

# Chronic ethanol administration decreases fatty acyl-CoA desaturase activities in rat liver microsomes

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The  $\Delta^9$ -desaturase system in liver microsome from rats treated chronically with ethanol was studied. Stearoyl-CoA desaturase activity decreased by 80% and palmitoyl-CoA desaturase activity was not detectable in microsomes from ethanol-fed rats, while activities of electron transport components such as NADH-cytochrome *c* and NADH-ferricyanide reductases remained unchanged. However, chronic ethanol administration resulted in an adaptive induction of the activity of NADPH-cytochrome *c* reductase and the contents of cytochrome *b*<sub>5</sub> and P-450. The activity of the terminal component (cyanide-sensitive factor; CSF) of the desaturase system was greatly depressed by ethanol treatment. The NADH/NAD ratio in microsomes of ethanol-fed rats increased over 2-fold. These results suggest that, during chronic ethanol ingestion, decreased activities of  $\Delta^9$ -desaturases are due mainly to a decreased content of the terminal component of the desaturase system.

*Chronic ethanol administration*

*Fatty acyl-CoA desaturase*

*Cyanide-sensitive factor*

## 1. INTRODUCTION

It is well known that, during chronic exposure to ethanol, biological membranes appear to adapt readily to the fluidizing effect of ethanol by altering their membrane lipid composition. For example, prolonged intake of ethanol results in adaptive modifications of fatty acid unsaturation and membrane fluidity of microsomes from rat liver [1,2] and some organisms [3,4]. From the clinical point of view, in addition, alcohol exerts its anesthetic potency and CNS (central nervous system) depressant activity by interacting with the lipid portion of the neural membranes [5,6]. Ethanol administration to rats produces fatty liver via accumulated fatty acids in hepatic triacylglycerols [7–9], which appear to be due to reduced fatty acid oxidation. Although ethanol is oxidized through the cytosolic NADH-dependent alcohol dehydrogenase and aldehyde dehydrogenase, the microsomal ethanol-oxidizing system (MEOS) has been shown to contribute significantly to ethanol oxidation in livers of ethanol-adaptive rats [10]. We have here investi-

gated whether chronic ethanol feeding may cause an adaptive modification of fatty acid desaturase system in liver microsomes which is involved in the formation of unsaturated fatty acids.

## 2. MATERIALS AND METHODS

### 2.1. Diet treatment

Male Sprague-Dawley rats of 115–125 g body weight were fed for 10 weeks on a nutritionally adequate liquid diet (17% sucrose, 33% oil mixture, 19% casein, 31% ethanol) in which ethanol provided 31% of the total calories [11]. Control rats received the same diet, but with sucrose isocalorically substituted for ethanol, i.e., 48% sucrose, 33% oil mixture, 19% casein. Both types of rats were administered as 80 kcal/head every 24 h.

### 2.2. Preparation of microsomes

After the treatment, rats were killed by decapitation. Livers were immediately collected and homogenized in 0.25 M sucrose containing 10 mM Tris-HCl (pH 7.2), 5 mM EDTA, 1 mM dithiothreitol.

The homogenate was centrifuged at  $10000 \times g$  for 20 min, the pellet discarded and the supernatant centrifuged again at  $105000 \times g$  for 60 min. Microsomes obtained were washed once by suspending in 10 mM Tris-HCl buffer (pH 7.2). Washed microsomes were immediately frozen at  $-90^\circ\text{C}$  before use. Protein content of microsomes was measured as in [12], using bovine serum albumin as standard.

### 2.3. Enzyme assays

The activities of palmitoyl-CoA and stearoyl-CoA desaturases were assayed, using 20 nmol [ $^{14}\text{C}$ ]-palmitoyl-CoA or [ $^{14}\text{C}$ ]stearoyl-CoA, 50 nmol NADH, 0.1 M potassium phosphate (pH 7.2), 400  $\mu\text{g}$  microsomes in a final volume of 0.5 ml at  $37^\circ\text{C}$  as in [13,14].

The NAD(P)H-cytochrome *c* and NADH-ferricyanide reductase activities were determined using 20 nmol cytochrome *c* or 500 nmol potassium ferricyanide, 100 nmol NADH or NADPH, 20–100  $\mu\text{g}$  microsomes, and 0.1 M potassium phosphate buffer (pH 7.2) in a total volume of 1.0 ml at  $37^\circ\text{C}$  [15,16]. The activities were calculated using respective extinction coefficients of  $19.6 \text{ mM}^{-1} \cdot \text{cm}^{-1}$  [17] and  $1.02 \text{ mM}^{-1} \cdot \text{cm}^{-1}$  [18].

### 2.4. Analysis of hemoprotein content

Cytochrome *b*<sub>5</sub> was reduced by the addition of 100 nmol NADH and the content of cytochrome *b*<sub>5</sub> was determined by measuring the reduced minus oxidized difference spectrum, taking the extinction coefficient of the cytochrome between 424 and 409 nm as  $185 \text{ mM}^{-1} \cdot \text{cm}^{-1}$  [19].

