PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 155, 67-70 (1977)

Effects of Nicotinic Acid on Induction of Hepatic Drug Metabolizing Enzymes by Chronic Ethanol Administration[1] (39747)

D. J. TUMA,[2] M. F. SORRELL,[3] J. A. VANDERHOOF, AND A. J. BARAK

Liver Study Unit, Veterans Administration Hospital, Omaha, Nebraska 68105, and the Departments of Medicine and Biochemistry, University of Nebraska Medical Center, Omaha, Nebraska 68105

Introduction. Chronic ethanol feeding produces a proliferation of the hepatic smooth endoplasmic reticulum (1-3), enhances the activities of various drug-metabolizing enzymes (1, 4-6) as well as glucose-6-phosphatase (7), and increases the content of cytochrome *P*-450 (2, 6). Recent studies by Villeneuve *et al.* (8) indicated that hepatic cytochrome *P*-450 induced by chronic ethanol administration differs from cytochrome *P*-450 obtained from control, phenobarbital-, and 3-methylcholanthrene-treated rats.

Since previous studies in this laboratory (9) have shown that the chronic intake of nicotinic acid, a commonly administered serum lipid-lowering agent, potentiated the hepatic steatosis due to chronic ethanol feeding, this study was conducted to determine whether nicotinic acid also potentiated the inductive effects of ethanol on drug-metabolizing enzymes. The influence of nicotinic acid on the inductive effects of phenobarbital on drug metabolism (10, 11) was also investigated.

Materials and methods. Experiment 1. Male, Sprague-Dawley rats (approximately 150 g) were maintained for 1 week on the nutritionally adequate, semiliquid control diet of DeCarli and Lieber (12). After this equilibrium period, the rats were divided into four groups of seven and fed as follows: group 1, the control diet; group 2, the control diet supplemented with nicotinic acid (0.5 mg/ml); group 3, the ethanol diet (12), which was identical with the control diet except that ethanol was substituted isocalorically for carbohydrate to provide 36% of the total calories; group 4, the ethanol diet supplemented with nicotinic acid (0.5 mg/ml). On a daily basis the animals in groups 1, 2, and 3 were fed that amount of diet consumed by rats in group 4 for a period of 28 days. The amount of nicotinic acid received daily by the rats (approximately 150 mg/kg) was about two to three times that used to treat hyperlipemia in man (13). After 28 days, the nonfasted rats were killed and their livers were removed. Cytochrome *P*-450 content (14) and aniline hydroxylase (15) and glucose-6-phosphatase (16) activities were measured in freshly prepared liver homogenates.

Experiment 2. Male, Sprague-Dawley rats weighing 150 g were divided into four groups (six rats per group) and placed on a Purina Rat Chow diet as the control diet for 1 week. After this period, the rats received the following dietary regimen: group 1, the control diet; group 2, the control diet plus nicotinic acid supplemented in the drinking water at a concentration so that each rat received approximately 150 mg/kg/day; group 3, the control diet plus a 0.1% solution of sodium phenobarbital as drinking water; group 4, the control diet plus a 0.1% solution of sodium phenobarbital as drinking water which was supplemented with nicotinic acid (approximately 150 mg/kg/day). The daily intake of phenobarbital of rats in groups 3 and 4 was 99 ± 8 and 89 ± 12 mg/kg/day, respectively. On a daily basis the rats in groups 1, 2, and 3 were fed that amount of diet consumed by rats in group 4 for a period of 28 days. After the experimental period, the nonfasted rats were killed and their livers

[1] This work was supported in part by the Veterans Administration and the Swanson Center for Nutrition, Inc., Omaha, Nebraska.

[2] Send reprint requests to Dean J. Tuma, Ph.D., Liver Study Unit, Veterans Administration Hospital, 4101 Woolworth Avenue, Omaha, Neb. 68105.

[3] This investigator is a recipient of Academic Career Development Award AM 70316 from the National Institutes of Arthritis, Metabolism and Digestive Diseases.

ISSN 0037-9727

were removed. As in experiment 1, cytochrome *P*-450 content and aniline hydroxylase and glucose-6-phosphatase activities were measured in freshly prepared liver homogenates. Since it has been demonstrated that chronic ethanol feeding restricts the growth rate of rats (17, 18), the results of the various enzyme assays in this study were expressed and compared on the basis of activity/total liver/100 g body weight.

Student's *t* test was used for estimating the significance of difference between group means; the level of confidence employed was 95%.

Results. The results from experiment 1 are shown in Table I. The body weights of the ethanol-fed rats were lower than the isocalorically fed controls; nicotinic acid administration did not affect the body weights in either group. Nicotinic acid treatment increased the liver weights in the ethanol-treated rats but slightly lowered them in the controls. In accordance with previous studies (4–7), chronic ethanol feeding increased the content of cytochrome *P*-450 and elevated the activity of the drug-metabolizing system, aniline hydroxylase, as well as another microsomal enzyme, glucose-6-phosphatase. Nicotinic acid administration alone did not affect the level of cytochrome *P*-450 or aniline hydroxylation but slightly lowered the activity of glucose-6-phosphatase. However, the administration of nicotinic acid further elevated the ethanol-induced increase of cytochrome *P*-450 content and aniline hydroxylase activity, but not glucose-6-phosphatase activity. Thus, it appears in this case that nicotinic acid potentiated the inductive effects of ethanol on microsomal drug metabolism.

