ACTIVITIES OF HEPATIC MICROSOMAL ELECTRON TRANSPORT SYSTEM IN PROLONGED ETHANOL-TREATED RATS

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In previous studies, the authors reported that chronic ethanol feeding caused an increase in the amount of cytochrome P-450 and stimulated the *p*-hydroxylation of aniline, but had no effect on the N-demethylation of aminopyrine or the side-chain oxidation of hexobarbital by liver microsomes (1) (2). Holtzman et al. (3) pointed out that the N-demethylation of ethylmorphine by liver microsomes was more closely related to NADPH cytochrome P-450 reductase activity than to the amount of cytochrome P-450. On the other hand, it has recently been revealed that cytochrome P-450 reductase activity decreases in the presence of aniline and increases in the presence of aminopyrine (4).

Thus in the present study, activities of main components of hepatic microsomal electron transport system in prolonged ethanol-treated rats were determined in the presence of the drug substrate.

Male Wistar rats were used. Ethanol treatment and preparation of liver microsomes were the same as described previously (5). Rats of the ethanol group were given ethanol solution as drinking fluid *ad libitum* prior to sacrifice. All rats were fasted for 12 hr prior to sacrifice. Cytochrome P-450 and b_5 contents were determined by the method of Holtzman et al. (6) and of Barker and Smuckler (7) respectively. Assay for cytochrome P-450 reductase activity was essentially the same as the method of Gigon et al. (4). Cytochrome c reductase and NADPH oxidase activity was assayed according to Phillips and Langdon (8) and Gillette et al. (9) respectively. Microsomal protein content was determined by the method of Lowry et al. (10).

Activities of cytochrome P-450 reductase, cytochrome c reductase and NADPH oxidase of the ethanol group increased significantly in the absence of the drug substrate as compared with those of control rats. Activities of cytochrome P-450 reductase and cytochrome c reductase of the ethanol group in the presence of aminopyrine (3 mM) increased significantly, compared with those in the control group. With cytochrome P-450 reductase, however, there was no difference in the aminopyrine-enhanced rate of reduction of cytochrome P-450 between control and ethanol-treated rats. The magnitude of the stimulatory effect of aminopyrine was the same in both groups (Table 1). In the presence of aniline (2 mM) the rate of reduction of cytochrome P-450 of the ethanol group was similar to that of the controls and the NADPH cytochrome c reductase activity of the

TABLE 1. Cytochrome P-450 reductase activity of prolonged ethanol-treated rats in the absence and presence of aminopyrine.

Each experiment was carried out with microsomes from the same animal respectively.

Groups	Aminopyrine (3 mM)		Difference
	Absence	Presence	
Control (8)	2.64±0.16	4.20±0.44	1.56±0.55
Ethanol-treated (8)	3.62±0.50**	5.24±0.55**	1.47±0.31

Values are the mean values±standard deviation. The number of animals used for each value is in parentheses. P-450 reductase activity : $\Delta\text{Abs}_{450}/\text{sec/mg protein} \times 10^{-3}$

**significantly different from control, $p<0.01$

TABLE 2. Cytochrome P-450 reductase activity of prolonged ethanol-treated rats in the absence and presence of aniline.

Each experiment was carried out with microsomes from the same animal respectively.

Groups	Aniline (2 mM)		Difference
	Absence	Presence	
Control (8)	2.40±0.14	1.71±0.13	0.69±0.08
Ethanol-treated (8)	3.10±0.22**	1.96±0.24	1.14±0.14**

** significantly different from control, $p<0.01$

ethanol group was significantly higher than that of the controls. The magnitude of the inhibitory effect of aniline in the ethanol group was higher than that in the control group (Table 2). There was no difference in the activity of NADPH oxidase between the control and ethanol groups in the presence of the drug substrate.

In the present study, the rate of reduction of cytochrome P-450 of the ethanol group was higher than that of the controls in the presence of aminopyrine although there was no difference in the aminopyrine-enhanced rate of reduction of cytochrome P-450 between the controls and ethanol-treated rats. There was no difference in the rate of reduction of cytochrome P-450 in the presence of aniline between the controls and ethanol group. The cytochrome P-450 reductase activity of the ethanol group was more sensitive to aniline than that of the control rats. On the other hand, with drug hydroxylase activity *p*-hydroxylation of aniline increases significantly but N-demethylation of aminopyrine in the ethanol group does not increase (2). It is known that the rate of oxidation of aminopyrine by liver microsomes from 3-methylcholanthrene-treated rats is the same as the rate of reduction of cytochrome P-450 in the presence of aminopyrine while the rate of oxidation of aniline is about 10% that of aminopyrine (11). These findings suggest that the rate-limiting step of aminopyrine N-demethylation may be the rate of reduction of cytochrome P-450 while the reduction of the hemoprotein is not limiting in *p*-hydroxylation reaction of aniline.

At present, the relationship between microsomal hydroxylase activity and cytochrome

P-450 reductase activity in the ethanol-treated rats is difficult to interpret. The results obtained in the present study suggest that part of the cytochrome P-450 fraction which increased by chronic ethanol ingestion may be different qualitatively from that of the control rats.

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EFFECT OF 4-PENTENOIC ACID ON LIPIDS IN PLASMA AND LIVER OF RATS

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It has been reported that 4-pentenoic acid (4-PA) inhibited fatty acid oxidation in *in vitro* systems, but causes little inhibition of oxidative phosphorylation in mitochondria (1, 2, 3). Kleinman et al. (4) have suggested that *in vivo* perfusion of dog kidneys with 4-PA produces a natriuresis, kaliuresis, and bicarbonate diuresis independent of changes in renal hemodynamics and which appear to be due to inhibition of the metabolic energy sources for active transport. To consider the biological action of 4-PA, we have examined the effects of 4-PA on free fatty acids (FFA) and triglycerides (TG) in plasma and liver of rats, and also attempted to determine its effect on the peroxidation of polyunsaturated fatty acid.

Wistar strain male rats, weighing 150 to 250 g, were used as test subjects. 4-PA in saline solution or saline alone for control was injected i.p.. 4-PA was given once a day at a dose of 50 mg/kg for 3 days. Blood samples were taken from all rats after which the animals were exsanguinated. The liver was removed 24 hr after the final treatment. Plasma and liver homogenate (prepared in ice-cold 0.25 M sucrose, 1:9, w/v) were used as samples to be assayed for FFA and TG. Mitochondrial fraction was prepared by the method of Hogeboom (5). Protein was determined by the method of Lowry et al. (6).