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Significance of Serum Gamma Glutamyl Transpeptidase as a Marker of Alcoholism

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ISHII, H., F. OKUNO, Y. SHIGETA, S. YASURAOKA, Y. EBIHARA, T. TAKAGI AND M. TSUCHIYA. *Significance of serum gamma glutamyl transpeptidase as a marker of alcoholism.* PHARMAC. BIOCHEM. BEHAV. 13: Suppl. 1, 95–99, 1980.—To study the effect of chronic ethanol administration on the activity of gamma glutamyl transpeptidase (GGTP) in various tissues, female rats were pair-fed liquid diets with 36% of total calories either as ethanol or as isocaloric carbohydrate (controls). Six weeks of ethanol feeding resulted in a significant increase of cytochrome P450 content. Hepatic microsomal GGTP activity was almost doubled after ethanol feeding whether expressed per g of liver or per mg of microsomal protein. Furthermore, intestinal GGTP activity was significantly enhanced after ethanol, whereas there was no change in the enzyme activity in either kidney or pancreas. There was a concomitant elevation of plasma GGTP activity. Phenobarbital administration to rats resulted in an enhancement of GGTP activity in the liver whether given orally or intraperitoneally. In addition, intestinal GGTP activity after oral phenobarbital was also significantly increased, although its activity after intraperitoneal administration was not enhanced. These results suggest that enhanced hepatic and intestinal GGTP activities may contribute, at least in part, to an increased level of serum GGTP frequently seen in chronic alcoholics.

Gamma glutamyltranspeptidase Alcoholism Liver Intestine Microsomes Enzyme induction Phenobarbital

GAMMA glutamyl transpeptidase (GGTP) is an enzyme which catalyses the transfer of the gamma glutamyl group from peptides containing it to other peptides and to L-amino acids. In normal subjects this enzyme is concentrated mainly in the kidney and the pancreas, followed by the small intestine, with only one-tenth in the liver [14]. It is present in the form of a membrane-bound constituent of the microsomal fraction but is also present as a soluble form in the cytoplasm [23], and elevated serum activity of GGTP has been reported in hepatobiliary and pancreatic [20,22] diseases.

Moreover it has been found that the elevation of serum GGTP activity is frequently seen in alcoholics and heavy drinkers, and its measurement is a sensitive marker for the detection of alcoholism [8, 16, 26]. However the mechanism of this enhancement of serum GGTP activity in alcoholics is still a matter of controversy.

In the present paper, we attempted to clarify some of the mechanisms involved in enhanced serum GGTP activity in alcoholics.

METHOD

Female Wistar rats, weighing about 180 grams, were used for the study and housed in individual wire-bottom cages. The animals were fed a nutritionally adequate liquid diet [2] for 6 weeks (purchased from Bio Serv. Co. Inc., Frenchtown, NJ, Diet No. 711-A, C.). Carbohydrate, protein and fat provided 47%, 18% and 35%, respectively of the total calories. Pair-fed rats consumed the same diet except that carbohydrate was isocalorically replaced by ethanol, accounting for 36% of total calories.

In the second set of experiments, Wistar rats were given sodium phenobarbital (80 mg/kg, IP) for 4 days.

In the third set of experiments, rats were administered a 0.1% solution of sodium phenobarbital ad lib in the drinking water for 4 weeks. The amount of phenobarbital intake was approximately 100 mg/kg of body weight in each rat.

All of the animals were decapitated, and the blood was collected from the neck vessels. The liver was immediately perfused *in situ* via the portal vein with ice-cold physiological saline, quickly excised, weighed and used for experiments. All subsequent steps were performed at 2°C to 4°C. The liver was homogenized in 3 volumes of 0.25 M sucrose. Then the homogenates were centrifuged at 10,000 × G for 20 min, followed by ultracentrifugation at 105,000 × G for 60 min in a Beckman L5-50 ultracentrifuge. The pellets harvested by this procedure were used as microsomal fractions.

