Stimulation of cholesterol 7α-hydroxylase by phenobarbital in two strains of rats

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Abstract The effect of phenobarbital administration on the in vitro activity of cholesterol 7α-hydroxylase was investigated in two strains of rats. In rats of the Wistar strain the daily injection of phenobarbital (100 mg/kg per day ip for 5 days) produced a 33% increase in hepatic microsomal protein and a sixfold stimulation of specific activity of the enzyme. In rats of the Charles River colony (Sprague-Dawley derived) identical treatment with phenobarbital resulted in a 45% increase in hepatic microsomal protein and no change in the specific activity of cholesterol 7α-hydroxylase. Plasma phenobarbital concentrations were three to four times greater in the Wistar rats, suggesting that these strains differ also in their capacity to metabolize phenobarbital.

Supplementary key words microsomal enzyme

In 1968, Einarsson and Johansson studied the effect of phenobarbital administration on the activities of several hydroxylation systems participating in bile acid metabolism (1). They reported that the drug failed to stimulate the activity of cholesterol 7α-hydroxylase in hepatic microsomes of Sprague-Dawley rats. In contrast, Shefer, Hauser, and Mosbach (2), using Wistar rats, and Wada et al. (3), using Sprague-Dawley rats, observed a significant enhancement of microsomal cholesterol 7α-hydroxylase activity following the administration of phenobarbital. Since the results obtained by the three groups of investigators appeared to be inconsistent, we reinvestigated the effect of phenobarbital on cholesterol 7α-hydroxylase in Wistar rats and in Sprague-Dawley-derived rats of the Charles River colony.

Abbreviations: TLC, thin-layer chromatography.

EXPERIMENTAL PROCEDURES

Animals

Male rats of the Wistar strain (230–360 g) were obtained from the Otisville laboratory of the New York City Health Department. The Sprague-Dawley-derived animals (200–370 g) were purchased from the Charles River Breeding Laboratories, Wilmington, Mass.

All animals were fed Purina rat chow pellets ad lib. throughout the experiment. The experimental animals were injected at 9 AM daily ip with phenobarbital, 100 mg/kg body weight, dissolved in a 0.9% sodium chloride solution. The control animals were injected with saline. 2 hr after the fifth injection (11 AM) the animals were killed by cervical dislocation.

Plasma phenobarbital concentrations were determined as described by Butler, Mahaffee, and Waddell (4).

Microsomal cholesterol 7α-hydroxylase activity

The livers of the rats were removed, and the microsomal fractions were prepared and assayed for cholesterol 7α-hydroxylase activity exactly as described previously (2). The substrate of the enzyme, [4-^{14}C]cholesterol, was purchased from New England Nuclear Corp., Boston, Mass., and purified by TLC in the presence of unlabeled cholest-5-en-3β,7α-diol, cholest-5-en-3β,7β-diol, and cholest-5-en-3β-ol-7-one. The labeled cholesterol was eluted from the silica gel with acetone and contained less than 0.1% cholest-5-en-3β,7α-diol, as determined by TLC of the purified product.

RESULTS AND DISCUSSION

Table 1 summarizes the data on the effect of phenobarbital administration on (*1*) liver size and weight of microsomal protein per gram of liver, and (*2*) the activity

TABLE 1. Phenobarbital stimulation of cholesterol 7α-hydroxylase in two strains of rats

Strain	No. of Rats	Treatment	Body Weight	Wet Wt of Liver	Microsomal Protein	Cholesterol 7α-Hydroxylase Activity: Per mg of Microsomal Protein	Cholesterol 7α-Hydroxylase Activity: Per Liver
			g	*g*	*mg/g liver*	*pmoles/min*	
Charles River	4	None	271 (29.0)[a]	11.8 (0.89)	20.5 (2.16)	3.59 (0.49)	872 (74)
	8	Phenobarbital[b]	268 (21.3)	15.0 (1.25)	29.7 (2.62)	3.35 (0.58)	1293 (125)
Wistar	7	None	239 (24.9)	10.7 (0.63)	25.9 (2.84)	2.36 (0.32)	644 (109)
	8	Phenobarbital	260 (18.6)	13.5 (0.81)	34.4 (2.09)	14.81 (1.35)	6689 (818)