Cytochrome P-450 was assayed using the carbon monoxide difference spectra of dithionite-reduced microsomes [19]. The extinction difference of the hemoprotein was taken as  $91 \text{ mM}^{-1} \cdot \text{cm}^{-1}$  (450 minus 490 nm) for cytochrome P-450.

CSF (terminal component) activity was assayed by measuring spectrophotometrically the stearoyl-CoA-stimulated reoxidation rate of NADH-reduced cytochrome *b*<sub>5</sub> by monitoring the change in absorbance difference between 424 and 409 nm. The sample cuvette contained microsomes with 0.2 nmol cytochrome *b*<sub>5</sub>, 200 nmol fresh  $\text{Na}_2\text{S}$  to inhibit mitochondrial cytochrome oxidase, and 0.1 M Tris-HCl buffer (pH 7.2) in a final volume of 3.0 ml. The rate of reoxidation of cytochrome *b*<sub>5</sub> reduced by 2 nmol NADH at  $37^\circ\text{C}$  was calcu-

lated using  $185 \text{ mM}^{-1} \cdot \text{cm}^{-1}$  [19] as the extinction difference of the cytochrome between 424 and 409 nm by the method in [20] with a slight modification [21,22].

### 2.5. Analysis of NAD(P)H contents

The content of NAD(H) was determined as in [23]. Liver tissue was removed under pentobarbital (50 mg/ml/kg, i.p.) by the quick freeze method and was frozen in liquid nitrogen. For the determination of NAD content, the frozen tissue was homogenized with 5-fold volume of 0.6 N  $\text{HClO}_4$  and centrifuged at  $5000 \times g$  for 5 min at  $4^\circ\text{C}$ .  $\text{KH}_2\text{PO}_4$  (1 M) was slowly added to the supernatant with stirring and the solution was adjusted to pH 7.2–7.4. NAD content in the supernatant was measured using 100 mM ethanol, 2 units alcohol dehydrogenase, 1 ml supernatant and 50 mM pyrophosphate buffer (50 mM  $\text{Na}_4\text{P}_2\text{O}_7$ , 25 mM semicarbazide-HCl; pH 8.8) in a final volume of 2.0 ml at  $25^\circ\text{C}$ . The content was determined by the increase in absorbance at 340 nm.

For the determination of NADH content, the frozen tissue was homogenized in cooled 0.5 N KOH in ethanol. This homogenate was incubated for 5 min at  $90^\circ\text{C}$ , and then quickly cooled. After 5 min, the solution was neutralized by slowly adding triethanolamine-HCl-phosphate mixture (0.5 M triethanolamine, 0.4 M  $\text{KH}_2\text{PO}_4$ , 0.1 M  $\text{K}_2\text{HOP}_4$ ) until the pH was about 7.8. Ten min later, centrifugation was carried out at  $40000 \times g$  for 5 min. NADH content in the supernatant was measured using 0.2 mM pyruvate, 10 units lactate dehydrogenase, 2 ml supernatant in a total volume of 2.2 ml at  $25^\circ\text{C}$ . The content was determined by the decrease in absorbance at 340 nm using a Hitachi-356 two-wavelength double-beam spectrophotometer.

### 2.6. Materials

The following chemicals were obtained from commercial sources: [ $1\text{-}^{14}\text{C}$ ]palmitoyl-CoA and [ $1\text{-}^{14}\text{C}$ ]stearoyl-CoA (New England Nuclear, Boston, MA); palmitoyl-CoA and stearoyl-CoA (P-L Biochemicals, Milwaukee, WI); NADH, NADPH, horse heart cytochrome *c*, pyruvate, alcohol dehydrogenase and lactate dehydrogenase (Sigma, St. Louis, MO); potassium ferricyanide (Kishida Chemical Co., Osaka); Lieber/DeCarli liquid diets (Oriental Yeast Co. Tokyo). Sprague-Dawley rats were obtained from Shizuoka

laboratory animal center, Shizuoka. Other chemicals were of the highest purity available from commercial sources.

### 3. RESULTS AND DISCUSSION

Chronic ethanol ingestion has been shown to induce conspicuous structural and functional changes in liver microsomal membranes, including decreases of membrane fluidity [1] and modifications of fatty acid composition [2]. The latter report has suggested ethanol-induced alterations in fatty acid desaturase activities. Similar changes have been reported in some other organisms as model systems, such as *Tetrahymena pyriformis* [3] and *Escherichia coli* [24]. We have demonstrated that fatty acid desaturase activities decrease in microsomes from *Tetrahymena* cells chronically exposed to ethanol [4]. These changes have been interpreted as an adaptive response to ethanol because of the development of tolerance against ethanol.

