Biochimica et Biophysica Acta, 596 (1980) 331—337

BBA 78641

EFFECTS OF CHRONIC CONSUMPTION OF ETHANOL AND LOW-THIAMIN, LOW-PROTEIN DIETS ON THE LIPID COMPOSITION OF RAT WHOLE BRAIN AND BRAIN MEMBRANES

EZIO A. MOSCATELLI and PAUL DEMEDIUK

John M. Dalton Research Center, University of Missouri-Columbia, Columbia, MO 65211 (U.S.A.)

(Received June 13th, 1979)

Key words: Ethanol consumption; Dietary protein; Thiamin; Lipid composition; (Brain membrane)

Summary

Four groups of rats were used in a nutritionally-controlled study of effects of chronic ethanol consumption on brain membrane lipid composition.

Rats chronically consuming ethanol were fed high-nutrient or low-thiamin, low-protein diets. After 4 months, lipid analyses were performed on brains, brain microsomes and myelin from each group and from pair-fed, non-ethanol controls.

Among the effects of ethanol was an increase of the relative proportion of cholesterol in microsomal lipids while there was decrease of it in myelin. Ethanol also increased plasmenylethanolamine while decreasing phosphatidylethanolamine proportions in myelin and in whole brain lipids, decreased the total lipid phosphorus of whole brain, and elevated the proportion of phosphatidylserine in microsomal and whole brain lipids. Effects of poor diet generally did not interfere with ethanol effects except in the case of microsomal lipids, where it apparently prevented an ethanol-induced increase in proportion of cholesterol. These changes may be adaptive responses to the fluidizing effect of ethanol on membranes.

Introduction

Sustained ethanol abuse has been known for some time to produce neuropathological changes in brain, including abnormalities in myelin and other membranes [1—5]. Most of what is known has been learned from postmortem study of brains of human alcoholics. Although nutritional deficiency is a major

contributor to most neurological damage ascribed to chronic alcoholism [2,6—8], no controlled nutritional animal studies have been done in this area.

Membrane alterations severe enough to be seen histopathologically would be expected to be reflected in membrane lipid changes. Little information is available regarding effect of chronic consumption of high levels of ethanol on brain lipids. Lesch et al. [9—12] reported decreased total lipids in brains of patients who died of 'hepatocerebral degeneration'. The most conspicuous specific changes reported were decreased cerebrosides in cerebral white and grey matter, pons-medulla, and cerebellum, and decreased glycerophosphatides and cholesterol in regions rich in white matter. Dietrich has suggested that cerebellar plasmalogen distribution may be altered in the alcoholic human [13], and has reported decreased cerebellar plasmalogen in alcohol-consuming rats [14]. On the membrane level, Sun et al. [15] have described increased synaptosomal phospholipid acyl transferase activity after chronic ethanol administration to rats, while lowered polyunsaturated fatty acid levels were recently reported in phospholipids of the crude synaptosomal fraction from ethanol-dependent mice [16]. A significant increase in cholesterol has been demonstrated in erythrocyte and synaptosomal membranes from mice chronically consuming ethanol [17].

In this laboratory, interest in a general exploration of changes in neural membrane lipids following chronic ethanol consumption led to development of a rat model of human chronic ethanol abuse [18]. This report describes use of a modification of this earlier model for an 18-week nutritionally-controlled investigation of ethanol-induced lipid compositional changes in brain myelin and microsomes. The model used will be described in detail in a separate report describing neuropathological changes in brains of these animals (Moscatelli, E.A., Amromin, G., Switzer, M.K. and Blaker, W.D., unpublished).

Methods

Nutrition and ethanol administration. 60 male albino Wistar rats (Charles Rivers) initially weighing 239—306 g were individually housed over aspen bedding in 40 screen bottom metabolic cages (HB-11M) with HB-66 tunnel feeders (Hoeltge Inc., Cincinnati, OH) for daily determination of food and fluid consumption. Rats were randomly divided into four diet groups designated EA, EB, NA, and NB. Two synthetic diets, A and B, were commercially prepared by ICN, Cleveland, OH. Diet A consisted of 25% casein, 10% corn oil, 40% sucrose, 20% cellulose, 5% of a salt mixture and 3% ICN Vitamin Diet Fortification Mixture. Diet B contained 10% casein, 10% corn oil, 55% sucrose, 20% cellulose, 5% salt mix and 3% Vitamin Mixture. Both diets were completely deficient in thiamin, permitting regulation of thiamin consumption by addition of thiamin to the drinking fluids. In our nomenclature, A and B indicate the diet received, while E and N indicate the presence and lack of ethanol respectively.

Fluids presented were prepared with tap water, 95% (v/v) ethanol (Commercial Solvents, Terre Haute, IN), sucrose and fructose (reagent grade, Fisher Scientific Co., Fair Lawn, NH). Sugar solutions were prepared (w/v) in the

ethanol/water (v/v) solutions. Environmental conditions, such as temperature and lighting, were those normally found in an animal laboratory.

Ethanol-consuming groups were presented drinking fluid ad libitum, and fluid consumption NA and NB were isocalorically matched to EA and EB, respectively, by presentation of calculated amounts of 22% aqueous sucrose.

