EFFECT OF X-RAY IRRADIATION ON ACTIVITY OF KEY ENZYMES OF HEME SYNTHESIS AND DEGRADATION IN RAT LIVER

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KEY WORDS: δ-aminolevulinate synthetase; hemoxygenase; tryptophan pyrrolase; cytochrome P-450; x-ray irradiation.

A marked fall in the concentration of cytochrome P-450, one cause of postradiation inhibition of activity of the microsomal oxygenation system [1, 11], responsible for metabolism of a wide range of drugs, toxins, carcinogens, and steroid hormones, is observed in the liver of animals irradiated with ionizing radiation. Inhibition of activity of this enzyme system may contribute to further disturbances of metabolism and also may significantly modify the toxicity and pharmacokinetics of drugs in the irradiated organs. It has been postulated that the postradiation fall in the cytochrome P-450 level may be connected with intensification of destruction of this hemoprotein as a result of intensification of lipid peroxidation [2, 15]. However, the effect of radiation on cytochrome P-450 biosynthesis remains virtually unstudied.

Considering the important role of heme metabolism in the biosynthesis of cytochrome P-450 [7], in which the greater part (60-70% of the total) of the heme synthesized in the liver is utilized, it was decided to study the effect of x-ray irradiation on activity of δ-aminolevulinate synthetase and hemoxygenase – the velocity-limiting enzymes of synthesis and catabolism of heme, and also on the level of "free" (unutilized) heme in rat liver.

EXPERIMENTAL METHOD

Male Wister rats weighing 140-180 g were used. The animals were subjected to whole-body irradiation on the RUM-13 x-ray apparatus (dose rate 0.5 Gy/min, voltage 180 kV, current 15 mA, filters 0.5 mm Cu + 1 mm Al). The rats were deprived of food for 20-24 h beforehand, decapitated, and the liver was perfused in situ with 1.15% KCl.

To determine δ-aminolevulinate synthetase activity the liver was homogenized in 9 volumes of 10 mM Tris-HCl buffer, pH 7.4, containing 0.9% NaCl. The reaction was triggered by addition of 1 ml of 5% homogenate to 1 ml of incubation medium containing 200 mM glycine, 20 mM EDTA, 0.4 mM pyridoxal-5-phosphate, and 300 mM Tris-HCl, pH 7.2 [6]. Incubation continued for 1 h at 27°C. The δ-aminolevulinic acid formed was determined by the method in [12]. δ-Aminolevulinate synthetase activity was expressed in nanomoles δ-aminolevulinic acid/mg protein/h. Hemoxygenase activity was determined in liver microsomes by the method in [9] and expressed in nanomoles bilirubin formed/mg protein/h.

The level of "free" heme in the animals' liver was judged by the degree of saturation of tryptophan pyrrolase with heme [3], which was estimated from the ratio between activity of holo-tryptophan pyrrolase and total tryptophan pyrrolase activity (with saturation of the enzyme with heme). Total tryptophan pyrrolase activity and activity of holo-tryptophan pyrrolase were determined by the method in [4].

All spectrophotometric measurements were made on the Hitachi-556 spectrophotometer (Japan). The protein concentration was determined by the method in [13]. The experimental results were subjected to statistical analysis. The significance of differences was estimated by Student's test.

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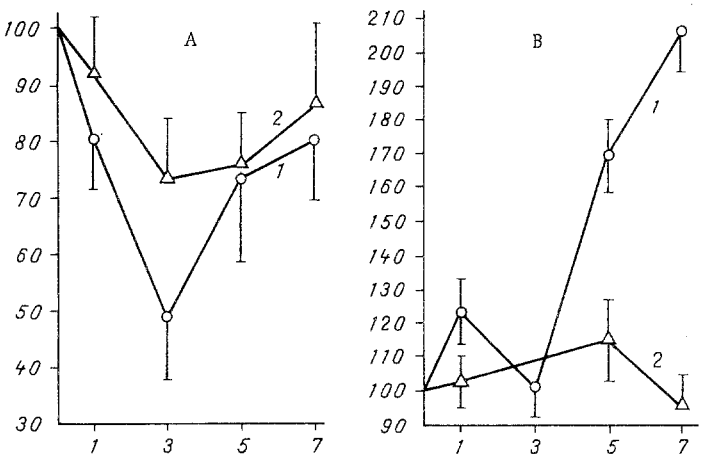

Fig. 1

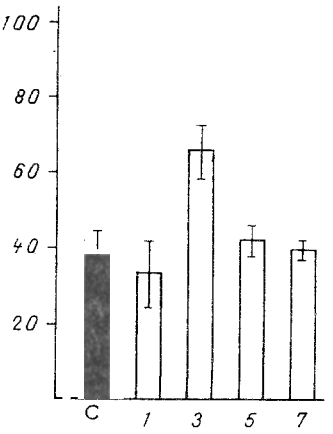

Fig. 2

Fig. 1. Changes in δ-aminolevulinate synthetase (A) and hemoxygenase (B) activity in liver of rats irradiated with x-rays in doses of 7 Gy (1) and 5 Gy (2). Abscissa, time after irradiation (in days); ordinate, activity (in % of control).

