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EFFECT OF 16α-CYANOPREGNENOLONE ON THE HYDROXYLATION OF LITHOCHOLIC ACID BY RAT LIVER MICROSOMES

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Abstract—The effect of 16α-cyanopregnenolone (PCN)* on the microsomal hydroxylation of tritium-labelled lithocholic acid was studied in rats. The 6β-hydroxylation increased about twice and the 7α-hydroxylation three to four times. The ability of PCN to prevent lithocholic acid-induced cholelithiasis in rats is discussed.

PRETREATMENT with the potent catatoxic steroid 16α-cyanopregnenolone (PCN)* protects rats against many toxicants.[1] This effect has usually been explained by the induction of drug metabolizing hepatic microsomal enzymes. PCN-treatment increases liver weight and stimulates smooth-surfaced endoplasmic reticulum proliferation in rat liver cells,[2] enhances NADPH-cytochrome *c* reductase activity and cytochrome P-450 content[3,4] and increases the capacity of liver microsomes to hydroxylate some drugs[4] and progesterone.[5] We studied previously the influence of PCN on the microsomal metabolism of 4-androstene-3,17-dione and progesterone and on some enzymes involved in the synthesis and metabolism of bile acids.[6] Several hydroxylations of 4-androstene-3,17-dione and progesterone were stimulated severalfold and the 7α-hydroxylation of taurodeoxycholic acid was increased about twice. On the other hand the 7α-hydroxylase and the 12α-hydroxylase involved in the biosynthesis of bile acids, were not stimulated.

Recently it was reported that PCN prevents the formation of biliary concrements by lithocholic acid in rats.[7] This effect might be mediated by an enhanced metabolism of lithocholic acid. To test this possibility the influence of PCN on the hydroxylation of lithocholic acid in rat liver microsomes was studied.

EXPERIMENTAL

Materials. Tritium-labelled lithocholic acid (specific radioactivity, 16·7 μCi/mg) was obtained from New England Nuclear Corp,, Boston, Mass., U.S.A. NADPH was obtained from Sigma Chemical Co., St Louis, Mo. 16α-Cyanopregnenolone was a generous gift from Dr. J. Babcock.

Animals and *preparation of homogenates.* Five albino male rats of the Sprague–Dawley strain weighing about 150 g were injected i.p. with 5 mg PCN (in 0·5 ml of propylene glycol–dimethylsulfoxide, 1 : 1, v/v) twice daily for 5 days. Control rats were

* Systematic names and non-standard abbreviations: PCN, 16α-cyanopregnenolone; chenodexoycholic acid, 3α,7α-dihydroxy-5β-cholanoic acid; lithocholic acid, 3α-hydroxy-5β-cholanoic acid; deoxycholic acid, 3α,12α-dihydroxy-5β-cholanoic acid.

injected in the same way with vehicle only. The animals had free access to water and a commercial pellet diet. The animals were killed by a blow on the head about 12 hr after the last injection. The livers were excised quickly, chilled and weighed. Liver homogenates, 20% (w/v), were prepared in a modified Bucher medium,[8] pH 7·4, with a Potter–Elvehjem homogenizer equipped with a loosely fitting pestle. The homogenate was centrifuged at 20,000 *g* for 15 min. The supernatant was centrifuged at 105,000 *g* for 1 hr and the microsomal fraction obtained was suspended in Bucher medium in a volume corresponding to that of the 20,000 *g* supernatant. The protein concentration of the microsomal fraction was determined according to Lowry *et al.*[9]

Incubations with tritium-labeled lithocholic acid. Fifty μg of tritium-labeled lithocholic acid, dissolved in 50 μl of 95% aqueous ethanol (v/v), was added to a mixture of 1 ml of microsomal fraction and 2 ml of Bucher medium fortified with 3 μmoles of NADPH. In incubations with liver homogenates of PCN-treated rats only 0·5 ml of microsomal fraction was used. The incubation was carried out for 20 min and was terminated by the addition of an equal volume of 95% aqueous ethanol (v/v). After filtration the mixture was acidified with 0·2 N HCl and extracted with ether. The ether extract was washed with water until neutral and the solvent was evaporated. The residue was dissolved in 0·5 ml of chloroform–methanol (2:1, v/v) and applied to precoated silica gel plates (250 μ, Merck, Darmstadt, Germany) together with suitable reference compounds as internal standards. S 11 (trimethylpentane-ethyl acetate–acetic acid, 10:10:2, by vol.) was used as solvent system.[10] The compounds were located by exposing the chromatoplates to iodine vapor.[11] The iodine was allowed to evaporate at room temperature and the appropriate zones were scraped off and extracted with methanol. Aliquots of the extracts were taken for measurement of radioactivity.

Radioactivity measurements. Radioactivity was assayed in a Packard liquid scintillation spectrometer, model 3003.

Statistical analysis. Student's *t*-test was used and the significance level was set at 0·01.

RESULTS

The liver weights of the PCN-treated rats (5·5 $\pm$ 0·1 g/100 g body wt) were increased by about 35 per cent ($P < 0{\cdot}001$) compared to control rats (4·1 $\pm$ 0·2 g/100 g body wt). The concentration of microsomal protein was slightly elevated in PCN-treated animals compared to control animals (6·0 $\pm$ 1·6 and 5·5 $\pm$ 0·2 mg/ml, respectively) but this difference was not significant.