The results from experiment 2 are shown in Table II. No significant differences in body weights among the rats in the four groups were observed. The liver weights were increased in the phenobarbital-treated rats, but nicotinic acid feeding did not affect liver weights in either the phenobarbital or control groups. The well-known inductive effects of phenobarbital (10, 11) were demonstrated by the observed elevation of hepatic cytochrome *P*-450 content and aniline hydroxylase activity; however, unlike the case with ethanol, nicotinic acid did not potentiate these inductive effects of phenobarbital. Glucose-6-phosphatase activity was unchanged in the livers of the variously treated animals in this experiment.

Discussion. The elevation of cytochrome *P*-450 levels (2, 6) and the increases in the activity of the microsomal enzymes, aniline hydroxylase (6), and glucose-6-phosphatase (7) in livers of rats chronically fed ethanol were confirmed in this study. This investigation also shows that nicotinic acid administration potentiated the ethanol-induced increases in cytochrome *P*-450 and aniline hydroxylase activity. This enhancing effect of nicotinic acid seemed to be unique in the ethanol-treated animals because this drug

TABLE I. EFFECT OF CHRONIC ETHANOL AND NICOTINIC ACID ADMINISTRATION ON CYTOCHROME *P*-450 AND MICROSOMAL ENZYMES IN THE LIVER.

Diet	Body weight (g)	Liver weight[a]	Cytochrome *P*-450[b]	Aniline hydroxylase[c]	Glucose-6-phosphatase[d]
Control	266 ± 9	3.4 ± 0.1	270 ± 26	306 ± 88	71.9 ± 8.8
Control + nicotinic acid	255 ± 11[e]	3.1 ± 0.2*	298 ± 44[e]	352 ± 104[e]	57.9 ± 5.7*
Ethanol	224 ± 11**	3.5 ± 0.2[e]	408 ± 109*	788 ± 28**	94.3 ± 14.0*
Ethanol + nicotinic acid	223 ± 13[f]	4.1 ± 0.3***	602 ± 54***	1142 ± 243****	101.9 ± 16.4[f]

[a] Values expressed as g/100 g body wt, mean ± SD.
[b] Values expressed as nmole/100 g body wt, mean ± SD.
[c] Activity expressed as nmole of aniline metabolized/min/100 g body wt, mean ± SD.
[d] Activity expressed as μmoles of phosphate released/min/100 g body wt, mean ± SD.
[e] Not significant compared to control value.
[f] Not significant compared to ethanol value.
* $P < 0.01$, compared to control value.
** $P < 0.001$, compared to control value.
*** $P < 0.01$, compared to ethanol value.
**** $P < 0.05$, compared to ethanol value.

TABLE II. EFFECT OF PHENOBARBITAL AND NICOTINIC ACID ADMINISTRATION ON CYTOCHROME *P*-450 AND MICROSOMAL ENZYMES IN THE LIVER.

Diet	Body weight (g)	Liver weight[a]	Cytochrome *P*-450[b]	Aniline hydroxylase[c]	Glucose-6-phosphatase[d]
Control	326 ± 27	4.0 ± 0.6	163 ± 34	150 ± 29	81.1 ± 5.4
Control + nicotinic acid	316 ± 13[e]	3.9 ± 0.4[e]	182 ± 15[e]	191 ± 37[e]	69.0 ± 14.7[e]
Phenobarbital	316 ± 13[e]	5.3 ± 0.9*	764 ± 66*	469 ± 41*	78.6 ± 16.1[e]
Phenobarbital + nicotinic acid	327 ± 21[f]	5.1 ± 0.4[f]	700 ± 98[f]	438 ± 42[f]	74.3 ± 13.7[f]

[a] Values expressed as g/100 g body wt, mean ± SD.
[b] Values expressed as nmole/100 g body wt, mean ± SD.
[c] Activity expressed as mean nmole of aniline metabolized/min/100 g body wt, mean ± SD.
[d] Activity expressed as μmole of phosphate released/min/100 g body wt, mean ± SD.
[e] Not significant compared to control value.
[f] Not significant compared to phenobarbital value.
* $P < 0.001$.

did not influence cytochrome *P*-450 levels or aniline hydroxylation in the control or phenobarbital-treated rats. The microsomal enzyme, glucose-6-phosphatase, which is not involved directly in drug metabolism, was essentially unaffected in all cases by nicotinic acid.