Fig. 2. Changes in saturation of tryptophan pyrrolase with heme in liver of rats irradiated with x-rays in a dose of 7 Gy. Abscissa, time after irradiation (in days); ordinate, percentage saturation of tryptophan pyrrolase with heme. C) Control.

EXPERIMENTAL RESULTS

Normally δ-aminolevulinate synthetase and hemoxygenase activity in the rat liver was 0.282 ± 0.014 and 3.74 ± 0.16 nanomole/mg protein/h respectively. X-ray irradiation in a minimal lethal dose (7 Gy) caused marked changes in δ-aminolevulinate synthetase and hemoxygenase activity in the rat liver (Fig. 1A, B). For instance, a marked fall in δ-aminolevulinate synthetase activity was observed only 24 h after irradiation (to 81% of normal), and the greatest fall took place on the 3rd day (to 49% of normal), i.e., it preceded the maximal fall of the cytochrome P-450 level observed on the 5th day after irradiation in this dose [1, 11]. Partial normalization of δ-aminolevulinate synthetase activity occurred on the 5th-7th day. Hemoxygenase activity in the initial period after irradiation (24 h) increased very slightly and for a short time, after which (on the 5th-7th day) there followed a phase of prolonged and more than twofold increase in the activity of this enzyme (Fig. 1B), coinciding mainly with the phase of a marked fall in cytochrome P-450 level [1, 11].

Irradiation of the animals in a dose of 5 Gy, which had virtually no effect on the cytochrome P-450 level, did not induce any significant changes in hemoxygenase activity (Fig. 1B) and caused a much smaller decrease in δ-aminolevulinate synthetase activity (Fig. 1A) than irradiation in a dose of 7 Gy.

The opposite changes found in δ-aminolevulinate synthetase and hemoxygenase activity are evidence that synthesis of heme is inhibited and its catabolism intensified in the liver of rats irradiated with a lethal dose of ionizing radiation. However, as experiments to determine the degree of saturation of tryptophan pyrrolase with heme showed, these disturbances of heme synthesis and catabolism do not lead to a fall in the "free" heme level in the liver of the irradiated animals. Furthermore, the increase in the degree of saturation of tryptophan pyrrolase with heme found on the 3rd day after irradiation, from 38.4 to 65.6% (Fig. 2) indicates a substantial rise in the level of unutilized heme in the rats' liver during this period. The increase in the steady-state concentration of "free" heme during the period of marked inhibition of heme synthesis may be connected with a fall in the rate of heme utilization in hemoprotein biosynthesis.

In turn, elevation of the "free" heme level may be one cause of the postradiation changes found in δ-aminolevulinate synthetase and hemoxygenase activity, for unutilized heme is a regulator of the activity of these enzymes [5, 10, 14]. The observed induction of hemoxygenase was possibly due also to increased accessibility of other heme-containing substrates for this enzyme and, in particular, as a result of intensification of autolysis and hemolysis and of lipid peroxidation in the irradiated organism. There is some evidence [8] that one of these substrates of hemoxygenase is an inactive form of cytochrome P-450. The possibility therefore cannot be ruled out that the increase in hemoxygenase activity in the liver of irradiated animals may lead to intensification of degradation not only of "free" heme, but also of cytochrome P-450.

It is difficult to give an unambiguous estimate of the role of this decrease in δ-aminolevulinate synthetase activity discovered in postradiation changes in the cytochrome P-450 level because this effect is observed against the background of elevation of the "free" heme level.

The results are thus evidence that significant disturbances of heme metabolism take place in the liver of irradiated animals; however, they do not give the final answer to the question of the role of the observed effects in the fall in the microsomal cytochrome P-450 level.

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GEL FILTRATION STUDY OF CYTOPLASMIC cAMP RECEPTORS IN THE KIDNEYS OF RATS OF DIFFERENT AGES

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The ability of the kidney to participate in the maintenance of water and electrolyte homeostasis in albino rats develops most rapidly during the period when maternal feeding ceases. During this same period the animal develops the ability to respond to regulatory influences of peptide hormones such as ADH, parathyroid hormone, and calcitonin. Sensitivity to these hormones is determined both by maturity of the receptor–adenylate cyclase complex and by the state of the intracellular mechanisms participating in realization of the cAMP effect [2, 6]. An important, and in many cases the sole, contribution to cytoplasmic cAMP reception is made by regulatory subunits of cAMP-dependent protein kinases of various types [5]. The role of cAMP-dependent protein kinases in realization of the hormonal effects and their structure in adult animals has now been comprehensively studied [1, 5, 8, 9]. However, there is extremely little information on the ontogenetic changes in protein-kinase complexes. The study of the molecular weight of receptor complexes with the property of specific reception of cAMP, and formed in the cytosol of the kidneys of animals with an immature and hormonally competent kidney, with different cAMP concentrations in the medium, can contribute to our understanding of the pathways of ontogeny of the intracellular mechanisms of action of cAMP.

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