The rough and smooth microsomal fractions were obtained from another fraction of liver homogenates from the first set of experiments. The microsomal subfractions were obtained by using a discontinuous sucrose density gradient according to Bergstrand *et al.* [1]. The purity of the rough

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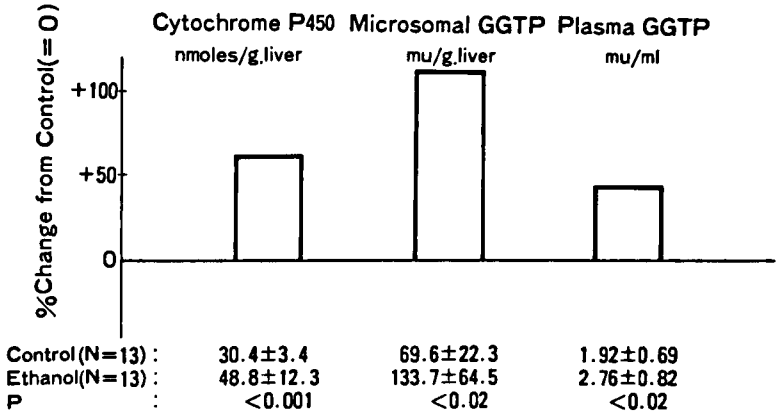

FIG. 1. Effect of chronic ethanol administration on hepatic microsomal cytochrome P450 content and the activities of hepatic microsomal and plasma GGTP in rat.

and smooth microsomal membranes was monitored electron microscopically.

Tissue homogenates of the kidney, pancreas and small intestine were prepared for the determination of GGTP. For the intestinal preparation, the small intestine, removed from the mesentery 10 to 15 cm below the pyloric ring, was used. After washing with ice-cold saline, its mucosa was removed by scraping gently with a glass edge on an iced glass plate.

The activity of GGTP was measured according to Orlowski's method [15], with gamma glutamyl-p-nitroanilide as a glutamyl group donor. One unit of activity was expressed as the amount of enzyme required to liberate one micromole of p-nitroaniline from the substrate in one minute at 37°C. Hepatic cytochrome P450 content was measured by the method of Omura and Sato [13], using a Shimazu MSP-5000 recording spectrophotometer. Protein concentration was determined according to Lowry *et al.* [10]. In all experiments, significance of the results was evaluated by the Student's *t*-test.

TABLE 1

EFFECT OF CHRONIC ETHANOL ADMINISTRATION ON THE GGTP ACTIVITIES OF VARIOUS TISSUE HOMOGENATES IN RAT

	Control (13)	Ethanol (13)	*p*
Liver			
mg/g of tissue	138.7 ± 2.5	227.8 ± 32.4	<0.01
Kidney			
u/g of tissue	464.3 ± 51.9	460.8 ± 47.0	N.S.
Pancreas			
u ± g of tissue	65.7 ± 7.0	69.9 ± 5.3	N.S.
Duodenum			
u/g of tissue	1.7 ± 0.4	2.5 ± 0.2	<0.02

Data express mean ± SEM.
N.S. means not significant.
Figures in parentheses indicate number of animals used for the experiment.

RESULTS

Effect of Chronic Ethanol Feeding on Cytochrome P450 Content and GGTP Activity

After 6 weeks of ethanol feeding a significant increase in cytochrome P450 content was observed in hepatic microsomal fractions of ethanol-fed animals, as shown in Fig. 1. Futhermore, hepatic microsomal GGTP activity was almost doubled when expressed per gram of liver (Fig. 1). This difference remained unchanged whether expressed per mg of microsomal protein or 100 g of body weight. Concomitantly, plasma GGTP activity was significantly elevated in ethanol-fed rats (Fig. 1).

Table 1 shows the effect of chronic ethanol feeding on GGTP activity in hepatic, renal, pancreatic and intestinal tissues, indicating that chronic ethanol feeding results in an increase in GGTP activity only in the liver and the duodenum, but not in the kidney or the pancreas.

TABLE 2

EFFECT OF CHRONIC ETHANOL ADMINISTRATION ON GGTP ACTIVITY IN ROUGH AND SMOOTH MICROSOMAL MEMBRANES

	Rough Membrane	Smooth Membrane
GGTP		
(mu/mg protein)		
Control	0.25 ± 0.03	1.06 ± 0.03
Ethanol	0.63 ± 0.08*	2.41 ± 0.05*
(mu/g liver)		
Control	3.33 ± 0.61	10.70 ± 0.94
Ethanol	6.03 ± 0.80*	20.27 ± 4.72*

Each value represents Mean ± SEM obtained from 4 animals.
*($p<0.01$) compared to respective controls.

