Vol. 99, No. 1, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
March 16, 1981 Pages 142-148

# GAMMA-GLUTAMYLTRANSFERASE ACTIVITY OF LIVER PLASMA MEMBRANE: INDUCTION FOLLOWING CHRONIC ALCOHOL CONSUMPTION

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Received January 20,1981

## Summary

Chronic alcohol consumption to rats for 6 weeks results in an enhancement of gamma-glutamyltransferase activity both in the serum and liver. These alterations are associated with an increased hepatic content of reduced glutathione. Upon isolation of liver homogenates by discontinuous sucrose gradient ultracentrifugation, an induction of gamma-glutamyltransferase activity could be demonstrated in the plasma membranes of the hepatocytes of alcohol-fed rats when compared to their pair-fed controls. These findings suggest that increased serum gamma-glutamyltransferase activities commonly observed in alcoholism can be attributed at least in part to an induction of plasma membrane gamma-glutamyltransferase activity.

Gamma-glutamyltransferase (GGT; EC 2.3.2.2) is located in a variety of organs including renal brash border membranes (1) and plasma membranes of the hepatocytes (2). This enzyme is a component of the gamma-glutamyl cycle and is considered to play a significant role for the transport of extracellular amino acids through the outer membranes of the cells (1). For this process intracellular reduced glutathione (GSH) is required (1). Chronic alcohol consumption leads to alterations of hepatic amino acid metabolism (3) and to striking elevations of serum GGT activities. Previous studies in man (4-7) and rats (8-11) have shown that chronic alcohol consumption results in an increased hepatic activity of gamma-glutamyltransferase, whereas controversial results have been obtained in rats for reduced glutathione (GSH) both with an increased (12) and unaltered (13) hepatic content.

This study was therefore undertaken to investigate the effect of chronic alcohol consumption on GGT activities in serum, liver homogenates and liver plasma membranes as well as on the hepatic content of reduced glutathione.

0006-291X/81/050142-07$01.00/0

## Materials and Methods

Female Sprague-Dawley rats were purchased for Zentralinstitut für Versuchstierzucht Hannover (West Germany) with a starting body weight of 220-250 g. They were divided into two groups of 10 rats each and pair-fed nutritionally adequate liquid diets containing either ethanol (36% of total calories) or isocaloric carbohydrates (dextrin) as controls for 6 weeks (14). They were sacrificed by decapitation, and blood was collected from the neck vessels. The livers were immediately perfused *in situ* through the portal vein with ice-cold 0.15 M KCl, and a 25% liver homogenate was prepared (15) for the determination of the hepatic GGT activity and glutathione content. In another set of experiments, 4 pairs of rats were treated as described above, except that the livers were perfused with ice-cold 1mM $NaHCO_3$ (pH 7.5), and liver plasma membranes were prepared following homogenization. The liver plasma membrane fractions consisted with two forms, one exhibited bile canaliculi enriched plasma membranes and the other one bile canaliculi free plasma membranes. The isolation was performed by discontinuous sucrose density gradient ultracentrifugation as described by Song et al (16) and Yousef et al (17).

Gamma-glutamyltransferase (EC 2.3.2.2) activity was measured in serum, liver homogenates and plasma membranes by spectrophotometric assay according to the method of Szasz et al (18). The hepatic content of reduced glutathione (GSH) was assayed in 25% liver homogenates with the Ellman's reagent according to the procedure of Mitchell et al (19). Protein concentration of liver homogenates and plasma membranes was determined by the method of Lowry et al (20).

## Results

Chronic alcohol consumption for 6 weeks resulted in a significant increase of serum gamma-glutamyltransferase (GGT) activity when compared to their pair-fed controls (Table 1). Similarly, this regimen led to a striking enhancement of GGT activity in liver homogenates of alcohol fed rats, irrespectively whether the activity was expressed per g of liver wet weight, per g of liver protein or per 100 g of body weight. Moreover, compared to control animals the hepatic content of reduced glutathione (GSH) of alcohol-fed rats was increased by 29% ($p<0.01$) and 43% ($p<0.001$) when expressed per g of liver wet weight and per 100 g of body weight, respectively (Table 1). When expressed per g of liver protein, however, the hepatic content of reduced glutathione in alcohol fed animals was slightly but not significantly greater than in controls due to the increased hepatic protein concentration observed after chronic alcohol consumption (Table 1).

