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The role of receptors in radiation hormesis

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Summary. The response of the receptor of plant origin, phytochrome and the receptor of rat lung cytoplasmic membrane adenylate cyclase to chronic, growth- and development-stimulating low-dose γ-radiation (0.36 and 0.036 cGy/day) has been studied. The influence of radiation results in sensitization of receptors to specific effectors and receptor-mediated stimulation of corresponding enzymes.

Introduction

Based on the literature data and our observations, the conclusion has been made [7] that the low-dose γ-radiation-induced stimulation of cell division, growth and development of organisms, increase of resistance to unfavourable environment, is not an accidental phenomenon but follows a common low, just as the harmfull effect of high doses of γ-radiation on biota. This phenomenon, called hormesis, has been analyzed in detail by Luckey [15] and discussed lively [21, 19, 6, 16].

Taking into account the opposite effects of high and low doses of γ-radiation, the hypothesis was put forward that the molecular-cellular mechanisms underlying these processes should also be different [8]. At present it is widely believed that the ionization of molecules is the key mechanism of the damaging action of high-dose γ-radiation, leading to direct or indirect destruction of cell nucleus informational molecules.

According to our hypothesis, the determining event in the stimulatory effects of low-dose γ-radiation is the excitation of receptor molecules incorporated in condensed ordered membrane structure [9].

According to refs. [1, 2], single acts of excitation in these structures may form solitons and plasmons, capable of changing the receptor conformation as specific receptors do. The excited receptor would increase the activity of the corresponding enzymes and become more sensitive to the action of specific effectors. It was proposed that these mechanisms underlie the stimulation of normal physiological processes.

The aim of the present work was to obtain experimental support for our hypothesis. The experiments were carried out using plant tissue where the excitation of the receptor-phytochrome with red light (660 nm) leads to the synthesis of phenylalanine ammonia lyase (PAL) and carotenoids, and plasma membrane from rat lung cell where adenylate cyclase (Ac) receptor regulates synthesis of cyclic adenosine-3,5-monophosphate (c-AMP) [23].

Materials and methods

The experiments were carried out in a chamber, $2 \times 2 \times 2$ m, which is schematically shown in Fig. 1. A lead container-collimator with a ^{137}Cs source of γ-radiation with an activity of 3×10^8 Bq was placed on the table in the chamber. With the source in the working position, the collimator generated a taper of the irradiated space with a spatial angle of about $\pi/2$ steradian.

Our calculations and the measurements using a scintillation radiometer with the sodium-iodide crystal indicated that at points 1, 2 separated from the source by a distance of 40 cm and 1 m 26 cm, respectively (Fig. 1), the dose absorbed by biological object per day was 0.36 and 0.036 cGy, i.e. 500 and 50 times higher than the irradiation level in control objects (7.2×10^{-4} cGy/day) which were placed in the same chamber behind the additional lead wall, 5 cm thick, (Fig. 1 K) and which were protected from below against scattered radiation by a lead sheat, 14 mm thick. The temperature at all points of the chamber was held at $22 \pm 2°$ C.

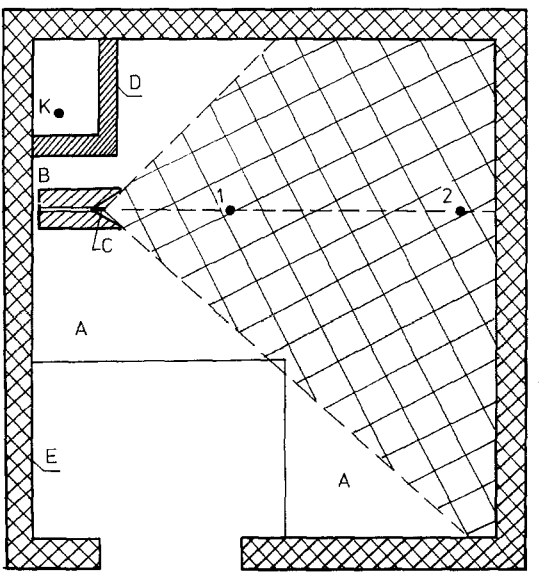

Fig. 1. Scheme of the chamber for chronic γ-irradiation. 2×2 m. A – table for experiments. B – lead container-collimator. C – radiation source (^{137}Cs, $3 \cdot 10^8$ Bq). D – lead wall, 5 cm thick. E – brick wall of the chamber, 10 cm thick. K – location of control specimens, 1, 2 – zones where experimental specimens were placed. The irradiated area is indicated by cross-hatching

The upper cover of the chamber was made of glass above which an illuminator consisting of luminescent lamps of ЛБ type was placed. At the required moment the samples were evenly illuminated with 300 lx ± 10%, which was controlled with a luxmeter.

