

The Effect of Longterm Alcohol Exposure on Liver Choline Dehydrogenase Activity

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SUMMARY

Rats were exposed to an ethanol-containing drinking mixture for 54, 84 and 105 days. It was shown that a progressive increase in liver choline dehydrogenase activity occurred which became statistically significant at 105 days. This may be the mechanism by which ethanol increases choline requirement in mammals. Studies of this type might further our understanding of alcohol-related liver disease, and are particularly pertinent in countries such as Zambia in which alcohol consumption is high and dietary choline intake is likely to be low.

INTRODUCTION

Choline is an essential substrate for the formation of phospholipids in hepatocytes, a stage in the formation of lipoproteins (Lucas and Ridout, 1967). Choline is phosphorylated by the enzyme choline kinase and either enters the pathway leading to phosphatidylcholine or is oxidised through aldehyde to betaine by the choline oxidase system of liver cell mitochondria, the rate-limiting enzyme of which is choline dehydrogenase. As choline is not reformed to any great extent from betaine (Stetten, 1941), the major source of the substrate is dietary (Wells, 1954), although a certain amount of phosphatidylcholine can be synthesised from serine. However, Wells (1954) pointed out that this alternative pathway is not capable of providing sufficient phospholipid to maintain lipoprotein transport.

There is evidence that choline deficiency is responsible for impairment of liver transport of fats, (Haines, 1965; Haines and Mookerjee, 1965; Lombardi, Pani and Shlunk, 1968). Though other possible causes have been considered they enjoy

little supportive experimental evidence. Stetton and Grail (1943) concluded that any effect of choline on rates of synthesis or degradation of fatty acids accumulating as triglycerides in the liver are chiefly synthesised at the site. Wells (1954) suggests that the phospholipotropic effect of choline is exerted by the intact molecule, not by the labile methyl groups, having shown that choline homologs retain phospholipotropic activity, though they are not methyl donors.

The role of ethanol in influencing choline metabolism is pertinent for two particular reasons —

- (1) Both choline deficiency and high levels of ethanol administration result in fatty liver.
- (2) Ethanol administration has been claimed to increase the choline requirement by some mechanism not involving calories, (Klutskin and Krehl, 1954).

Providing a toxic level is not reached choline can be infused into experimental animals indefinitely without ill effect, and under normal conditions the effects of choline deficiency are rarely seen (Lucas and Ridout, 1967). This suggests that though excess choline can be removed by oxidation this pathway is relatively inactive when choline intake is low. These findings suggest that substrate concentration controls choline oxidation under normal conditions; such a mechanism would allow available choline to be utilised in phospholipid synthesis since during a period of choline shortage the oxidation pathway would be relatively inactive.

Accepting that the effect of ethanol on choline uptake and utilisation is complex (Tuma et al, 1937), we have studied the longterm effect of ethanol exposure on rat liver choline dehydrogenase activity

as it is feasible that induction of choline dehydrogenase, with consequent diversion of choline into the choline oxidation pathway, could be responsible for the increase in choline requirements during ethanol administration and hence some of the manifestations of alcoholic liver disease.

METHODS

Three groups of five rats were exposed to a water/ethanol (9: 1 v/v drinking mixture for 54, 84 and 105 days respectively). Fifteen control rats drank water. The rats were matched for weight, age, sex (male) and strain (S/D) and were allowed free access to standard rat food.

ENZYME ASSAY

2.5 g of liver from each rat was homogenised in 7.5 ml of 0.44 M sucrose by seven strokes of a Potter-Elvehjem glass and Teflon homogeniser. Mitochondrial disruption was achieved by subjecting the homogenate to ultrasound for 1 minute at 26×10^3 c.p.s.; amplitude 8μ peak-peak (Wilken, 1965). Samples were kept on ice throughout. The homogenates were centrifuged for 30 minutes at 3°C and 3400 g (r_{av} 12 cm), and stored at -20°C . The assay for choline dehydrogenase was based on that of Wilken (1970). A final reaction volume of 3 ml contained: Tris/HCl buffer pH 7.50, 50 μmol ; potassium phosphate buffer pH 7.50, 30 μmol ; mannitol 117 μmol ; MgSO_4 15 μmol ; NAD 3 μmol ; ^{14}C -choline chloride 30 μmol (7×10^5 d.p.m. μmol^{-1}). The reagents were warmed to 30°C in water bath following which the reaction was started by adding 1.5 ml of supernatant to the reagents. 1.5 ml of 0.44 M sucrose was added to blanks. All samples were assayed in duplicate. After incubation for 80 minutes at 30°C the reaction was terminated by precipitating out the protein with 1 ml of perchloric acid 9.4 per cent (w/v), this being subsequently neutralised with 0.15 ml of 10 M KOH (determined by titration). Centrifugation for 10 minutes on a bench centrifuge (g not critical) at 10°C allowed approximately 3.5 ml of supernatant to be collected, which was subsequently desiccated to one tenth of the original volume under vacuum with silica gel as the desiccant. As ^{14}C -choline is sensitive to radiation decomposition as a solid, care was taken to avoid drying down completely. A row of 10 spots (10 μl) from each supernatant concentrate was made at the origin line of Whatman No. 1 chromatography paper, spread 2 cm apart. The papers were developed by ascending chromatography in butan-1-01/ethanol/acetic acid/water (8 = 2 = 1 = 3 v/v) until the solvent front had travelled 20 cm, they were then dried in a current of air. The position and definition of radioactive material on each paper was determined by viewing the paper in a Pullan spark chamber using

