocytes decreased after irradiation (Table 1); the degree of decrease was comparable with the degree of decrease of adenylate cyclase activity observed at the same times (Fig. 1). Phosphodiesterase activity of the thymus lymphocytes showed phasic changes after irradiation: Initially it was higher than normal (1 and 3 h after irradiation), but after 24 h it was lower.

The early postradiation increase in phosphodiesterase activity in the thymocytes of rats was discovered by Zhivotova et al. [2]. These workers' findings agree with results obtained during the present investigation of lymphocytes of the mouse thymus, and this fact points to the absence of species specificity of the early increase in phosphodiesterase activity observed in the thymus cells.

After irradiation the cyclic AMP concentration in the splenic lymphocytes (Table 2) was unchanged in the early periods (1 and 3 h after irradiation) but was lower after 24 h. The cyclic AMP concentration in the

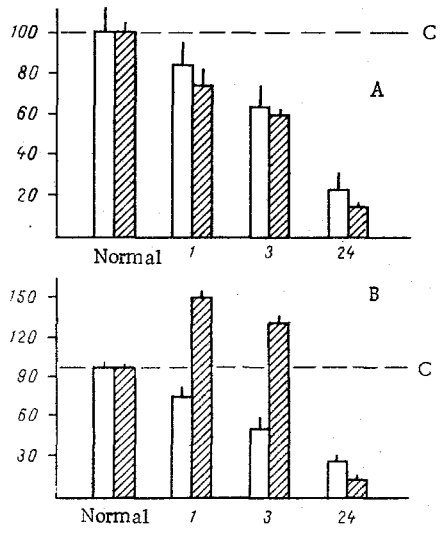

Fig. 1. Comparison of adenylate cyclase and phosphodiesterase activity in lymphocytes of spleen (A) and thymus (B) in SHK mice after irradiation in a dose of 800 rad. Abscissa (on both diagrams) time after irradiation (in h); ordinate, activity of enzyme (in % of normal). Unshaded column denotes adenylate cyclase; shaded column phosphodiesterase. C) Control line.

lymphocytes of the thymus 1 h after irradiation was reduced by 25% and 3 h after irradiation by 60%; later it rose but did not reach the normal level.

Irradiation was found not to lead to any significant increase in the proportion of cells taking up the vital stain Nigrosin (dying cells): 7-10% normally, 9-17% 1 h, 7-12% 3 h, and 14-18% 24 h after irradiation.

During the first 3 h after irradiation the cell composition of the isolated suspensions was similar to normal. Changes in the cell composition of the suspension of spleen cells in favor of small lymphocytes were observed 24 h after irradiation. By this time the cells of the spleen and thymus showed profound destructive changes. Disturbances in the cyclic AMP system observed in the late stages (24 h) after irradiation could thus be due to morphological changes in the cells.

In the splenic lymphocytes, in which the decrease in adenylate cyclase and phosphodiesterase activity in the early periods (1 and 3 h) after irradiation took place parallel so that their ratio remained approximately the same as normally, the steady-state cyclic AMP concentration remained at the normal level. In the thymus cells changes in adenylate cyclase and phosphodiesterase activity were in opposite directions: Adenylate cyclase activity was lower and phosphodiesterase activity higher than normally. The greater intensity of hydrolysis evidently also determined the steady-state cyclic AMP concentration, which fell below the control level in the thymus cells. To explain the changes in the steady-state cyclic AMP level in the cell activity of the enzymes determined in experiments in vitro was compared. Such a comparison is evidently possible because adenylate cyclase activity was determined by the use of cyclic AMP in a concentration close to that actually existing in the cell (10^{-3} M). Changes in phosphodiesterase activity were studied by the use of cyclic AMP in a concentration also close to that found in the cell ($2 \cdot 10^{-7}$ M). Changes in the activity of this enzyme of a similar character also were found when higher concentrations (10^{-4} M) were used.

Besides the steady-state concentration of cyclic AMP, its level also was determined in the spleen and thymus cells after addition to them of isoproterenol, a stimulator of adenylate cyclase. The cyclic AMP concentration (Table 2) in the irradiated cells after exposure to isoproterenol was lower than normally. After treatment with isoproterenol, cyclic AMP is known to accumulate in the lymphocytes mainly in the nucleus

[7, 8, 12]. The postradiation change in the cyclic AMP level in the lymphocytes, which was increased by isoproterenol, is therefore difficult to account for unequivocally by the use of the same approaches as used to study changes in the steady-state concentration of the cyclic nucleotide.

The findings described above suggest that the cyclic AMP system or, at least, the system regulating the cyclic AMP concentration in the lymphocytes, is damaged by ionizing radiation.

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RHODOPSIN RESYNTHESIS IN RATS WITH HEREDITARY RETINAL DYSTROPHY

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Rhodopsin resynthesis was studied in vivo in the retina and optic cup of two strains of rats with hereditary dystrophy: Campbell albino rats and Hunter rats with pigmented eyes. Wistar and MSU rats, respectively, were used as the controls. The rate of reduction of rhodopsin after its decolorization in the retina in the affected animals was shown to be much slower than in healthy animals and to decrease as the disease developed. In the period of marked morphological changes, only 50% of the decolorized pigment was reduced during 2 h of dark adaptation (the time for complete regeneration of rhodopsin in healthy rats). In Campbell and Hunter rats the breakdown and resynthesis of rhodopsin take place not only in the retina, but also in the layer of fragments of outer segments of the photoreceptors, located between cells of the pigmented epithelium and the retina.

KEY WORDS: rhodopsin resynthesis; retina; hereditary retinal dystrophy.

One of the manifestations of hereditary retinal dystrophy – a serious disease leading to blindness – is a change in the quantity of visual pigment (rhodopsin) in the retina [3, 5]. If animals with retinitis pigmentosa are kept in darkness, development of the disease is considerably retarded [3, 4], evidence of serious disturbances in the systems responsible for resynthesis of rhodopsin after its decomposition under the influence of light. This is confirmed by changes observed in the activity of enzymes concerned in rhodopsin resynthesis [5]. A characteristic feature of retinitis pigmentosa in rats is the formation of a layer of "fragments" of the

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