DOES CHRONIC ALCOHOL CONSUMPTION REALLY INDUCE HEPATIC MICROSOMAL GAMMA-GLUTAMYLTRANSFERASE ACTIVITY?

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Summary

Ethanol feeding of rats for 6-7 weeks was accompanied by elevated plasma and liver γ-glutamyltransferase (GT) activities compared to enzyme activities in control animals fed carbohydrates isocalorically. Similar differences in hepatic GT activity were present after feeding these diets for 1 or 4 weeks. In all instances the difference between ethanol-fed and control rats was due to a reduction of GT activity in the animals fed the control diet, while ethanol-fed rats had GT activities close to pre-experimental values. Since neither treatment influenced the subcellular distribution of hepatic GT activity, no evidence for induction of microsomal GT activity from a pretreatment level due to ethanol was present.

Alcoholic hepatic disease in man is regularly accompanied by increased γ-glutamyltransferase (GT) activity in plasma (1). GT is located both in the microsomal and in the plasma membrane fraction of the liver (2-4). Increased plasma levels of GT could represent abnormal leakage due to membrane damage, more or less unspecifically related to alcohol consumption. On the other hand, since ethanol is regarded as an inducer of microsomal enzyme activities (5,6), increased circulating GT levels could be caused by an "overflow" if ethanol increased GT in hepatic microsomes. This mechanism has been proposed for increased GT activities after other enzyme inducing drugs (3,7,8). A slight, statistically insignificant increase of GT has been reported in one experiment where ethanol was given in amounts below those otherwise used to demonstrate increases of microsomal enzyme activity after ethanol (8). We therefore decided to use the Lieber-DeCarli model of ethanol feeding (9), which provides the rats large daily amounts of ethanol, to see whether plasma GT increased with or without accompanying changes in liver enzyme activity. While this work was in progress, Teschke et al. (10) published results from quite similar experiments. Their results correspond to ours, but additional observa-

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tions presented in this report resulted in a quite different interpretation.

Materials and Methods

Male Wistar rats were obtained from MØllegaard Hansens Avlslaboratorier A/S, Ejby, Denmark. They were housed in separate plastic boxes, maintained on a 12 h light - 12 h dark cycle. The rats were pair-fed an all liquid diet (Bio-Mix, No 711, Bio-Serv, Inc., New Jersey, USA) according to DeCarli and Lieber (10). Ethanol constituted 35 per cent of the total calories given in the experimental groups while carbohydrates replaced this amount of calories in the control groups. The additional calories were made up by fat (35%), protein (18%) and carbohydrates (12%) in both groups. The mean daily intake of ethanol ranged from 12.3 to 14.2 g/kg in all experimental groups.

Two experiments were performed. In the first ethanol and control diet were given for 6 to 7 weeks and the animals were killed at the end of this period. In the second experiment animals were sacrificed after 0 (pre-experimental group), 1, 4 or 6 to 7 weeks on the diets. The rats were fasted for twelve hours before exsanguination under light ether anesthesia. Blood was drawn and 0.1 volume of a 3.13% solution of trisodium citrate dihydrate was added. Plasma was either analysed at once or stored at -80 °C until analysis. Fresh liver homogenates were subjected to fractionation of subcellular organells by the method of DeDuve et al.(11), otherwise the livers were stored at -80 °C before homogenization in 0.25 M sucrose. Preparations for GT measurement contained in addition 1% deoxycholate. This was found to increase the reproducibility of the GT activity measurements without altering the level of the activity. γ-Glutamyltransferase (EC 2,3,2,2) was measured at 37 °C as previously described (12). NADPH-cytochrome C reductase (EC 1,6,2,4) was measured at 30 °C in liver homogenates according to Phillips and Langdon (13). The enzyme activities are expressed in international units (μmol/min). Protein was determined as described by Lowry et al. (14).

Results

In all experiments the body weight of the animals at sacrifice was between 260 and 330 g. The weight curves are given in Fig. 1.

Ethanol intake for 6 to 7 weeks was accompanied by higher GT activities both in rat liver and plasma when compared to controls fed carbohydrates isocalorically (Table 1). The difference between the two groups was somewhat more marked when hepatic enzyme activity was referred to 100 g rat instead of g liver (Table 1). This was due to a higher liver to body weight ratio in ethanol consuming rats (0.030) than in control rats (0.026), as reported earlier for rats consuming the Lieber-DeCarli diets (15). When the activity was expressed per mg protein, the difference was

Table 1. Effect of chronic ethanol treatment on γ-glutamyltransferase activity in liver and plasma.