Polaroid Land film type 20C with an exposure of 10 minutes at aperture 4. The pattern of chromatographic migration of choline, betaine and betaine aldehyde for our system was verified using standard samples of these molecules. The separated spots were cut into strips; one containing the choline spots and one containing the combined betaine and betaine aldehyde spots (betaine is formed by oxidation of betaine aldehyde by the enzyme betaine aldehyde oxidase). The strips were eluted over a period of 1 hour with 15 ml of water. Spark chamber photographs taken after elution showed no apparent residual activity. Samples were evaporated to dryness under infrared lamps and redissolved in 1 ml water. Ten ml Bray's solution was added to each vial which was counted in an Intertechnique ABAC SL40 liquid scintillation counter. The radio activities were corrected for quench by the use of an external standard.

The activity of choline dehydrogenase was determined from the proportion of the total radioactivity contained in the betaine and betaine aldehyde chromatograph spots, and was expressed as n.mol of substrate metabolised per minute per mg of protein.

Choline dehydrogenase in the pellet was assayed in the same manner.

Succinate dehydrogenase in the supernatant fraction from each liver was assayed by the technique described by Singer (1974).

Protein Assay

The protein content of the liver homogenate supernatant and pellet was assayed by the technique of Oyama and Eagle (1956). The protein standard was bovine serum albumin supplied by Sigma Chemical Co. U.S.A. batch 38C/8195.

Background Radioactivity

The background radioactivity was 50 d.p.m. compared with 10^5 d.p.m. for choline and 10^3 d.p.m. for betaine and betaine aldehyde; it was therefore disregarded.

The effect of CN

A pilot study was performed in which the assay was performed in the absence and presence of CN at a concentration of 10^{-4}M ; no difference was found, demonstrating that choline is oxidised freely by choline dehydrogenase in this preparation and that the oxidation does not depend on an intact electron transport chain.

RESULTS

There was no significant difference between controls and the ethanol treated groups for mean

group weight, total group liver weight, total energy consumption, supernatant protein content or pellet protein content.

Table 1 shows that a progressive rise in supernatant choline dehydrogenase activity occurred with increasing time of exposure to ethanol, this being statistically significant at 105 days exposure. Pellet choline dehydrogenase activity was low and showed no rising trend. There was no increase in the activity of succinate dehydrogenase with increasing exposure to ethanol.

Histological examination of liver samples

Professor J. Ball, Manchester University, examined liver specimens from control and ethanol treated rats. All specimens from rats receiving 105 days exposure to ethanol showed gross fatty change microscopically; all parts of the lobules being involved. Those receiving 54 and 84 days exposure showed a lesser degree of fatty change. Control liver specimens contained relatively little fat.

DISCUSSION

The rise in cytoplasmic choline dehydrogenase activity with an absence of an increase in the activity of succinate dehydrogenase suggested that exposure to ethanol did not cause a non-specific increase in membrane-bound oxidising enzyme activity, and that choline dehydrogenase activity was increased by longterm exposure to ethanol. The definite manner in which choline dehydrogenase activity increased with time of exposure to ethanol rules out the possibility that contamination of the homogenate with ethanol — resulted in artificially altered enzyme levels. These

results demonstrate that chronic ethanol exposure increases liver choline dehydrogenase in rat. Increased oxidation of choline in vivo might account for the increased choline requirement previously observed when ethanol is administered and, furthermore, could be the reason for some of the manifestations of longterm ethanol abuse in humans, particularly when dietary choline intake is low. It is perhaps reasonable to suggest that in Zambia our efforts to reduce the incidence of alcohol related liver disease should focus not only on discouraging prolonged heavy drinking but also on the provision of a diet rich in co-factors such as choline which are essential for normal hepatocellular function. Furthermore, it might further our understanding of the pathology of alcohol-related liver disease in Zambia if a dietary choline was undertaken, and interpreted alongside studies of liver histology and biochemistry of the type described in this paper.

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TABLE 1

Mean activity \pm SD of choline dehydrogenase in supernatant and pellet, and succinate dehydrogenase in supernatant. The units units of enzyme activity are n.mol of substrate metabolised per mg of protein per minute.

Group	n	Choline dehydrogenase (supernatant)	Choline dehydrogenase (pellet)	Succinate dehydrogenase
Control	15	18.69 \pm 7.95	3.24 \pm 1.44	42.62 \pm 11.21
54 days ethanol	5	25.16 \pm 9.33 †	3.89 \pm 2.19 †	48.92 \pm 9.88 †
84 days ethanol	5	27.02 \pm 12.70 †	3.45 \pm 1.72 †	44.63 \pm 10.21 †
105 days ethanol	5	29.22 \pm 7.20 *	2.98 \pm 1.50 †	43.50 \pm 8.43 †

* 0.025 p 0.02; † not significant (controls vs ethanol treated groups by two-tailed student's t-test with Bessel correction for small sample size).