After 18 weeks all rats were killed by decapitation. Brains were immediately removed and stored at −70° C for use in the present study.

Preparation of subcellular fractions. Brains from five animals per group were halved, one half of each being used for whole brain analyses while corresponding halves were homogenized and subjected to subcellular fractionation into myelin and microsomes. Individual brain-halves were homogenized in approximately 20 vols. of 0.32 M sucrose in a motor-driven Potter-Elvehjem homogenizer. Microsomes were isolated by the method of Sun and Horrocks [20], while myelin was isolated from the 13 500 × *g* pellet by the method of Norton and Poduslo [21]. All procedures were carried out at 0—4° C.

Isolation and analyses of lipids. Membrane fractions were dispersed in water and the lipids extracted by the addition of 5 vols. of chloroform/methanol (2 : 1, v/v) containing 0.001% 2,6-ditertbutyl-4-methyphenol. The remaining brain halves were weighed, homogenized in 5 ml water and similarly extracted. The lipid-containing lower phases were stored under N_2 at −20° C until used.

Aliquots containing approx. 10 μg phosphorus were chromatographed according to the two dimensional thin-layer chromatography system of Rouser et al. [22]. After spraying with 40% (v/v) H_2SO_4-0.8% (w/v) potassium dichromate and charring, ultraviolet-visualized spots were aspirated and assayed for phosphorus as specified by Rouser.

Plasmenylethanolamine levels were determined by a modification of the reactional two dimensional TLC method of Horrocks and Sun [23]. Plates coated with silica gel G were developed in chloroform/methanol/water (140 : 60 : 8, v/v), dried under N_2, exposed to HCl vapors for 3 min, redried and developed in the second dimension with the same solvent mixture. The HCl-resistant and HCl-labile ethanolamineglycerophospholipid spots were visualized, aspirated, and assayed for phosphorus as above.

Cerebrosides were assayed by first isolating them from lipid aliquots by silica gel G TLC using chloroform/methanol/water (140 : 60 : 8, v/v) as the developing solvent. Spots were visualized by exposure to I_2 vapors; appropriate spots were aspirated and the lipids were extracted from the silica gel by adding 2 ml chloroform/methanol (2 : 1, v/v) followed by 0.5 ml water. After centrifugation, the lower phase was removed, evaporated to dryness under N_2 and assayed by the colorimetric anthrone method of Yamamoto and Rouser [24].

Cholesterol was determined after isolation from lipid aliquots on silica gel G TLC with chloroform/benzene (2 : 1, v/v) as the developing solvent. Spots visualized by spraying with 0.2% (w/v) Rhodamine G in 95% ethanol were aspirated and extracted as described in the preceding paragraph. Cholesterol content was determined by the colorimetric *o*-phthalaldehyde method of Zlatkis and Zak [25].

Total lipid phosphorus levels of whole brain extracts were determined by spotting aliquots on silica gel H TLC plates; then visualizing, aspirating and assaying for phosphorus as previously detailed.

Significance levels of changes were determined by analysis of variance for all analytical data.

Results

Data describing average daily intakes of ethanol and nutrients, as well as weekly growth, will be reported in detail elsewhere, together with descriptions and photographs of histologic findings (Moscatelli, E.A., Amromin, G., Switzer, M.K. and Blaker, W.D., unpublished). Briefly, average ethanol consumptions of EA and EB groups were 3.6 and 2.4 g/day, respectively, while consumption of calories as ethanol by the EA group was 41.5%.

Whole brain lipid analysis

Total lipid phosphorus levels expressed as μmol per g wet wt. brain are as follows: NA = 53.8 ± 0.9, NB = 50.8 ± 1.1, EA = 47.9 ± 1.4, EB = 43.6 ± 2.5. The decreases seen in the ethanol consuming groups (EA and EB) are statistically significant at the 95% confidence level as determined by the *Q* Method for analysis of variance. Lipid class composition presented in Table I exhibits changes in relative levels of ethanolamineglycerophospholipids, phosphatidylserine, and phosphatidic acid. Plasmenylethanolamine levels increased in parallel with decreases in phosphatidylethanolamine levels in the two sets of ethanol-treated animals (EA and EB), while phosphatidylserine levels increased in all three experimental groups. Phosphatidic acid decreased only in brain lipids of the low-thiamin, low-protein, ethanol-consuming rats (EB).

Microsomal lipid analyses

The most striking changes in microsomal lipid class composition were found in cholesterol levels (Table II). Brain microsomes from the low thiamin, low

TABLE I

LIPID COMPOSITION OF WHOLE BRAIN

Results are expressed as relative molar amounts based on total lipid phosphorus as 100, and shown as mean ± S.E. Each group comprised 5 rat brain-halves. tr, trace.

