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CYCLIC AMP METABOLISM IN THE LIVER OF NEWBORN RATS AFTER IRRADIATION DURING ORGANOGENESIS

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The level of adenylate cyclase (AC) and phosphodiesterase (PDE) activity and the reserves of cyclic AMP in the liver of newborn rats were investigated in normal animals and after irradiation on the 9th day of embryonic development. After irradiation in a dose of 50 R the basal AC and PDE activity was reduced, but there was no change in the steady-state cyclic AMP content in the tissues. The adrenalin-stimulated AC activity showed only a tendency to fall after irradiation. It is suggested that at critical moments of development, when the hormonal inducer is present in the liver of irradiated animals the conditions may be created for an increase in the cyclic AMP reserves.

KEY WORDS: adenylate cyclase; phosphodiesterase; cyclic AMP; rat liver; ontogeny.

The role of cyclic AMP in the regulation of growth, proliferation, and morphological and enzymic differentiation has now been established [4-6, 8, 11]. Since these processes are disturbed by the action of ionizing radiation of the body, it is interesting to study the state of the cyclic AMP system in order to explain certain radiobiological effects.

There is evidence of the high radiosensitivity of the period of major organogenesis in the embryonic development of animals. Irradiation during this period, depending on dose, leads to a lower prenatal mortality than in the preimplantation period, so that it is possible to obtain progeny for investigation, although substantial disturbances of future development take place [9]. There is no information in the literature on cyclic AMP metabolism in newborn animals following irradiation of embryos in the period of organogenesis.

The basal cyclic AMP level and activity of enzymes of its metabolism, namely adenylate cyclase (AC) and phosphodiesterase (PDE), in the liver of newborn rats were investigated after irradiation on the 9th day of embryonic development.

EXPERIMENTAL METHOD

Wistar rats in heat were detected by vaginal smears and mated with males in the ratio of 2:1. The following morning was taken as the beginning of pregnancy for females found to have spermatozoa. The animals were irradiated on the 9th day of pregnancy in a dose of 50 R on the GUBÉ (cobalt-60) apparatus, with a dose rate of 23 R/min. The liver of newborn rats was used for the experiments.

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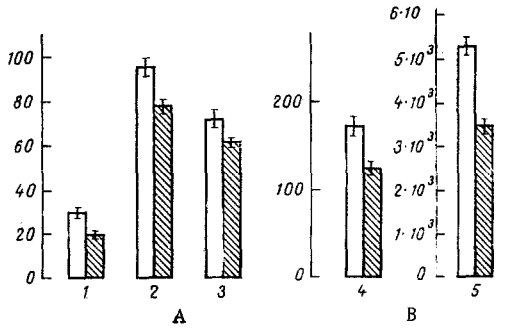

Fig. 1. Level of AC and PDE activity (in pmoles reaction product/min/mg protein) in liver of newborn rats under normal conditions and after irradiation on 9th day of embryonic development. A) AC activity: 1) in absence of stimulators; 2) in presence of adrenalin (0.1 mM), 3) in presence of NaF (10 mM); B) PDE activity: 4) in substrate concentration 1 μM; 5) in concentration 0.5 mM; difference significant ($P \leq 0.05$). Unshaded columns – control; shaded – experiment.

The liver was quickly frozen in liquid nitrogen and a weighed sample of about 70 mg was extracted with a mixture of ethanol and water in the ratio of 1:1 on a boiling water bath for 4 min. The pooled extract from three successive extractions was dried on a rotary evaporator. The dry residue was dissolved in 50 mM Tris-HCl, pH 7.5, containing 4 mM EDTA and was used after centrifugation for the determination of cyclic AMP by the method in [3] as modified in [10]. Binding protein was isolated from rabbit muscles [10]. Charcoal of Norit brand was used to adsorb cyclic AMP not bound with protein. Cyclic AMP-8-^{3}H was added to the homogenizer to allow for losses during processing.

AC activity was determined in the unpurified fraction of membranes obtained after centrifugation of the homogenate at 43,000g, as described previously by the writers [1].

PDE activity was determined in a liver homogenate made up in 50 mM Tris-HCl, pH 7.5. The composition of the incubation medium was: 50 mM Tris-HCl, pH 7.5 or 8.0, 4 mM $MgCl_2$, cyclic AMP-8-^{3}H (1.8×10^5-1.36×10^5 cpm); 0.5 mM or 1 μM cyclic AMP, 5 mM 5'-AMP, homogenate (as protein) 100 or 10-20 μg to the higher and lower concentrations of substrate respectively. Incubation for 10 min ended with heating on a boiling water bath for 3 min. The reaction product 5'-AMP-^{3}H was then isolated on Silufol-IV-254 disks by thin-layer chromatography [2].

Protein was determined as [9]. Radioactivity was measured with the SL-30 (Intertechnique, France) scintillation counter.

EXPERIMENTAL RESULTS

As Fig. 1 shows, basal AC activity (activity in the absence of any modulators) in the liver of irradiated animals was reduced by 34.6%. PDE activity also was reduced by about the same degree. The results were identical for two forms of PDE – with high and low affinity for substrate.

The changes observed in the activity of these enzymes are evidence of the slowing of cyclic AMP metabolism in the liver of the newborn animals after irradiation on the 9th day of embryonic development. It must be emphasized that the effect was demonstrated for a dose as low as 50 R. An attempt to study the effect of 200 R was unsuccessful, for this dose, if given to embryos on the 9th day of development, caused their death. It was therefore difficult to obtain progeny from the irradiated animals, in agreement with data in the literature [12].

The slowing of cyclic AMP metabolism in the liver of the irradiated animals was not accompanied by any changes in the basal cyclic AMP level. For instance, the liver of the control animals contained 1.00 ± 0.03 pmoles and the liver of the irradiated animals 0.93 ± 0.03 pmoles cyclic AMP/mg wet weight of tissue. The explanation is evidently that changes in the activity of the enzymes synthesizing and hydrolyzing cyclic AMP in the liver of the irradiated animals were of unequal magnitude.

Cyclic AMP synthesized in response to the action of stimulators is more important for functional activity in the critical period of ontogeny. So far as the AC of the liver is concerned, this refers to specific stimulators of hormonal nature, namely adrenalin and glucagon. These hormones take part in enzymic differentiation, i.e., the formation of new enzymes in the perinatal period [4].

The stimulating action of adrenalin on AC of the membrane fraction of the neonatal rat liver was investigated under normal conditions and after irradiation. As Fig. 1 shows, no significant difference was found in the activity of adrenalin-stimulated AC; there was only a tendency for it to fall in the irradiated animals. A similar picture was observed, incidentally, during activation of the enzyme by a nonspecific stimulator, namely fluoride ions.

Calculation of the activating action of adrenalin on AC showed some stimulation of the response to adrenalin after γ-ray irradiation. The percentage of activation was 321.0 ± 27.3 for the control and 417.0 ± 17.3 for the irradiated animals.

The experimental data show that irradiation of an embryo with a dose of 50 R on the 9th day of development had no adverse effect on the formation of β-adrenoreceptors or on the appearance of hormonal activation of AC. It can tentatively be suggested that in the critical stages of ontogeny, i.e., when the corresponding inducers act on the tissue, AC will synthesize cyclic AMP in the liver of irradiated animals in an amount closely similar to that in the control. A decrease in PDE activity, which as was stated above is observed in the liver of irradiated animals, will lead to the accumulation of cyclic AMP in the tissue. This in turn, evidently through cyclic AMP-dependent protein-kinases, will influence the processes controlled by this cyclic nucleotide.

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