EFFECT OF WHOLE-BODY γ-RAY IRRADIATION ON THE RATE OF STEROID HORMONE HYDROXYLATION BY RAT LIVER MICROSOMES

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The rate of hydroxylation of androgens was shown to be significantly reduced by γ-ray irradiation in doses of over 600 R. The rate of hydroxylation of estrogens showed little change. During irradiation under these conditions the content of cytochrome P-450 in the rat liver microsomes fell. The decrease in the rate of hydroxylation of steroid hormones by rat liver microsomes under the influence of whole-body γ-ray irradiation evidently depends on quantitative changes in cytochrome P-450.

KEY WORDS: hydroxylation; steroids; γ-ray irradiation.

Oxidative hydroxylation of xenobiotics and steroid hormones is carried out by a system of oxidases located in the endoplasmic reticulum of the liver and using reduced NADP as the donor of reducing equivalents for molecular oxygen [1, 2, 4]. These reactions take place in the terminal region of the NADPH-dependent chain, on cytochrome P-450 [4, 9]. Under the influence of ionizing radiation the rate of hydroxylation of various xenobiotics by liver microsomes is reduced [5-7].

The object of this investigation was to study the effect of whole-body γ-ray irradiation on the rate of hydroxylation of various steroid hormones and on the content of cytochromes P-450 and b_5 in rat liver microsomes.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 120-150 g, receiving the standard animal house diet. The animals were irradiated with ^{60}Co γ rays on the GUBÉ-1500 apparatus in doses of 100, 400, 600, 800, and 1000 R. The investigation was carried out 1, 3, 6, and 9 days after irradiation.

The animals were decapitated and the liver washed out with cold 1.15% KCl solution. A previously minced weighed sample of tissue was then homogenized in a glass—Teflon Potter's homogenizer in isolated medium in the ratio of 1:3 (w/v). The microsomal fractions were sedimented by centrifugation of the postmitochondrial supernatant (9500g) on the VAC 601 centrifuge at 105,000g for 1 h. The residue was resuspended in 1.15% KCl and diluted to a concentration of 20-30 mg protein/ml. The protein content in preparations of the microsomes was determined by Lowry's method [8].

The stoichiometry of the hydroxylation reactions, in which for every mole of NADPH oxidized, 1 mole of molecular oxygen is absorbed and 1 mole of oxidized substrate is formed, formed the basis for the study of the rate of steroid hormone metabolism as reflected in the rate of oxygen utilization [1, 3]. The rate of disappearance of oxygen from the incubation medium at 30°C was measured on the LP-7 polarograph with a stationary platinum electrode of the covered type. The incubation mixture contained (in 1 ml) 100 mM Tris-HCl, pH 7.4, 15 mM $MgCl_2$, and 2-3 mg of microsomal proteins. The reaction was started by the addition of 1 mM NADPH. To inhibit the peroxidation reaction, EDTA was added to the medium in a final concentration of 1 mM.

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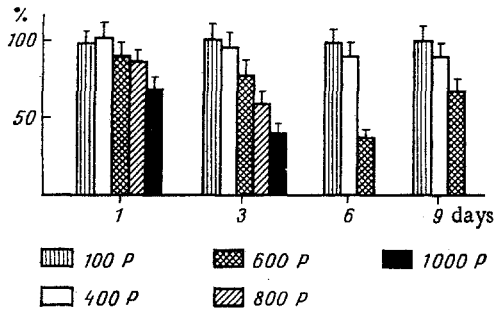

Fig. 1

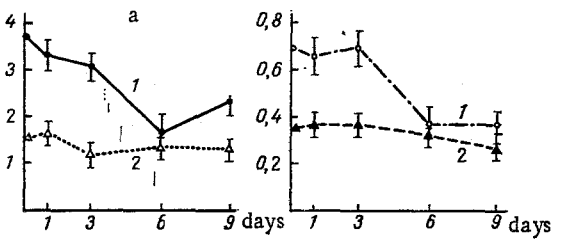

Fig. 2

Fig. 1. Effect of various doses of γ-ray irradiation on rate of hydroxylation of testosterone. Final testosterone concentration 10^{-4} M. Ordinate, uptake of O_2 (in %); abscissa, time after irradiation (in days).