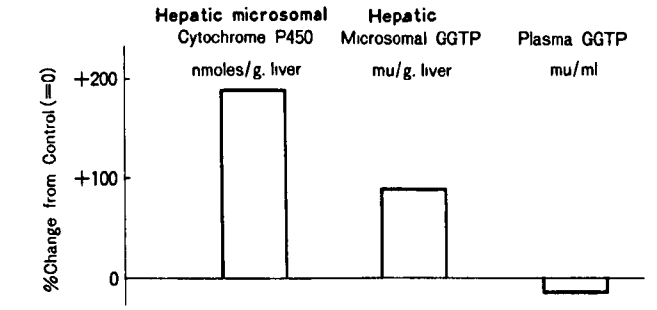

FIG. 2. Effect of intraperitoneal phenobarbital administration on hepatic microsomal cytochrome P450 content and the activities of hepatic microsomal and plasma GGTP in rat.

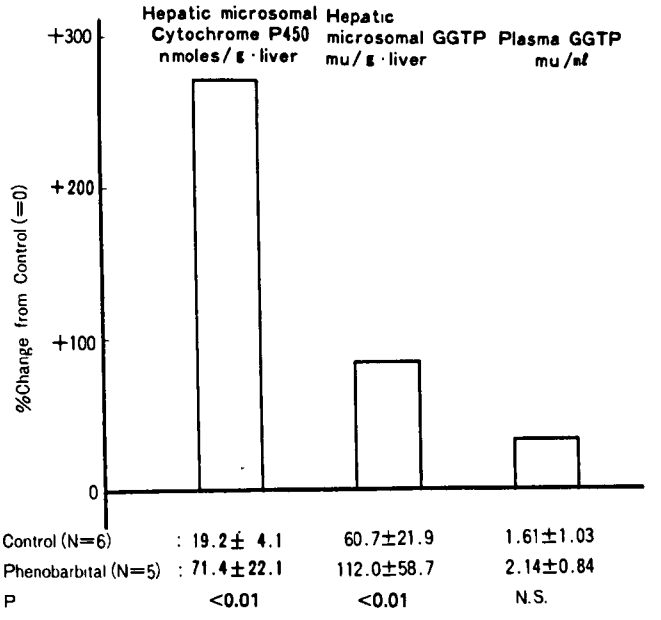

FIG. 3. Effect of oral phenobarbital administration on hepatic microsomal cytochrome P450 content and the activities of hepatic microsomal and plasma GGTP in rat.

Effect of Chronic Ethanol Feeding on the Submicrosomal Localization of GGTP Activity

In controls, the rough microsomes accounted for 20% of the GGTP activity and the smooth microsomes for 80% when expressed per mg of microsomal protein. In the control livers, 24% of the GGTP activity was recovered from the rough microsomes and 76% from the smooth, when expressed per g of liver (Table 2). Chronic ethanol feeding resulted in an enhancement of GGTP activity in both microsomal fractions. However, the increase occurred predominantly in the smooth microsomal membranes.

Effect of Intraperitoneal or Oral Phenobarbital Administration on Cytochrome P450 Content and GGTP Activity

In another set of experiments with phenobarbital administration, microsomal cytochrome P450 content increased significantly in phenobarbital-treated rats, whether the drug was administered intraperitoneally or orally (Figs. 2, 3). Furthermore, hepatic microsomal GGTP activity was enhanced significantly by either route of phenobarbital administration, whether expressed per g of liver or mg of microsomal protein. However, plasma GGTP activity was not significantly increased, either in the animals given phenobarbital orally, or in the rats given phenobarbital intraperitoneally. Furthermore, four weeks of oral administration of phenobarbital resulted in an enhancement of intestinal GGTP activity, whereas there was no significant increase in the enzyme activity in the intestine of the rats treated intraperitoneally with phenobarbital (Table 3). However, no significant changes of GGTP activity were seen either in the renal or pancreatic tissue, whether phenobarbital was administered intraperitoneally or orally.

TABLE 3

EFFECT OF ETHANOL OR PHENOBARBITAL ON THE INTESTINAL GGTP ACTIVITY

Experimental groups		GGTP Activity (u/g tissue)	
Control	(13)	1.7 ± 0.4	$p<0.02$
Ethanol	(13)	2.5 ± 0.2	
Control	(3)	2.2 ± 0.2	p: NS
Phenobarbital (IP)	(5)	2.6 ± 0.5	
Control	(6)	1.9 ± 0.4	$p<0.05$
Phenobarbital (PO)	(5)	3.7 ± 0.3	

Data express Mean ± SEM. NS means not significant.
Figures in parentheses indicate number of animals used for the experiments.