To study the subcellular site of increased hepatic gamma-glutamyltransferase activity due to chronic alcohol consumption, two types of liver plasma membranes were isolated by discontinuous

Table 1

EFFECT OF CHRONIC ETHANOL CONSUMPTION ON THE ACTIVITIES OF GAMMA-GLUTAMYLTRANSFERASE (GGT) IN SERUM AND LIVER AND ON THE HEPATIC CONTENT OF REDUCED GLUTATHIONE (GSH)

| Assay | Control diet | Alcohol diet | p |
|---|---|---|---|
| Serum GGT | | | |
| U/l | 2.33 ± 0.16 | 4.64 ± 0.67 | <0.01 |
| Liver GGT | | | |
| U/g of liver wet weight | 0.08 ± 0.01 | 0.14 ± 0.01 | <0.001 |
| U/g of liver protein | 0.78 ± 0.10 | 1.19 ± 0.09 | <0.01 |
| U/100 g of body weight | 0.35 ± 0.03 | 0.71 ± 0.08 | <0.001 |
| Liver GSH | | | |
| U/g of liver wet weight | 4.57 ± 0.08 | 5.90 ± 0.18 | <0.001 |
| U/g of liver protein | 46.3 ± 2.3 | 50.8 ± 1.6 | NS |
| U/100 g of body weight | 20.5 ± 0.9 | 29.3 ± 1.4 | <0.001 |
| Liver protein | | | |
| mg/g of liver wet weight | 98 ± 6 | 118 ± 5 | <0.05 |
| mg/100 g of body weight | 463 ± 32 | 583 ± 34 | <0.05 |

Female Sprague-Dawley rats were fed for 6 weeks nutritionally adequate liquid diets containing either ethanol (36% of total calories) or isocaloric dextrin as controls. Gamma-glutamyltransferase activity was determined in the serum and the 25% liver homogenate. The hepatic content of reduced glutathione (GSH) and protein was assayed in the 25% liver homogenate. The values are derived from 10 experimental animals in each group and are given as means ± SEM. The significance was assessed by the Student's t-test.

sucrose density gradient ultracentrifugation. As expected, gamma-glutamyltransferase activity was recovered in both bile canaliculi enriched and bile canaliculi free plasma membranes (Table 2). The specific activity of gamma-glutamyltransferase in bile canaliculi enriched plasma membranes was considerably greater than in bile canaliculi free plasma membranes. Compared to liver plasma membranes, the activity of gamma-glutamyltransferase in the liver homogenate was rather low. Chronic alcohol consumption results in an enhancement of gamma-glutamyltransferase activity not only in the liver homogenate (Table 1 and 2) but also in liver plasma membranes (Table 2). Following prolonged ethanol intake, gamma-glutamyltransferase activity was more than doubled in both the bile canaliculi enriched plasma membranes and in the bile canaliculi free ones.

## Discussion

The present study demonstrates an increase of serum and hepatic gamma-glutamyltransferase (GGT) activities after chronic alcohol

Table 2

EFFECT OF CHRONIC ETHANOL CONSUMPTION ON GAMMA-GLUTAMYL-TRANSFERASE ACTIVITIES OF LIVER HOMOGENATES AND LIVER PLASMA MEMBRANES

| Assay | Control diet | Alcohol diet |
|---|---|---|
| Liver homogenate | | |
| U/g of protein | 0.74 | 1.27 |
| Liver plasma membranes | | |
| -bile canaliculi enriched (U/g of protein) | 7.43 | 17.32 |
| -bile canaliculi free (U/g of protein) | 1.52 | 3.27 |