Fig. 2. Effect of whole-body γ-ray irradiation in a dose of 600 R on rate of hydroxylation of steroid hormones (a) and content of microsomal cytochromes (b). a. 1) Testosterone; 2) estriol. b: 1) Cytochrome P-450; 2) cytochrome b_5. Ordinate: a) uptake of O_2 (in nmoles•min^{-1}•mg^{-1}); b) content of cytochromes (in nmoles•mg^{-1}); abscissa, time after irradiation (in days).

TABLE 1. Effect of Irradiation on Rate of Hydroxylation of Steroid Hormones by Rat Liver Microsomes (M ± m)

Steroid	Rate of hydroxylation, nmoles $O_2 \cdot min^{-1} \cdot mg$ protein^{-1}		P
	control	experiment	
Testosterone	3,8± 0,3	1,6± 0,2	<0,01
Ethyltestosterone	3,5± 0,2	2,1± 0,2	<0,05
Methyltestosterone	2,8± 0,3	1,4± 0,2	<0,05
Testosterone propionate	3,2± 0,3	2,2± 0,2	<0,05
Estradiol	2,4± 0,4	1,9± 0,3	>0,05
Estriol	1,4± 0,2	1,3± 0,2	>0,05
Estradiol dipropionate	1,9± 0,2	1,7± 0,3	>0,05

Legend. Rats received whole-body γ-ray irradiation in a dose of 600 R. Rate of hydroxylation measured on sixth day after irradiation. Final hormone concentration 10^{-4} M.

The content of cytochromes b_5 and P-450 in the microsomal preparations was determined with an SF-14 double-beam differential spectrophotometer. Microsomes were added to cuvettes containing 4 ml of 100 mM Tris-HCl, pH 7.4, in an amount of 1-2 mg protein. The zero line was recorded, after which a few crystals of dithionite were added to the right-hand cuvette, and the recording taken after 60 sec. The difference in optical density (E) between the minimum at 408 nm and the maximum at 425 nm, calculated per milligram protein, was used as the index of the content of cytochrome b_5. To determine cytochrome P-450, carbon monoxide was passed into the left-hand cuvette for 60 sec, after which dithionite was added and the differential spectrum was recorded in the 400-490 nm region. The difference between the values of E at the absorption maximum at 450 nm and the minimum at 490 nm, calculated per milligram protein, was used as the index of the content of cytochrome P-450 [9].

EXPERIMENTAL RESULTS AND DISCUSSION

Investigation of the effect of different doses of γ-ray irradiation on the rate of hydroxylation of the steroid hormones showed that irradiation in doses of under 600 R had no effect on the rate of steroid hydroxylation. The results of the study of the effect of different doses of γ-ray irradiation on the rate of hydroxylation of testosterone are given in

Fig. 1. A statistically significant decrease in the rate of testosterone hydroxylation after irradiation in a dose of 1000 R was observed on the first day, whereas after doses of 800 and 600 R this effect was observed on the third day. Smaller doses had no significant effect on the rate of testosterone hydroxylation. The study of the effect of irradiation on the rate of hydroxylation of different steroid hormones showed that the degree of inhibition of the rate of oxygen utilization depends on the steroid studied. As Fig. 2a shows, on the sixth day after whole-body γ-ray irradiation in a dose of 600 R the rate of testosterone hydroxylation was reduced by 2-2.5 times. The rate of hydroxylation of estriol, however, was almost unchanged after irradiation. Similar results were obtained for other steroids also. It will be clear from Table 1 that after irradiation in a dose of 600 R the rate of hydroxylation of androgens was significantly reduced but changes in the rate of hydroxylation of the estrogens tested were not significant.

During investigation of the content of cytochromes P-450 and b_5 after irradiation in a dose of 600 R (Fig. 2b) it was found that the minimum of the content of cytochrome P-450 was observed on the sixth day after irradiation, i.e., at times when the rate of hydroxylation of androgens reached a minimum. The changes in the cytochrome b_5 level, on the other hand, were not significant.

One of the factors in the reduction of the rate of steroid hydroxylation is evidently the quantitative change in cytochrome P-450.

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