RADIATION MODULATION OF ACTIVITY OF SOME ENZYME SYSTEMS OF ISOLATED PLASMA MEMBRANES DURING EARLY ONTOGENY

L. V. Slozhenikina, T. E. Ushakova,
L. P. Mikhailets, and A. M. Kuzin

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Activity of adenylate cyclase (ADCase), cyclic AMP phosphodiesterase (PDE), and 5'-nucleotidase was investigated in plasma membranes from the liver of 20-day rat embryos, either normal or exposed to ionizing radiation. Irradiation of the plasma membranes with gamma-rays in doses of between 0.1 and 100 kR was shown to reduce ADCase activity, and to have a more marked effect, in the case of larger doses, when accompanied by stimulation by isoproterenol. The 5'-nucleotidase and PDE activity was unchanged under the influence of doses up to 100 kR.

KEY WORDS: Gamma-irradiation; adenylate cyclase; cyclic AMP phosphodiesterase; 5'-nucleotidase, rat liver, ontogeny.

An important role in the development and formation of the radiation effect is ascribed to disturbance of the regulatory properties of enzymes of cell membranes. A special place among biological membranes is occupied by the plasma membrane. Because of the presence of an adenylate cyclase complex, this membrane is responsible for the formation of cyclic AMP, the universal regulator of a wide variety of processes. The presence of the enzyme cyclic AMP phosphodiesterase (PDE), which hydrolyzes cyclic AMP [5], has also been demonstrated in the plasma membrane. Recently increased interest has been shown in the study of the action of ionizing radiation in plasma membranes and, in particular, of the activity of enzymes of cyclic AMP metabolism [8, 10]. However, no information of this sort is available for the early period of development of the organism, when radiosensitivity is increased.

This paper gives data on the effect of gamma-irradiation of plasma membranes from the rat liver in the prenatal period of development of adenylate cyclase (ADCase), PDE, and 5'-nucleotidase activity.

EXPERIMENTAL METHOD

The plasma membranes were isolated from the liver of 20-day Wistar rat embryos [9]. The membranes were irradiated in doses of 100 to 1000 R on the GUPOS ^{137}Cs apparatus (dose rate 365 R/min) and in doses of 10 to 100 kR, on the RKH apparatus (^{60}Co, dose rate 7.8 kR/min). The incubation medium for determination of ADCase activity contained (in mM): glycyl-glycine buffer, pH 7.5, 50; theophylline 10, $MgCl_2$ 5, ATP 1, creatine phosphate 25, and creatine kinase (300 μg/ml), and when necessary it also contained 0.2 mM isoproterenol. The cyclic AMP thus formed was determined by the method described previously [4], in the modification of Tovey et al., [11]. Cyclic AMP-binding protein was isolated from rabbit muscles [11]. BDE activity was determined [1] in the presence of 0.2 μM cyclic AMP. To determine 5'-nucleotidase activity medium of the following composition was used (in mM): Tris-acetate buffer, pH 7.6, 100, 5'-AMP 4, $MgCl_2$ 4. Inorganic phosphorus [2] and protein [7] were determined. Radioactivity was measured by the SL-30 liquid scintillation counter (Intertechnique, France).

EXPERIMENTAL RESULTS

The results showed (Fig. 1) that irradiation of the plasma membranes from the liver of 20-day rat embryos with gamma-rays leads to changes in ACDase activity. For instance, the basal (in the absence of stimu-

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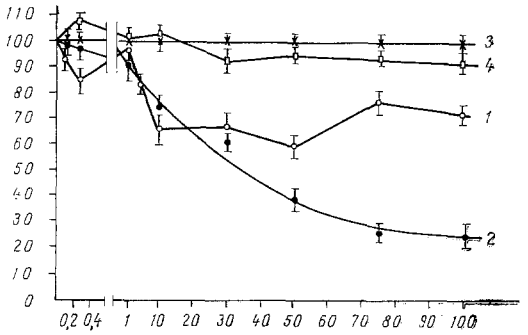

Fig. 1. Effect of gamma-ray irradiation on enzyme activity of plasma membranes. Abscissa, dose of irradiation (in kR); ordinate, enzyme activity, (in % of control); 1 and 2) ADCase in absence and presence of isoproterenol (0.2 mM) respectively; 3) 5'-nucleotidase; 4) PDE. Specific activities (per milligram protein) of enzymes of unirradiated membranes; 1) 27.81 ± 3.32 pmoles cyclic AMP/min; 2) 197.45 ± 11.87 pmoles cyclic AMP/min; 3) 34 μmoles P_{inorg}/h; 4) 33.45 pmoles 5'-AMP/min.