Female Sprague-Dawley rats were fed for 6 weeks nutritionally adequate liquid diets containing either ethanol (36% of total calories) or isocaloric dextrin as control. Gamma-glutamyl-transferase activity was determined in the 25% liver homogenate and in liver plasma membranes. The data in liver homogenates were expressed as U/g of liver protein and those in plasma membranes as U/g of plasma membrane protein. The values are means of 4 experimental animals each group.

consumption which is associated with an enhanced hepatic content of reduced glutathione (Table 1). Following discontinuous sucrose density gradient ultracentrifugation of liver homogenates, gamma-glutamyltransferase activity was recovered in liver plasma membranes (Table 2). When compared to control rats, an enhancement of gamma-glutamyltransferase activity was found in bile canaliculi enriched and bile canaliculi free plasma membranes after prolonged alcohol intake. These findings suggest that increased serum gamma-glutamyltransferase activities commonly observed in alcoholism can be attributed at least in part to an induction of plasma membrane gamma-glutamyltransferase activity.

The data of the present experiments clearly show that gamma-glutamyltransferase activity is localized in the plasma membranes of the hepatocyte (Table 2), agreeing thereby with other reports (21, 22). In addition, a variety of studies have previously indicated the presence of gamma-glutamyltransferase activity in the microsomal fraction of the hepatocyte (8-10). The possibility exists that during subcellular fractionation the microsomal membranes become contaminated by plasma membranes (21, 22) which contain gamma-glutamyltransferase activity (Table 2). It is therefore unknown whether the gamma-glutamyltransferase activity recovered in the

microsomal fraction may actually represent an enzyme originating from microsomal membranes, liver cell plasma membranes or both sources.

Chronic alcohol consumption led to an increase of gamma-glutamyltransferase activity not only in the microsomal fraction of the hepatocyte (8-10) but also in the plasma membrane fraction as demonstrated in the present study (Table 2). Since the degree of contamination of the microsomal fraction by liver plasma membranes has not yet been established, it is unknown to what extent liver plasma membranes contribute to the enhanced gamma-glutamyltransferase activity of the microsomal fraction in alcohol-fed rats.

The increase of hepatic gamma-glutamyltransferase activity following chronic intake of alcohol was attributed to a direct effect of ethanol by some (8) but not by others (23). In the latter study the decreased carbohydrate content of the alcohol diet by itself has been incriminated in the increase of hepatic gamma-glutamyltransferase activity (23), a viewpoint opposed by others (8-10). Indeed, a diet with a low carbohydrate content by itself has little if any effect on hepatic gamma-glutamyltransferase activity (11). However, when ethanol was added to the latter diet, a striking increase of hepatic gamma-glutamyltransferase activity was observed, suggesting that ethanol rather than dietary imbalance plays a major role for the enhancement.

In one experimental study with rats, chronic alcohol consumption was shown to result in a reduced hepatic activity of gamma-glutamyltransferase (24), contrasting to the enzyme induction of gamma-glutamyltransferase activity observed in other reports (8-11) and the present study (Table 1 and 2). Moreover, chronic alcohol consumption in man increases hepatic gamma-glutamyltransferase activity (4-7), findings subsequently confirmed by another group (25).

Chronic alcohol consumption to rats for 6 weeks results in a slight increase of hepatic reduced glutathione content (Table 1), confirming thereby previous studies (12). However, no alterations have been reported in rats fed up to 4 months with alcohol in another study (13). It is conceivable that alterations of hepatic glutathione content and of gamma-glutamyltransferase activities observed following chronic alcohol consumption may explain at least in part changes of hepatic amino acid and protein metabolism commonly observed under these experimental conditions (3, 26).

In conclusion, chronic alcohol consumption leads to an induction of gamma-glutamyltransferase activity of liver plasma membranes which may be responsible for enhanced serum gamma-glutamyltransferase activities commonly observed in alcoholism.

## Acknowledgements

This study was supported in part by a grant from Deutsche Forschungsgemeinschaft, Bad Godesberg, Federal Republic of Germany. The expert technical assistance of Ms. U. Hennigs and H. Landmann is greatfully acknowledged.

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