INDUCTION OF HEPATIC MICROSOMAL GAMMA-GLUTAMYLTRANSFERASE ACTIVITY FOLLOWING CHRONIC ALCOHOL CONSUMPTION

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Summary

Chronic alcohol consumption for 4-5weeks results in an enhancement of serum gamma-glutamyltransferase activity in rats. Concomitantly, an increase of hepatic gamma-glutamyltransferase was observed. Upon subcellular fractionation of liver homogenates by ultracentrifugation, an induction of gamma-glutamyltransferase activity could be demonstrated in the microsomal fraction of the hepatocyte. These findings suggest that increased serum gamma-glutamyltransferase activities commonly observed in alcoholism can be ascribed at least in part to an induction of microsomal gamma-glutamyltransferase activity.

High serum gamma-glutamyltransferase (GGT) activities are commonly found in alcoholics (1), but the mechanism of this phenomen remained to be established. Following the ingestion of alcohol, the metabolism of ethanol proceeds not only via cytosolic alcohol dehydrogenase but also in the microsomal fraction of the hepatocyte which comprises the endoplasmic reticulum (2-5). Chronic ethanol consumption results in a proliferation of the smooth endoplasmic reticulum of the hepatocyte (6) which is associated with an induction of various microsomal enzymes including the microsomal ethanol oxidizing system and other drug metabolizing enzymes (2,7-11). Since GGT activity is located primarily in the microsomal fraction of the hepatocyte (12), the question arose whether chronic ethanol consumption might result in an induction of microsomal GGT activity which in turn could explain increased serum levels

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of GGT activities following prolonged alcohol consumption.

Materials and Methods

Female Sprague-Dawley rats were purchased from Zentralinstitut für Versuchstierzucht Hannover (West Germany) and with a starting body weight of 1oo-13o g they were pair-fed nutritionally adequate liquid diets containing either ethanol (36 % of total calories) or isocaloric carbohydrates (dextrin) as controls for 4-5 weeks (13). The animals were decapitated, and the blood was collected from the neck vessels. The livers were immediately perfused in situ through the portal vein with ice-cold o.15 M KCl, and a 25 % liver homogenate was prepared with the same solution. The homogenate was centrifuged at 1o,ooo g for 3o min, and the resulting supernatant was used for the subsequent preparation of washed microsomes and of liver cytosol (1oo,ooo g supernatant) with an ultracentrifuge as described previously (14,15).

Gamma-glutamyltransferase (EC 2,3.2.2)activity was measured in the serum by spectrophotometric assay at 4o5 nm according to the method of Szasz (16) in an incubation medium (final volume 3.2 ml) containing gamma-glutamyl-p-nitranilid at a final concentration of 4 mM, 4o mM glycylglycine and 185 mM tris buffer, pH 8.25 (Boehringer Mannheim GmbH). Unless otherwise stated, GGT activity in hepatic subcellular fractions was determined under the same assay conditions as described above except that deoxycholate (1.o mg per assay) was added to the incubation medium. The activities of alanine aminotransferase (EC 2.6.1.2) and leucine arylamidase (EC 3.4.1.1) were measured in serum and liver according to the methods of Bergmeyer and Bernt (17) and Nagel et al. (18), respectively. Protein was determined with the biuret method according to Weichselbaum (19).

Each measurement was carried out in duplicate. The results were compared with the corresponding values of the pair-fed control. The means (±SEM) and individual differences were calculated, and their significances were assessed by the Student's t-test.

Results

Chronic alcohol consumption for 4-5 weeks results in a significant increase of serum gamma-glutamyltransferase (GGT) activity when compared to their pair-fed controls (Table 1). Conversely, the serum activities of other hepatic enzymes such as alanine aminotransferase and leucine arylamidase remained unchanged under these experimental conditions (Table 1). Of particular interest was the finding that the increase of serum GGT activity was associated with a marked enhancement of hepatic GGT activity (Table 1).

Table 1

EFFECT OF CHRONIC ETHANOL CONSUMPTION ON THE ACTIVITIES OF GAMMA-GLUTAMYLTRANSFERASE, ALANINE AMINOTRANSFERASE AND LEUCINE ARYLAMIDASE IN SERUM AND LIVER

Female rats were pair-fed for 4-5 weeks nutritionally adequate liquid diets containing either ethanol or dextrin as controls. The enzyme activities were determined in the serum and the hepatic homogenate and are expressed in units per ml serum and per g wet weight of liver, respectively. The values are derived from 12 experimental animals.

Enzyme activity	Control rat	Ethanol fed rat	P
Gamma-glutamyltransferase			
serum (units/l)	1.11 ± o,53	3.87 ± o.4o	< o.oo1
liver (units/g)	o.35 ± o.o2	o.56 ± o.1o	< o.o25
Alanine aminotransferase			
serum (units/l)	79.4 ± 17.o	73.3 ± 3.4	N.S.
liver (units/g)	29.5 ± 3.7	45.2 ± 6.9	< o.o5
Leucine arylamidase			
serum (units/l)	27.6 ± 2.8	28.8 ± 4.1	N.S.
liver (units/g)	o.65 ± o.1	o.59 ± o.1	N.S.