ulators) activity fell somewhat (by 15%) after a dose of 250 R. A considerable fall (by 35%) occurred after irradiation of the membranes in a dose of 10 kR. A further increase in the dose of irradiation to 100 kR led to no significant changes. Isoproterenol-stimulated ADCase activity showed exponential type of dose dependence, and amounted to 20% of the control with a dose of 100 kR. The high level of inhibition of hormone-stimulated ADCase activity compared with the basal level at high doses of irradiation (50-100 kR) is noteworthy. It is evidence of the greater vulnerability to irradiation either of the coupling element of the adenylate cyclase complex or of its regulatory subunit of the β-adrenoreceptor. Inhibition of ADCase activity has been demonstrated [8] during irradiation of plasma membranes from the liver of adult animals with high doses. However, these data do not clear the matter up completely, for we know that ADCase activity is inhibited during keeping of the preparation of 0°C [3]. Under the experimental conditions used by the authors cited, however, because of the low dose rate (429 R/min), irradiation continued for a long time. It is therefore quite possible to suggest that the inhibition observed may not have been attributable to the action of ionizing radiation. Since the irradiation in the present experiments was short in duration (in a dose of 100 kR it did not last more than 13 min), the time factor of inactivation can be ruled out and it must be assumed that the effect in this case was attributable to irradiation. When the present results are compared with those described in the literature [10], the higher radiosensitivity of ADCase from embryonic liver compared with adults will be noted. For instance, a dose of 10 kR inhibited basal activity of ADCase by 35% (Fig. 1), whereas approximately the same inhibition in the adult rat liver was observed after a dose of 36 kR.

The results of investigation of PDE and 5'-nucleotidase activity of plasma membranes from the liver of 20-day rat embryos are also shown in Fig. 1. Irradiation of isolated membranes in doses of up to 100 kR caused no deviation in the activity of the enzymes studied. Irradiation of plasma membranes from the adult rat liver with high doses, incidentally, likewise did not change 5'-nucleotidase activity [8, 10].

Enzyme systems bound with the plasma membranes thus react differently to ionizing radiation. Data which would explain this difference in radiosensitivity are not yet available. Since the problem of the role of lipids in the function of 5'-nucleotidase has not yet been settled [6], it can be tentatively suggested that irradiation affects the lipid component of the plasma membranes, and this is accompanied by changes in lipid-dependent enzymes, a group to which ADCase belongs.

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CHARACTERISTICS OF STEROID HORMONE BINDING SYSTEMS IN HEPATOCYTE PLASMA MEMBRANES

Yu. P. Denisov, P. V. Sergeev,
N. P. Saksonov, and G. V. Shutko

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Parameters of binding of cortisone and estradiol to plasma membranes of rat hepatocytes were studied by the method of liquid scintillation radiometry. The presence of two systems for the binding of these hormones in the membranes was demonstrated. One system is specific (saturable) and binds hormones in physiological concentrations. The capacity and affinity of this system for cortisone are significantly higher than for estradiol. The binding parameters within the temperature range from 4 to 37°C for cortisone and estradiol respectively are: Dissociation constant 2.1-3 and 2.7-4.5 nM, number of binding sites 2-2.4 and 0.14-0.18 nmoles/mg protein. Experiments with p-chloromercuribenzoate demonstrate the role of proteins in the working of this system. The second (unsaturable) system is nonspecific and its function is determined by the lipid component of the membranes. The affinity of corticosteroids for hepatocytes is probably due to the activity of the (saturable) specific system of the plasma membranes of these cells.

KEY WORDS: plasma membranes; steroid hormones; binding systems; binding parameters.

An important role in the realization of the action of steroid hormones on the cell is ascribed to its membranous structures and, in particular, the membranes of the intracellular organelles. Meanwhile one of the factors limiting the entry of steroids into the cell, transport through the plasma membrane, still remains virtually unknown. Only recently has evidence been obtained that the action of steroid hormones is mediated through their specific binding to the plasma membranes [1, 5-7]. In the present writers' opinion, this binding in hepatocyte membranes is due to the functioning of a membrane system which has been called the "system of preference for corticosteroids" [2].

The investigation described below was devoted to a study of certain parameters of the functioning of this system.

EXPERIMENTAL METHOD

Plasma membranes were isolated by the method of Dorling and Le Page [3] from the liver of female albino rats weighing 150-200 g. The purity of the membrane fraction was verified by determining activity of the marker enzyme 5'-nucleotidase [4] and electron-microscopically. Steroid hormones (cortisone-1,2-^{3}H and estradiol-6,7-^{3}H, from IRA) were incubated with a suspension of membranes (protein concentration 200 μg/ml) for 1 h. After sedimentation of the membranes on the VAC-601 centrifuge the supernatant was sampled in doses of 0.1 ml for scintillation analysis on the Intertechnique (France) apparatus. The SH-groups of the membrane proteins were blocked by p-chloromercuribenzoate (PCMB). The results were subjected to statistical analysis by Student's method.

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