We have here investigated fatty acid desaturase activity which may take part in a modification of membrane lipid fatty acid composition in liver from ethanol-exposed rats. In the ethanol formula for ethanol-fed rats, carbohydrate (sucrose) was isocalorically replaced by ethanol to the extent of 31% of the total calories. In liver microsomes from ethanol-fed rats,  $\Delta^9$ -desaturase activities drastically decreased; palmitoyl-CoA desaturase activity was undetectable and stearoyl-CoA desaturase activity decreased by 80% (table 1). Authors in [25] have also demonstrated that microsomal

Table 1

Alterations in activities of  $\Delta^9$ -desaturases in liver microsomes from chronically ethanol-administrated rats

Activity	Control (n = 6)	Ethanol-fed (n = 5)
Palmitoyl-CoA desaturase	0.34 ± 0.05	0.14 ± 0.04 <sup>a</sup>
Stearoyl-CoA desaturase	0.69 ± 0.04	n.d.

Details for the enzyme assays are described in section 2. n, number of experiments; <sup>a</sup>P < 0.01 compared to values of control rats. n.d., not detectable. Values are expressed as nmol/min per mg protein

$\Delta^9$ -desaturase (palmitoyl-CoA desaturase) activity is drastically reduced in ethanol-fed rats. This finding would be able to explain the reduction of unsaturated fatty acids in liver membranes from ethanol-fed rats. In HeLa cells [26] and *Tetrahymena* cells [4] chronically administered ethanol, the content of unsaturated fatty acids, especially palmitoleate and oleate, is greatly depressed. In addition, authors in [1] have reported that chronic ethanol ingestion evokes a decrease in liver microsomal membrane fluidity. Table 2 presents alterations in activities of electron transport components in liver microsomes prepared from ethanol-fed rats. The comparison of control with ethanol-fed groups showed a small but significant increase activity of NADPH-cytochrome c reductase, involving the transport of electrons from NADPH to cytochrome P-450, whereas activities of NADH-ferricyanide and NADH-cytochrome c reductases remained unchanged. However, activity of the terminal component (cyanide-sensitive factor; CSF) of the desaturase system decreased to one-fifth of the control. These results suggest that, by chronic exposure of rats to ethanol, diminished activities of  $\Delta^9$ -desaturase are due principally to the reduction in terminal component activity.

Table 2

Alterations in activities of electron transport components in liver microsomes from chronically ethanol-administrated rats

Activity	Control (n = 6)	Ethanol-fed (n = 5)
(μmol/min per mg protein)		
NADH-ferricyanide reductase	4.29 ± 0.68	4.16 ± 0.22
NADH-cytochrome c reductase	1.17 ± 0.03	1.23 ± 0.04
NADPH-cytochrome c reductase	0.107 ± 0.003	0.139 ± 0.021 <sup>a</sup>
(nmol/min per mg protein)		
CSF activity	1.36 ± 0.07	0.33 ± 0.07 <sup>a</sup>

Details for the enzyme assays are described in section 2. n, number of experiments; <sup>a</sup>P < 0.01 compared to values of control rats. CSF (terminal component) activity represents stearoyl-CoA-stimulated reoxidation rate of NADH-reduced cytochrome b<sub>5</sub>

Table 3

Alterations in contents of microsomal hemoproteins, NADH and NAD in liver from chronically ethanol-administrated rats

Content	Control	Ethanol-fed
	(nmol/mg protein)	
Cytochrome <i>b</i> <sub>5</sub>	0.47 ± 0.02(6)	0.59 ± 0.02(5) <sup>a</sup>
Cytochrome P-450	0.92 ± 0.06(6)	1.27 ± 0.05(5) <sup>a</sup>
	(nmol/g tissue)	
NADH	87 ± 21(7)	160 ± 39(8) <sup>a</sup>
NAD	741 ± 5(7)	740 ± 49(8)
NADH/NAD ratio	0.118 ± 0.027(7)	0.216 ± 0.051(8) <sup>a</sup>

Details for content analysis are described in section 2. Values in parentheses: number of experiments.

<sup>a</sup>*P* < 0.01 compared to values of control rats

The effects of chronic ethanol consumption on the contents of microsomal hemoproteins, NADH and NAD in rat liver are summarized in table 3. Chronic ethanol administration provoked small rises in contents of liver microsomal hemoproteins; a 1.26-fold increase of cytochrome *b*<sub>5</sub> and a 1.38-fold increase of cytochrome P-450. The enhancement of cytochrome *b*<sub>5</sub> content in ethanol-fed rats may be due to the increase in electron transport to cytochrome P-450, of which content increased in relation to ethanol oxidation [27]. Chronic ethanol intake resulted in a 2-fold elevation of NADH content in rat livers but had no effect on the content of NAD. The increase of NADH content would result from its enhanced generation by alcohol dehydrogenation and the decreases of oxidation rate of NADH [7] and of activity of NADH-dependent enzymes such as fatty acyl-CoA desaturases. The high NADH level would stimulate the biosynthesis of triacylglycerol in liver from ethanol-fed rats. In this work, evidence has been represented that liver microsomes from ethanol-fed rats accomplish an adaptive induction of cytochrome P-450, thereby leading to the decreases in both NADPH-cytochrome *c* reductase activity and cytochrome *b*<sub>5</sub> level.

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