To study the subcellular site of increased hepatic GGT activity due to chronic ethanol consumption (Table 1), rat liver homogenates were further subfractionated. Following ultracentrifugation of the 1o,ooo g supernatant, cytosolic GGT activity in the 1oo,ooo g supernatant was significantly increased in livers of alcohol fed rats compared to those of their pair-fed controls (Table 2). Nevertheless, the activity of cytosolic GGT activity was rather low in both groups of animals (Table 2) compared to the activities found in the liver homogenate (Table 1).

Table 2

EFFECT OF CHRONIC ETHANOL CONSUMPTION ON GAMMA-GLUTAMYL-TRANSFERASE ACTIVITIES IN THE CYTOSOL AND IN MICROSMOES OF RAT LIVER

Twelve rats were pair-fed nutritionally adequate liquid diets containing either ethanol or dextrin as controls for 4-5 weeks. Gamma-glutamyltransferase activites were determined in subcellular fractions of the liver. The results obtained for the cytosol and for the microsomal fraction are expressed per g liver wet weight and per g microsomal protein, respectively.

Subfractions	Gamma-glutamyltransferase activity		P
	Control rat	Alcohol fed rat	
	units / g		
Cytosol	0.043 ± 0.008	0.053 ± 0.013	< 0.05
Microsomes	2.67 ± 0.52	5.43 ± 0.45	< 0.01

Indeed, during subcellular fractionation GGT activity was recovered predominantly in the microsomal fraction of the hepatocyte. Furthermore, microsomal GGT activity was found to be significantly increased in the microsomal fraction of livers obtained from alcohol-fed rats when compared to those of control animals (Table 2).

The question arose whether ethanol per se might affect the activity of microsomal GGT. Therefore, ethanol was added in vitro to hepatic microsomes, and GGT activity was measured and compared to the results obtained under experimental conditions in which ethanol was replaced by physiological saline. Addition of ethanol resulted in a small increase of GGT activity in microsomes of alcohol-fed rats but not in those of their pair-fed controls (Table 3). When ethanol was replaced by deoxycholate, a pronounced enhancement of microsomal GGT activity could

Table 3

EFFECT OF IN VITRO ADDITION OF ETHANOL AND DEOXYCHOLATE ON THE ACTIVITY OF MICROSOMAL GAMMA-GLUTAMYLTRANSFERASE

Washed microsomes were prepared from rats fed for 4-5 weeks either the ethanol containing liquid diet or the control diet in which ethanol was replaced by dextrin. Hepatic microsomal gamma-glutamyltransferase activity was determined in the absence and presence of ethanol (final concentration 50 mM) or deoxycholate (1 mg/assay). The results are means of four experiments and are expressed in units per g microsomal protein.

Assay conditions	Microsomal gamma-glutamyltransferase activity	
	Control rat	Alcohol fed rat
	units / g	
No addition	2.68	3.32
+ Ethanol	2.63	3.67
+ Deoxycholate	3.61	4.47

be demonstrated in both alcohol-fed animals and controls (Table 3).

Discussion

The results of the present study demonstrate an increase of serum gamma-glutamyltransferase (GGT) activity following chronic alcohol consumption which is associated with an enhancement of hepatic GGT activity (Table 1). Subfractionation of liver homogenates revealed that GGT activity is strikingly enhanced in the microsomal fraction obtained from alcohol-fed rats when compared to their pair-fed controls (Table 2). These data suggest that increased activities of serum GGT following chronic alcohol

intake can be attributed to enhanced activities in the liver.

The primary event of chronic ethanol consumption appears to be an induction of microsomal GGT activity in the liver (Table 2). This is followed by an enhanced action of ethanol on the microsomal membrane itself since ethanol liberates GGT activity from the microsomal membrane of livers obtained from animals fed alcohol for several weeks (Table 3). GGT might then be released into the bloodstream, resulting in an increased serum activity (Table 1).

The observation that chronic ethanol consumption results in an induction of microsomal GGT activity is in keeping with the known inductive effect of chronic alcohol administration on other microsomal enzymes and components. Previous studies have indeed shown that chronic intake of alcohol induces the activities of microsomal NADPH-cytochrome P-450 reductase (8), NADPH-cytochrome c reductase (8), NADH-cytochrome b_5 reductase (10), microsomal drug metabolizing enzymes (7,8) and the microsomal alcohol oxidizing system (2,9-11) as well as increases the content of cytochrome P-450 (7,8) and cytochrome b_5 (10).

The data of the present study obtained in animals are similar to the results derived from alcoholics who also show striking elevations of serum GGT activities (1). Increased serum GGT activities have been widely used as a sensitive index for the detection of alcoholism (1,20). This study therefore shows that the enhancement of serum GGT activities after chronic alcohol consumption is associated with increased GGT activities in the liver which in turn can be ascribed to an induction of microsomal GGT activity.

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