DISCUSSION

There have been several reports that serum GGTP activity is increased in patients with alcoholic liver disease or in chronic alcoholics [8, 16, 26]. Among them, Rosalki *et al.* [16] reported that GGTP determination is the most sensitive and highly specific laboratory procedure for the detection of excessive alcohol consumption. This enzyme activity has also been reported to be enhanced in patients receiving enzyme-inducing drugs such as phenobarbital [17].

In our previous survey [25] we reported that more than 80% of 127 alcoholics showed elevated serum activity of GGTP. However, the mechanism of this enhancement in alcoholics remains to be elucidated. Alcohol, as well as phenobarbital, is known to result in a proliferation of hepatic endoplasmic reticulum, especially the smooth membranes, with increased cytochrome P450 content and enhanced ac-

tivities of glucose-6-phosphatase, microsomal ethanol oxidizing system and various drug metabolizing enzymes [4, 7, 9, 18]. It was reported in the earlier study [3] that hepatic GGTP activity increased significantly in rats given phenobarbital intraperitoneally, whereas there was no significant enhancement of the enzyme activity in the ethanol-treated rats.

In the present study, we have demonstrated that chronic ethanol administration to rats results in a significant increase in GGTP levels in hepatic microsomes and in plasma. In addition, both oral and intraperitoneal administration of phenobarbital resulted in a significant increase in hepatic microsomal GGTP activity associated with increased cytochrome P450 content. Some of these data have been reported previously [6].

The elevation of serum GGTP activity after chronic ethanol intake might be closely linked with a proliferation of hepatic endoplasmic reticulum. Teschke *et al.* [24] have suggested the same possibility. On the other hand, Rosalki [16] stated that the elevation of serum GGTP activity could presumably result from cellular damage to the liver induced by alcohol. Mørland *et al.* [11] have observed elevated plasma and hepatic GGTP activities after ethanol, but they attributed the difference in GGTP activity to a reduction in the enzyme activity in the control animals, possibly due to a higher dietary carbohydrate content. In that respect, more recently, Shaw *et al.* [21] have shown that when a nutritionally adequate diet with carbohydrate as 47% of calories, or the same diet with fat or ethanol substituted for carbohydrate was used, plasma and hepatic GGTP activities were increased after ethanol feeding, as compared to activity in animals fed a high carbohydrate diet. By contrast, no increase in GGTP activity was observed after a high fat diet, suggesting that an increase in GGTP activity after ethanol was not due to decreased carbohydrate content. Thus, the amount of ethanol ingested appears to be important for the enhancement of GGTP activity.

In the present study, we have shown that submicrosomal localization of GGTP is mainly in the smooth microsomal membrane. Moreover the enzyme activity after ethanol was enhanced predominantly in the smooth membrane. This tendency is in accord with the changes of microsomal drug metabolizing enzymes as well as glucose-6-phosphatase activity after chronic ethanol feeding, as previously reported [4, 5, 7, 18].

Another important aspect of the present study is that we have demonstrated enhanced activity of intestinal GGTP after ethanol ingestion (Table 3), whereas there was no such increase after intraperitoneal administration of phenobarbital. This finding is of interest in view of the effects of ethanol on the intestine, which are similar to those produced in the liver, i.e., mitochondrial abnormalities, and dilation and vesiculation of the smooth endoplasmic reticulum [19]. We wondered if this difference could be attributed to the differences in route of administration of ethanol (*per os*) and phenobarbital (intraperitoneal). Therefore, we have treated rats with phenobarbital orally, and found an increase in intestinal GGTP activity, in contrast to the results with intraperitoneal phenobarbital administration. However, neither route of administration of phenobarbital resulted in changes of plasma GGTP activity. Both ethanol and oral phenobarbital administration to rats resulted in an increase in hepatic and intestinal GGTP activities, whereas only ethanol resulted in a concomitant increase in plasma GGTP levels. These data suggest that the elevation of serum GGTP activity after ethanol ingestion could not be attributed only to the hepatic and/or intestinal enhancement of GGTP activity. Although further investigation is to be done, it is conceivable that ethanol affects the plasma membrane of the liver cell injuriously, whereas phenobarbital does not. In this respect, we have recently observed that hepatocytes isolated from rats fed ethanol chronically have altered membrane permeability, as shown by an increased number of cells stained with trypan blue, suggesting ethanol-induced fragility of the liver cell membrane [12].

At present, an enhancement of the activities of hepatic and intestinal GGTP, accompanied by an injury of liver cell membranes due to ethanol, may contribute, at least in part, to an increased level of serum GGTP seen after chronic ethanol consumption.

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