[a] Standard errors of the means are in parentheses.
[b] Phenobarbital injected daily for 5 days, 100 mg/kg ip, as described in Experimental Procedures.

of cholesterol 7α-hydroxylase per milligram of microsomal protein and per liver. In both strains the liver weights and the amounts of microsomal protein were enhanced by the drug. The *specific* activity of cholesterol 7α-hydroxylase was not stimulated by phenobarbital in the Charles River strain (Sprague-Dawley derived), and this is in accord with the data of the Swedish workers (1). In the Wistar rats the specific activity of the enzyme was enhanced sixfold, in agreement with our previous results (2). The total activity of cholesterol 7α-hydroxylase (per liver) was significantly increased in both strains, but the 48% increase in the Charles River animals can be ascribed to the increase in liver microsomal protein (45%), not to a specific stimulation of the enzyme by phenobarbital.

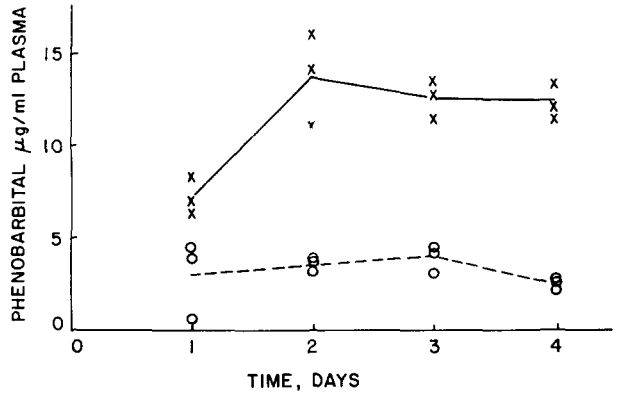

FIG. 1. Plasma phenobarbital concentrations in rats of the Wistar and Charles River strains during phenobarbital administration. 12 Wistar rats (×——×) and 12 Charles River rats (○— —○) weighing 250 g each were injected ip at 9 AM daily with phenobarbital (Na salt), 100 mg/kg rat. Groups of three rats from each strain were killed each day for 4 successive days at 11 AM, and their plasma was analyzed for phenobarbital (4). The lines represent the means of the drug concentrations in the plasma of each group of three animals.

Fig. 1 summarizes the plasma concentrations of phenobarbital in the two strains of rats during a 5-day study. Apparently, the plasma concentration of the drug was consistently higher in the Wistar rats. It is possible that increased drug levels are responsible in some way for the stimulation of 7α-hydroxylase activity in this strain. On the other hand, this difference in drug metabolism may merely indicate that the two strains of rats have different patterns of drug-induced enzyme activities.

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REFERENCES

1. Einarsson, K., and G. Johansson. 1968. Effect of phenobarbital on the conversion of cholesterol to taurocholic acid. *Eur. J. Biochem.* **6:** 293–298.
2. Shefer, S., S. Hauser, and E. H. Mosbach. 1968. 7α-Hydroxylation of cholestanol by rat liver microsomes. *J. Lipid Res.* **9:** 328–333.
3. Wada, F., K. Hirata, K. Nakao, and Y. Sakamoto. 1968. Participation of P-450 in 7α-hydroxylation of cholesterol. *J. Biochem.* (Tokyo) **64:** 415–417.
4. Butler, T. C., C. Mahaffee, and W. J. Waddell. 1954. Phenobarbital: studies of elimination, accumulation, tolerance and dosage schedules. *J. Pharmacol. Exp. Ther.* **111:** 425–435.