In experiments on phenylalanine ammonia-lyase (PAL) synthesis, 1 mm slices of resting potato tubers (*Solanum tuberosum L.*) were used. Preparation of potato slices and determination of PAL activity were carried out by the procedure described in [4] except that the slices were prepared in the weak green light, placed in a monolayer into Petri dishes, wrapped in black photographic paper and exposed to γ-radiation for 22 h. Slices kept in the dark without irradiation, and slices exposed to light of 300 lx ± 10% served as control. One unit of activity was defined as the amount of enzyme which produced an increase in absorption at 290 nm of 0.01 per hour in the standard assay. This change in absorption is equivalent to the formation of approx. 1 μg of trans-cinnamate per 1 ml of the reaction mixture.

In the experiments on synthesis of carotenoids, seedlings of mustard (*Brassica nigra L.*) were used. Mustard seeds were wrapped in filter paper, placed into glasses with tap water and allowed to germinate for 9 d in complete darkness in zones 1, 2 and K of the chamber (Fig. 1). Grinded leaf mass (0.5 g) was exposed to a dim safety light and a lot of substances were extracted with cold 80% acetone. The intensity of carotenoid synthesis was estimated according to [5] by the absorption maximum at 450 nm. The absorption spectra of the extracts were taken on a "Specord-UV vis" spectrophotometer.

Experiments aimed to study the effect of chronic lowdose γ-radiation on the activity of the adenylate cyclase (Ac) system were carried out on plasmatic cell membranes from lung tissue of Wistar line rats. 21-day-old males were placed to the chamber, in zones 2 and K (Fig. 1), 10 animals in each zone, where they were maintained on a standard diet at $t = 22 \pm 2°$ C and exposed to light for 9 h per day. On days 14 or 28 the animals were killed by decapitation, the thorax was dissected. The lung tissue was washed from blood by perfusing 20 ml of 0.9% cold saline through the pulmonary artery. Isolated tissues were chilled in liquid nitrogen and stored till the start of experiments.

The membrane fraction of the rat lung tissue was obtained at $t = 4°$ C. Preliminary defrosted and grinded lungs from one animal were homogenized in a buffer (1:6) in a homogenizer of the Polytron type. The buffer contained 20 m*M* *Tris*-(hydroxymethyl)aminomethan hydrochloride (*Tris*-HCl), 1 m*M* ethylenediamine-tetraacetic acid (EDTA), pH 7.5 at $t = 4°$ C. The homogenate was filtered through a nylon filter and centrifuged for 20 min at 2000 × g. The pellet was suspended in a Potter homogenizer in a buffer and then centrifuged under the same conditions. The obtained pellet was resuspended in a ratio 1:2 in buffer and used for Ac activity assay by the method described in [24] which is based on the enzymatic reaction of the formation of the labeled ^{14}C-cAMP from the labeled adenosinetriphosphate (^{14}C-ATP) followed by separation of nucleotides by thin-layer chromatography. The incubation medium for Ac activity assay contained in the final volume of 50 mkl: 50 m*M* *Tris*-HCl (pH 7.5 at $t = 37°$ C), 0.1 m*M* ATP, 20 m*M* creatine phosphate, 0.3 mg/ml creatine kinase, 5 m*M* $MgCl_2$, 2 m*M* cAMP, 10 m*M* theophylline, ^{14}C-ATP-(1–2)·10^6 imp/min and 20–30 μl of the membrane fraction, the protein content in which did not exceed 60–100 μg. The beta adrenergic agonist D,L-isoproterenol-hydrochloride at a concentration of 0.2 m*M*, and 10 m*M* NaF were used to stimulate the

Ac system. The cyclic ^{14}C-AMP was isolated by thin-layer chromatography using Silufol UV 254 plates preliminarily impregnated with sodium tetraborate (in 0.25 *M* solution of $Na_2B_4O_7 \cdot 10\ H_2O$). The amount of ^{14}C-cAMP was measured with a SL-30 liquid-scintillation counter ("Intertechnique", France). The activity of the enzyme was expressed in pmol ^{14}C-cAMP per min and per mg protein. Protein was estimated by the method of Peterson [18].

