HYDROXYLATION OF XENOBIOTICS AND STEROIDS IN THE ENDOPLASMIC RETICULUM OF THE RAT LIVER

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A polarographic method is suggested and can be recommended as a simple device for measuring the rates of oxidation of steroid hormones and xenobiotics. The rate of hydroxylation of steroids depends, it is suggested, on the polarity of the molecule of the oxidation substrate.
KEY WORDS: *liver microsomes; steroid hormones and xenobiotics; hydroxylation of steroids.*

The endoplasmic reticulum of the liver in animals and man is known to contain enzyme systems which will oxidize xenobiotics and steroid hormones [1, 2, 4, 6]. Hydroxylation is carried out by a respiratory chain which consists of free oxidation enzymes, utilizing reduced NADP as the donor of reducing equivalents for molecular oxygen [1, 8]. Oxygen, activated in the NADPH oxidation chain is utilized, first, in reactions of hydroxylation of endogenous substrates and xenobiotics; second, it is used in the formation of peroxides of unsaturated fatty acids.

The object of this investigation was to study the character of hydroxylation of some xenobiotics and steroid hormones and to choose the composition of an incubation medium for polarographic measurements in which there would be strict stoichiometry (1:1) between the quantity of oxygen assimilated and the quantity of hydroxylation product formed. The polarographic method of measuring the rate of hydroxylation of xenobiotics was proposed for use in the study of the rate of oxidation of steroid hormones by liver microsomes.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 130-150 g and kept on the standard animal house diet. Liver microsomes were obtained by the method described earlier [3]. The protein concentration in the microsomal samples was determined by Lowry's method [7]. The rate of oxygen assimilation was measured on the LP-60 (Czechoslovakia) polarograph with a standard platinum electrode of closed type. The incubation mixture (1 ml) contained 100 mmole Tris-HCl, pH 7.4, and 3-4 mg microsomal protein. The reaction at 30° was started by the addition of 1 mmole NADPH. To inhibit the peroxidation reaction, EDTA was added to the medium in a final concentration of 1 mM. To obtain maximal activation of the partial electron transport reactions, 16 mM Mg^{++} was added to the incubation medium. The final con-

TABLE 1. Rate of Hydroxylation of Xenobiotics (M ± m)

Xenobiotic	Final concentration (mM)	Formation of formaldehyde (in nmoles · $min^{-1} \cdot mg^{-1}$)	O_2 assimilation (in nmoles · $min^{-1} \cdot mg^{-1}$)
Ethylmorphine	1	12,3±0,3	9,5±0,2
Dimethylaniline	6	10,2±0,3	8,8±0,3
Amidopyrine	8	8,3±0,2	7,2±0,3
Aniline	3	0,9±0,2	0,8±0,1

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TABLE 2. Rate of Oxidation of Steroid Hormones by Liver Microsomes (M±m)

Steroid hormones 10^{-4}M	O_2 assimilation (in nmole·min^{-1}·mg^{-1}
Testosterone	3.8±0.1
Ethyltestosterone	3.5±0.1
Dehydroepiandrosterone	3.1±0.1
Methyltestosterone	2.8±0.2
Methylandrostenediol	2.4±0.2
Ethylandrostenediol	1.8±0.1
Androstenediol	1.6±0.2
Δ^4-Androstenediol	1.3±0.1
Estrone	3.2±0.2
Estradiol	2.6±0.1
Estradiol valerianate	2.3±0.1
Estradiol benzoate	2.2±0.1
Diethylstilbestrol	1.3±0.2
Ethinyl estradiol	1.2±0.1
Estriol	0.9±0.2
Progesterone	1.8±0.2
Prednisone	1.2±0.1
Cholesterol	1.1±0.1
Hydrocortisone	0.8±0.1

centration of the substances added is shown in Tables 1 and 2. The rate of hydroxylation was calculated as the difference between the rates of oxygen assimilation in the presence and absence of substrates. The rate of the demethylation reaction in ethylmorphine, dimethylaniline, amidopyrine, and aniline was measured by the formation of formaldehyde and p-aminophenol [5].

EXPERIMENTAL RESULTS AND DISCUSSION

Development of the method of studying the rate of metabolism of steroid hormones on the basis of the rate of oxygen assimilation was based on the stoichiometry of hydroxylation reactions, in which, for 1 mole of NADPH oxidized 1 mole of molecular oxygen is assimilated and 1 mole oxidation substrate is formed [1]. Preliminary experiments under the same conditions were carried out to measure the rate of formation of formaldehyde and the quantity of oxygen utilized in oxidation of the xenobiotics.

The results of determination of the demethylating activity of the microsomes by formaldehyde and oxygen formation are shown in Table 1. Ethylmorphine, dimethylaniline, amidopyrine, and aniline were used as hydroxylation substrates.

It will be clear from Table 1 that ethylmorphine was oxidized fastest, and that the rate of oxygen assimilation also was highest in this case. Dimethylaniline, amidopyrine, and aniline were demethylated rather more slowly. The ratio between the velocities of formaldehyde formation and oxygen assimilation in the different experiments remained constant and close to unity. It can be concluded from these results that the rate of oxygen assimilation under certain conditions reflects closely the velocity of the hydroxylation reaction. The method as developed was used to determine the rates of oxidation of some steroid hormones by liver microsomes of intact animals. As will be clear from Table 2, among among the group of androgens the relationship between the velocity of hydroxylation and the polarith of the molecule of the oxidation substrate was studied. For example Δ^4-androstenediol, with two hydroxyl groups at C_3 and C_{17}, was oxidized much more slowly than testosterone, with a hydroxyl group at C_{17} only. A similar relationship was found with the other hormones: estriol, with two hydroxyl groups, was hydrolyzed more slowly than estrone, which has no hydroxyl groups in its molecule. By comparing the velocities of hydroxylation of androgens, estrogens, corticosteroids, gestagens, and cholesterol, it was clear that the rate of oxidation depends on the polarity of the steroid molecule.

LITERATURE CITED

1. A. I. Archakov, Uspekhi Sovr. Biol., No. 2, 163 (1971).
2. P. V. Sergeev, R. D. Seifulla, and A. I. Maiskii, Molecular Aspects of the Action of Steroid Hormones [in Russian], Moscow (1971).
3. A. I. Archakov, I. I. Karuzina, I. S. Kokareva, et al., Biochem. J., 136, 371 (1973).
4. J. R. Gillette, Advances Pharmacol., 4, 220 (1966).
5. J. R. Gillette, Ann. New York Acad. Sci., 179, 43 (1972).
6. P. Hochstein and L. Ernster, Biochem. Biophys. Res. Commun., 12, 388 (1963).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem., 193, 265 (1951).
8. P. Strittmatter, in: Biological Oxidations, edited by T. Singer, New York (1968), p. 171.