Although no direct evidence is available to explain this potentiating effect of nicotinic acid on the induction of drug metabolism by ethanol, the results are consistent with the existence of a unique cytochrome *P*-450 in the ethanol-treated rat as proposed by Villeneuve *et al.* (8). These authors reported an ethanol-induced cytochrome *P*-450 which differs in catalytic activity from cytochrome *P*-450 in control, phenobarbital, and methylcholanthrene-treated rats. The data obtained in this study show that ethanol increased cytochrome *P*-450 content by 51%, whereas aniline hydroxylase activity was stimulated to a much greater extent (158%). Similar observations were also reported by Joly and Hetu (6). In contrast, others (4, 5) have shown that increases in benzphetamine demethylase and benzpyrene hydroxylase activities in ethanol-fed rats correlated with elevations in cytochrome *P*-450 levels. Furthermore, chronic phenobarbital treatment increased cytochrome *P*-450 levels 369% but aniline hydroxylation was enhanced by only 213% (Table II). The above data are in keeping with the findings of Villeneuve *et al.* (8) which indicate the presence of a distinct *P*-450, induced by ethanol with enhanced catalytic activity for aniline. This study further shows that nicotinic acid increased the cytochrome *P*-450 content in ethanol-treated rats (48%) to the same extent as it elevated aniline hydroxylase activity (45%). However, nicotinic acid did not influence either cytochrome *P*-450 levels or aniline hydroxylation in the control or phenobarbital-treated animals. These results suggest that nicotinic acid may play a role in enhancing the induction of a unique cytochrome *P*-450 which is induced by chronic ethanol administration.

The results of a previous study (9) demonstrating that nicotinic acid potentiates hepatic steatosis due to chronic ethanol intake, as well as the results of this study indicating an effect of nicotinic acid on drug metabolism in ethanol-treated rats, may have additional clinical significance. If these results can be extrapolated to man, these observations should be considered when alcohol users are treated for hyperlipidemia with nicotinic acid.

Summary. The content of cytochrome *P*-450 and activities of aniline hydroxylase and glucose-6-phosphatase were determined in the livers of rats fed ethanol and nicotinic acid singly and in combination for 4 weeks. Similar determinations were conducted in livers from rats administered phenobarbital and nicotinic acid alone and in combination for 4 weeks. Both chronic ethanol feeding and phenobarbital administration increased the content of cytochrome *P*-450 and aniline hydroxylase activity. Glucose-6-phosphatase activity was elevated by ethanol feeding but not by phenobarbital. Nicotinic

acid treatment increased the content of cytochrome *P*-450 and aniline hydroxylation in the ethanol-treated but not in the control or phenobarbital-treated animals. Nicotinic acid did not influence the activity of glucose-6-phosphatase in either the ethanol- or phenobarbital-treated rats. The results of this study are consistent with the existence of a unique cytochrome *P*-450 which is induced by ethanol feeding, and the induction of this hemoprotein is further enhanced by the concomitant administration of nicotinic acid with ethanol.

1. Rubin, E., Hutterer, F., and Lieber, C. S., Science **159,** 1469 (1968).
2. Ishii, H., Joly, J-G., and Lieber, C. S., Biochim. Biophys. Acta **291,** 411 (1973).
3. Oudea, M. C., Collette, M., and Oudea, P., Amer. J. Dig. Dis. **18,** 398 (1973).
4. Rubin, E., and Lieber, C. S., Science **162,** 690 (1968).
5. Joly, J-G., Ishii, H., Teschke, R., Hasumura, Y., and Lieber, C. S., Biochem. Pharmacol. **22,** 1532 (1973).
6. Joly, J-G., and Hetu, C., Biochem. Pharmacol. **24,** 1475 (1975).
7. Ishii, H., Joly, J-G., and Lieber, C. S., Metabolism **22,** 799 (1973).
8. Villeneuve, J-P., Mavier, P., and Joly, J-G., Biochem. Biophys. Res. Commun. **70,** 723 (1976).
9. Sorrell, M. F., Baker, H., Tuma, D. J., Frank, O., and Barak, A. J., Biochim. Biophys. Acta **450,** 231 (1976).
10. Conney, A. H., Pharmacol. Rev. **19,** 317 (1967).
11. Kuntzman, R., Ann Rev. Pharmacol. **9,** 21 (1969).
12. DeCarli, L. M., and Lieber, C. S., J. Nutr. **91,** 331 (1967).
13. Rivin, A. V., J. Amer. Med. Assoc. **170,** 2088 (1959).
14. Schoene, B., Fleishman, R. A., and Remmer, H., Eur. J. Clin. Pharmacol. **4,** 65 (1972).
15. Gang, H., Lieber, C. S., and Rubin, E., J. Pharmacol. Exp. Ther. **183,** 218 (1972).
16. Harper, A. E., *in* "Methods of Enzymatic Analysis" (H. U. Bergmeyer, ed.), p. 788. Academic, New York (1963).
17. Pirola, R. C., and Lieber, C. S., Pharmacology **7,** 185 (1972).
18. Banks, W. L., Kline, E. S., and Higgins, E. S., J. Nutr. **100,** 581 (1970).

Received December 8, 1976. P.S.E.B.M. 1977, Vol. 155.