Rats were given either control or ethanol diet for 6-7 weeks. The number of animals are given in parentheses. The mean values of each group are expressed ± S.E.M. Wilcoxon's test for paired samples was used to indicate the significance levels.

	Liver enzyme		Plasma enzyme
	(U/g liver)	(U/100 g rat)	(U/l)
Control-group (6)	0.137±0.010	0.383±0.023	4.37±0.41
Ethanol-group (6)	0.888±0.160	2.819±0.561	5.83±0.35
α	0.016	0.016	0.047

slightly less than when expressed per g wet weight, but still marked and statistically significant (results not shown).

The results obtained in the second experiment, in which the time course of enzyme activities in liver was followed, are presented in Fig. 2. Again a significant difference between hepatic GT in ethanol treated and control rats was found, and this was apparent already after 1 week. The results revealed that the difference was due to a fall in the activity of controls (35-50% of zero time value). In the ethanol-treated animals the enzyme activity remained close to the initial values. The lower part of Fig. 2 depicts the enzyme activity of a microsomal enzyme, NADPH-cytochrome C reductase, assayed in the same livers. This enzyme activity also declined with time in the control animals, but the fall was far less marked (75-90% of zero value) and occurred in both the experimental and the control group (Fig. 2).

GT activity was measured in the various subcellular fractions obtained from liver homogenates of control and experimental rats fed for 0, 1 and 4 weeks (Table 2). The GT activities recovered in the N-fraction (nuclei and plasma membranes), the M-fraction (mitochondria) and the P-fraction (microsomes) amounted to 76% of the activity of the homogenate, with relative activities of 66%, 8% and 26%, respectively (Table 2). The S-fraction (cytosol) contained less than 5% of the total homogenate. There was no obvious difference in the subcellular distribution of GT activity between

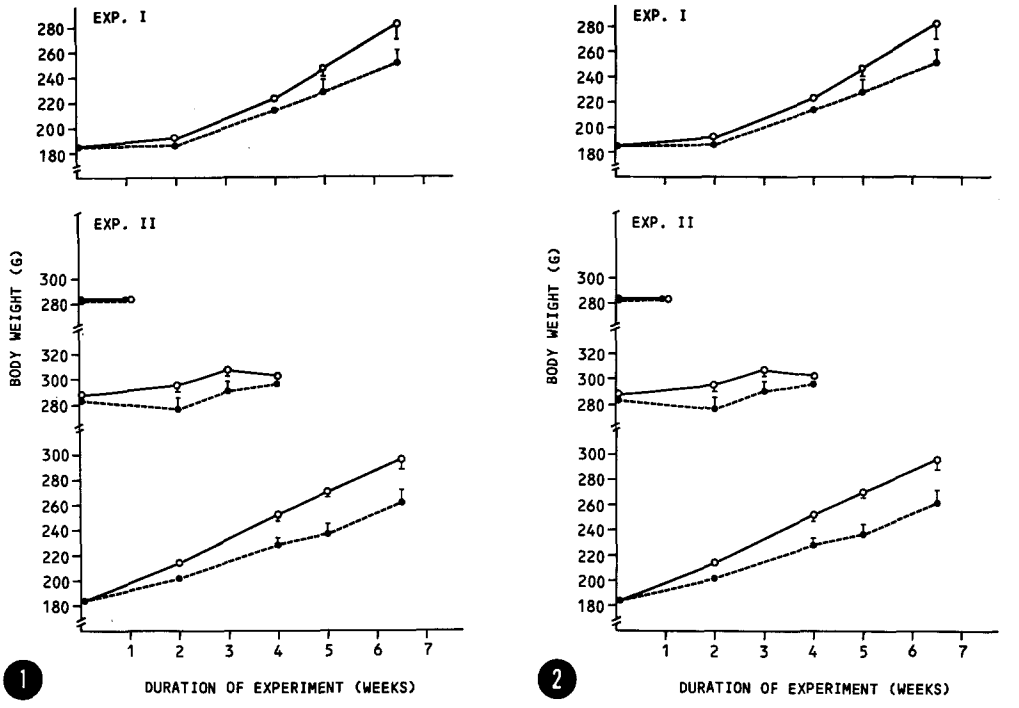

Fig. 1. Body weight of rats fed a diet in which ethanol constituted 36 per cent of the calories ●---●, or a control diet in which carbohydrates replaced ethanol isocalorically o——o. Each point represents the mean of 5 or 6 animals, the bars indicate S.E.M.