Results and discussion

The experiments with germination of resting seeds of *Lactuca sativa L.* and formation of flavonoids in etiolated seedlings of mustard gave indirect evidence that low-dose chronic γ-radiation has a stimulating effect on the receptor of plant origin phytochrome [10]. Based on the works which demonstrated that phytochrome when illuminated with visible light (660 nm) undergoes excitation and via intermediary messengers induces synthesis of PAL and carotenoid [22], we carried out experiments in order to determine whether low-dose chronic γ-radiation activates the phytochrome-dependent processes in plants. In the first set of experiments we studied the synthesis of PAL in slices of resting potato tubers. The results of four replications are presented in Table 1. It is seen that the receptors respond to the 22 h γ-irradiation at a dose rate of 0.36 cGy/day just as they do in the case of low-intensity visible light inducing synthesis of PAL.

In the second set of experiments, the activation of the phytochrome was estimated by the phytochrome-induced synthesis of carotenoids in etiolated seedlings of mustard. In Fig. 2 are shown the spectra obtained in one of the experiments. The fourfold repetition of the experiments gave the same results. It is clearly seen that whereas etiolated seedling grown in complete darkness contain a small amount of carotenoids (curve K), the amount of carotenoids in seedlings grown under similar conditions but exposed to γ-radiation at a dose rate of 0.036 cGy/day increases markedly (curve 2) ($p < 0.01$). A more intense irradiation (0.36 cGy/day) also induces stimulation of synthesis (Curve I), however, to a lesser extent, which is typical for hormesis. To make sure that phytochrome contributes to the increased synthesis of carotenoids observed under γ-radiation, the experiment was modified. After growth in complete darkness for 8 days, a part of seedlings were illuminated with visible light (300 lx), then they were

Table 1. The effect of low-dose chronic γ-radiation on synthesis of PAL

Experimental conditions	Activity of PAL		
	units per g of wet weight	% of control	P
Slices kept for 22 h in darkness (control)	10.1 ± 2.2	100	
Slices after 22 h γ-irradiation at a dose rate of 0.36 cGy/d	32.8 ± 1.71	335 ± 78	<0.05
Slices after 22 h illumination with visible light 300 lx $\pm$ 10%	38.1 ± 2.68	377 ± 64	<0.01

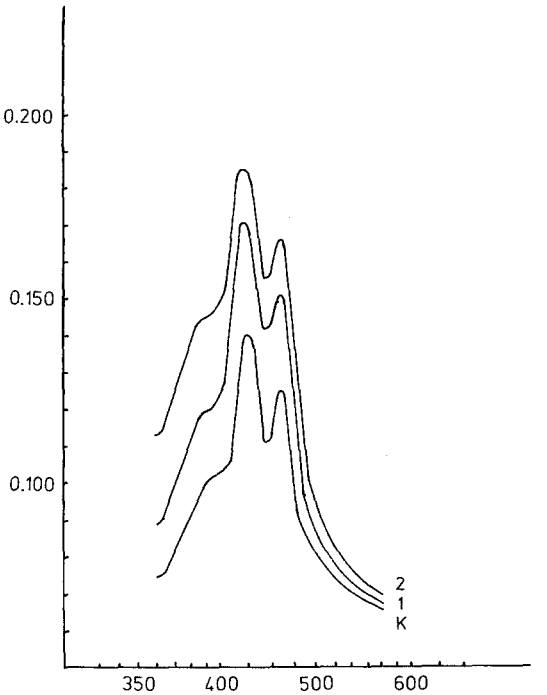

Fig. 2. Absorption spectra of carotenoids from mustard seedlings. On the abscissa – wavelength in nm. On the ordinate – optical density. The experiment was carried out during 9 days in complete darkness. K – control, 1 – 0.36 cGy/day, 2 – 0.036 cGy/day

Table 2. Intensity of synthesis of carotenoids in 9 d mustard seedlings under different irradiation conditions

Number of experiment	Experimental conditions	Intensity of carotenoid synthesis	
		optical density in the absorption maximum (450 nm)	in % of the control in darkness
1	Darkness (control)	0.140±0.005	100
2	Darkness, γ-irradiation, 0.36 cGy/day	0.170±0.009	121
3	Darkness, γ-irradiation, 0.036 cGy/day	0.180±0.008	129
4	Darkness for 8 days, illumination with visible light (300 lx) for 15 min and for 1 day again in darkness	0.185±0.007	132
5	Conditions as in (4) plus γ-irradiation at a dose rate of 0.036 cGy/day	0.230±0.01	164

allowed to stand one day more in the darkness. The results of threefold repeats of the experiments are presented in Table 2.