Upon incubation of tritium-labelled lithocholic acid with the microsomal fraction of liver homogenates from control rats, about 10 per cent of the substrate was converted into more polar products (Fig. 1). 3α,6β-Dihydroxy-5β-cholanoic acid was identified as the main metabolite by its chromatographic properties. A minor metabolite was identified as chenodeoxycholic acid. The ratio between 3α,6β-dihydroxy-5β-cholanoic acid and chenodeoxycholic acid was about 4:1. The 6β-hydroxylation was increased from 1·1 $\pm$ 0·2 to 2·1 $\pm$ 0·4 nmoles/mg protein and the 7α-hydroxylation from 0·3 $\pm$ 0 to 1·0 $\pm$ 0·2 nmoles/mg protein by PCN-treatment. Thus the 6β-hydroxylation was stimulated about twice ($P < 0{\cdot}005$) and the 7α-hydroxylation 3–4 times ($P < 0{\cdot}001$) by PCN-treatment. The ratio between 3α,6β-dihydroxy-5β-cholanoic acid and chenodeoxycholic acid was lowered from about 4:1 to 2:1.

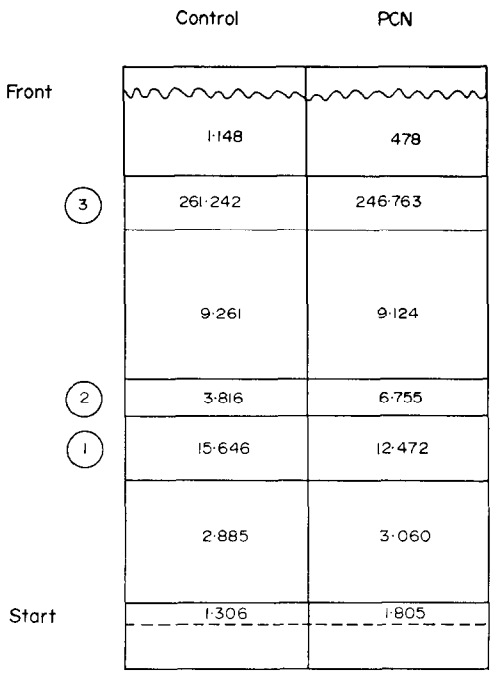

FIG. 1. Thin-layer chromatograms of extracts of incubations of tritium-labelled lithocholic acid with the microsomal fraction of liver homogenates from control rats (control) and from rats treated with PCN (PCN). The numbers on the chromatograms represent counts/min. Reference compounds were: (1) 3α,6β-dihydroxy-5β-cholanoic acid; (2) chenodeoxycholic acid; (3) lithocholic acid. Solvent, trimethylpentane–ethyl acetate–acetic acid (10:10:2, by vol.).

DISCUSSION

Lithocholic acid is formed from chenodeoxycholic acid by microbial enzymes during the intestinal passage (for a review, see ref. 12). It is poorly reabsorbed and only trace amounts can be detected in rat portal blood. Upon reaching the liver lithocholic acid is hydroxylated to yield chenodeoxycholic acid and 3α,6β-dihydroxy-5β-cholanoic acid.[13,14] The metabolism of lithocholic acid has also been studied *in vitro*.[15,16] In the microsomal fraction of rat liver homogenates fortified with NADPH lithocholic acid is predominantly 6β-hydroxylated and to a lesser extent 7α-hydroxylated. Previous work has shown that the 6β-hydroxylation involves the participation of a cytochrome P-450 and that it is stimulated by phenobarbital.[16] Recently, Lu *et al.*[4] gave evidence that PCN is a new type of inducer. It increases the content of cytochrome P-450 in the microsomes in the same way as phenobarbital. However the PCN-induced cytochrome P-450 seems to differ from the phenobarbital-induced cytochrome P-450 because the microsomal hydroxylase systems from phenobarbital- and PCN-treated rats have different substrate specificity. Our results give support to this suggestion. As mentioned above previous work has shown that phenobarbital stimulates the 6β-hydroxylation of lithocholic acid in rat liver microsomes. Our investigation shows that PCN also stimulates the 6β-hydroxylation of lithocholic acid and furthermore that the 7α-hydroxylation of lithocholic acid is enhanced to a greater

extent than the 6β-hydroxylation. Thus PCN-treatment gives a different pattern of metabolites than phenobarbital-treatment.

The feeding of 1% lithocholic acid has been shown to induce gallstones in rats.[17–21] The gallstones consisted predominantly of calcium salts of free and glycine conjugated lithocholic acid and 3α,6β-dihydroxy-5β-cholanoic acid. In a recent work Selye[7] demonstrated that PCN is able to prevent the lithocholic acid-induced cholelithiasis. Phenobarbital was less potent in this respect. No experimental data has been reported which might explain the mechanisms for this ability of PCN to influence the gallstone producing effect of lithocholic acid. Many possibilities may exist. A factor of importance might be the inducing effect of PCN on the microsomal hydroxylations of lithocholic acid.

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