Fig. 2. Liver γ-glutamyltransferase (GT) activity (upper part) and NADPH-cytochrome C reductase activity (lower part) in rats fed the ethanol ●---●, and control diet o——o, for various periods. Each point represents the mean of 5 or 6 animals, the bars indicate ± S.E.M.

the five groups of animals tested. Thus, the significant difference found in the total activity between the control and the ethanol-fed rats was also expressed in all subcellular fractions.

Discussion

The present results demonstrate significant higher activities of GT in plasma as well as in liver of rats fed ethanol as compared to the activities found in rats fed isocaloric amounts of carbohydrates. This indicates that higher plasma GT might be secondary to an increase in the liver. This observation is in

Table 2. Relative subcellular distribution of γ-glutamyltransferase activity in liver homogenates.

Fresh liver homogenates from animals given either control or ethanol diet for 0 (pre-experimental group), 1 or 4 weeks were prepared. Total GT activity of the various subcellular fractions are expressed in per cent of the total activity measured in the homogenate from the same animal. Each value represents the mean of 5 to 6 animals ± S.E.M.

Subcellular	0 week	1 week		4 weeks	
fraction	pre-exp.	control	ethanol	control	ethanol
Homogenate	100±19.4	100±7.8	100±14.4	100±8.1	100±21.1
N-fraction	53.3± 7.6	45.8±8.2	49.5± 9.1	46.7±3.4	49.3±12.1
M-fraction	4.9± 1.0	5.3±0.9	6.6± 1.1	7.8±1.0	6.1± 1.1
P-fraction	18.0± 3.7	19.1±2.0	21.5± 3.7	23.0±1.8	20.8± 3.8

accordance with that recently reported by Teschke et al. (10) who measured GT activity only after treatment for 4-5 weeks. We demonstrated the difference to be due to a decreased activity in the livers of the control rats, present already after one week. Moreover, the relative GT activities of the various subcellular fractions of the liver cells both from the ethanol and the control groups remained essentially unaltered. The plasma GT activity could therefore be correlated to the GT activity found in any subcellular fraction. Particularly, we did not find a relative increase in the GT activity of the microsomal fraction of the livers from ethanol-fed rats. Based on these observations we cannot interprete our results in accordance with Teschke et al. (10) i.e. that the increased GT activity of plasma upon long-term ethanol feeding is due to an increased activity in the liver as a consequence of an induction of microsomal GT activity. On the other hand, the results do not entirely exclude such a hypothesis, since e.g. some induction could have occurred from a new low level of enzyme activity. However, our findings cast doubt on the usefulness of this rat-model for the study of GT changes after ethanol intake in man.

A likely explanation of our results appears to be that some factor interfering with either GT activity or synthesis was present in the control diet, most probably in the carbohydrate fraction, since the control animals were fed more of this fraction than the experimental animals. Carbohydrate repression has been reported for several enzymes (16,17), also microsomal ones (18). This explanation is weakened by the fact that the zero time control rats had been fed a pellet diet in which carbohydrates (mainly as starches) constituted at least the same percentage as carbohydrates (mainly as maltose-dextrins) in the Lieber-DeCarli control diet. However, the qualitative difference in carbohydrates or in other factors between the pellet and the synthetic diet could be of importance, as indicated by the observations of Sobiech et al. (19).

The low GT activities in control animals could not be attributed to generally impaired nutritional status as these rats on the average increased their body weights somewhat more than ethanol consuming rats (Fig. 1). A lower weight gain in the experimental animals could on the other hand not explain the higher GT activity in these animals, since the enzyme activity was higher also when experimental animals and controls had grown to the same extent (1, 4 weeks, Fig. 1). Support for adequate feeding of both groups was obtained from the lack of induction of NADPH-cytochrome C reductase, since it has been shown that the "induction" of this enzyme disappeared if the feeding of Lieber-DeCarli diets allowed optimal weight gain of the experimental animals (20).

Our results raise the general question whether pair feeding with ethanol against carbohydrates represents a proper model when the effect of ethanol on enzyme activities is to be studied. It may be hazardous to use this model in experiments on microsomal enzymes if one cannot exclude carbohydrate repression.

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