It follows from the table that chronic γ-irradiation at a low dose rate (0.036 cGy/day) for 9 days exerts a similar stimulating effect on the phytochrome

Table 3. Effect of chronic γ-radiation (0.036 cGy/day) on the activity of Ac of plasmatic membranes from rat lung, pmol cAMP/(mg·min)

Duration and time of experiment	Total dose, replication	Basal activity	% of control	Isoproterenol-stimulated activity	% of increase	% of control	P	Activity in the presence of NaF	% of increase	% of control
14 days July 1989	control n=4	7.64±0.78	100	23.4±2.28	306	100	–	79.2±4.34	1076	100
	0.5 cGy n=4	12.3 ±1.25	160	45.4±5.09	370	194	<0.01	84.1±6.54	690	106
28 days April–May 1990	control n=7	11.3 ±1.96	100	32.9±4.14	291	100	–	119.4±5.33	1144	100
	1.0 cGy n=7	16.1 ±2.37	142	55.7±5.41	346	169	<0.001	149.6±9.81	981	124

as a 15 min illumination with the natural effector, the low-intensity visible light (300 lx). The γ-radiation-activated phytochrome responds more intensively to the natural effector (experiment 5).

The activation of the phytochrome-dependent processes observed upon exposure to low doses is, probably, essential for stimulation of growth and development of plants reported earlier for these conditions [11].

In the third set of the experiments we tried to determine whether the effects observed on plant receptors are peculiar to receptors of animal origin. We studied the activity of Ac from plasmatic membranes of rat lung, which is known to be controlled by the receptor of the Ac system [23].

Earlier it has been shown that Ac from isolated cytoplasmic membranes of liver cells exposed to acute γ-radiation is sensitive to doses of about 1–2 Gy [12]. In the present work we studied the effect of chronic low-dose γ-radiation (0.036 cGy/day) on basal activity of Ac and the activity of Ac in membranes treated with isoproterenol, a specific effector of the Ac system, and with fluorine ions which activate Ac via G-proteins [23]. The results obtained are presented in Table 3.

It is known from the data reported in literature that irradiation of rats with a lethal dose (8 Gy) decreases abruptly the basal activity of Ac in lymphoid cells and eliminates almost completely the response of the receptor to isoproterenol [23]. We studied the effect of the lethal dose (7.5 Gy) on the activity of Ac of membranes from rat lung and also found a decrease in its basal activity and the isoproterenol-stimulated activity. Chronic irradiation for 16 days at a much greater dose rate (12.9 cGy/day) also caused a reduction in the basal activity of Ac from 23.3±3.04 pmol/mg·min in the control to 11.3±1.03 pmol/mg·min in the experiment, and in the intensity of the response of the receptor to isoproterenol (42% of the control). The performed experiments indicate that the phenomenon of radiation hormesis, i.e. diametrically opposite response of the organism to high and low doses and dose rates of γ-radiation, also manifests itself in the functioning of the Ac system.

It has been shown earlier [20] that γ-irradiation of young rats with a total dose of 2.88 cGy for 24 h increases markedly the rate of growth and development.

The weight of the rats accounted for 120% of the control by the end of the experiment. Increasing the dose from 14.4 to 144 cGy did not affect the rate of weight increase. Only at doses exceeding 150 cGy this parameter decreased.

Comparison of these experiments indicates that the increase in the basal activity of Ac and the response of its receptor to the natural effector can be considered as beneficial for the normal physiological development of animals. Taking into account that the observed phenomena are induced by receptors via intermediate messengers [22, 23], it may be assumed that each of them can be activated by irradiation. However, the increased intensity of response of the receptors to specific effectors, the similarity of the results obtained with Ac and PAL, with the intermediate messengers being different, the absence of the effect on activation of Ac with sodium fluoride which is known to affect the G-proteins rather than the receptor, all are consistent with the hypothesis that at the basis of the observed effects is the radiation-induced activation of the receptor.

To summarize, one should note, first of all, a high sensitivity of the receptors of the membrane-bound enzymes, both of plant and animal nature, to chronic γ-radiation of low doses comparable with the increased background radiation. For the studied time intervals and dose rates γ-radiation induces excitation of the receptor (an enhanced susceptibility to natural effectors) rather than damage to the receptor and inhibition of its functions. These findings are in a full agreement with the works indicating a high sensitivity of different receptors of the central nervous system which also respond to low doses of γ-radiation with a physiologically normal reaction [14].

The results obtained confirm the hypothesis put forward by Kuzin [9] that the necessity of background radiation for the existence and development of the biota may be partially explained by its ability to maintain numerous receptors of the organism in a low-excited state necessary for reception of external effectors and for physiologically normal growth and development.

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