	NA	NB	EA	EB
Cholineglycerophospholipids	33.3 ± 0.8	31.9 ± 0.3	31.7 ± 1.1	31.1 ± 1.2
Ethanolamineglycerophospholipids	39.2 ± 0.6	38.4 ± 0.6	38.6 ± 0.6	37.6 ± 0.8
Phosphatidylethanolamine	16.3 ± 0.7	16.5 ± 0.3	15.2 ± 0.1 *	13.5 ± 0.8 *
Plasmenylethanolamine	22.2 ± 0.9	22.8 ± 0.9	25.4 ± 0.2 *	24.3 ± 0.6 *
Phosphatidic acid	2.7 ± 0.4	3.1 ± 0.3	3.1 ± 0.2	1.9 ± 0.2 *
Phosphatidylserine	11.6 ± 0.8	14.3 ± 0.3 *	15.3 ± 0.3 *	14.4 ± 0.6 *
Phosphatidylinositol	2.8 ± 0.1	3.5 ± 0.2	3.5 ± 0.4	2.8 ± 0.3
Lysophosphatidylethanolamine	tr	tr	tr	tr
Sphingomyelin	5.9 ± 0.2	5.4 ± 0.6	4.8 ± 0.3	6.7 ± 0.5
Material at TLC origin	2.5 ± 0.2	2.5 ± 0.1	2.1 ± 0.6	2.1 ± 0.4
Phosphorus recovery	98.2 ± 0.5	96.6 ± 1.0	98.4 ± 0.6	101.3 ± 0.3
Cholesterol	65.4 ± 1.5	63.3 ± 1.4	62.8 ± 1.2	64.3 ± 1.5
Cerebrosides	22.2 ± 0.9	24.1 ± 0.6	21.9 ± 1.1	21.2 ± 0.8

* Statistical significance at the 95% confidence level was determined as before.

TABLE II

MICROSOMAL LIPID COMPOSITION

Results are expressed as relative molar amounts based on total lipid phosphorus as 100, and shown as mean ± S.E. Each group comprised 5 rat brain fractions. tr, trace.

	NA	NB	EA	EB
Cholineglycerophospholipids	34.8 ± 0.7	33.7 ± 0.6	35.3 ± 0.4	37.0 ± 0.8
Ethanolamineglycerophospholipids	38.7 ± 0.4	40.3 ± 0.8	39.4 ± 0.4	36.5 ± 0.5 *
Phosphatidylethanolamine	15.9 ± 0.6	18.2 ± 0.2 *	15.8 ± 0.1	14.6 ± 0.4 *
Plasmenylethanolamine	22.7 ± 0.8	22.0 ± 0.6	23.9 ± 0.1	21.7 ± 0.5
Phosphatidic acid	1.4 ± 0.1	2.0 ± 0.4	2.1 ± 0.1	1.6 ± 0.4
Phosphatidylserine	10.6 ± 0.5	14.1 ± 0.2 *	12.3 ± 0.2 *	13.6 ± 0.6 *
Phosphatidylinositol	2.1 ± 0.2	3.3 ± 0.1 *	3.1 ± 0.1 *	2.6 ± 0.2
Lysophosphatidylethanolamine	tr	tr	tr	tr
Sphingomyelin	6.8 ± 0.3	6.5 ± 0.3	6.1 ± 0.2	6.9 ± 0.4
Material at TLC origin	1.4 ± 0.2	1.5 ± 0.2	1.7 ± 0.1	1.4 ± 0.2
Phosphorus recovery	99.5 ± 0.8	97.5 ± 0.5	99.1 ± 0.9	100.4 ± 0.7
Cholesterol	77.8 ± 1.2	65.8 ± 2.1 *	84.5 ± 2.1 *	72.2 ± 0.9 *
Cerebrosides	4.9 ± 0.2	4.8 ± 0.1	5.1 ± 0.4	4.9 ± 0.7

* Statistical significance was determined as before.

protein, non-ethanol-consuming group (NB) demonstrated a significant decrease in cholesterol, while a cholesterol increase was seen in the high-nutrient, ethanol-consuming group (EA). Membranes from animals which received a combination of poor diet and ethanol (EB) showed a cholesterol level significantly lower than that of controls, but not as low as seen in NB. Both ethanol and diet B produced increases in phosphatidylinositol, while phosphatidylserine increased in all three experimental groups.

Myelin lipid analyses

Cholesterol levels in myelin also showed clear changes, but the opposing

TABLE III

MYELIN LIPID COMPOSITION

Results are expressed as relative molar amounts based on total lipid phosphorus as 100, and shown as mean ± S.E. Each group comprised 5 rat brain fractions. tr, trace.

	NA	NB	EA	EB
Cholineglycerophospholipids	24.8 ± 0.9	25.6 ± 0.4	24.2 ± 0.7	25.8 ± 0.4
Ethanolamineglycerophospholipids	44.6 ± 0.8	47.3 ± 0.3	46.1 ± 0.6	43.7 ± 0.9
Phosphatidylethanolamine	12,7 ± 0.4	13.4 ± 0.4	10.2 ± 0.1 *	12.2 ± 0.4
Plasmenylethanolamine	31.9 ± 0.2	32.4 ± 0.3	35.0 ± 0.2 *	33.2 ± 0.8 *
Phosphatidic acid	3.9 ± 0.4	4.1 ± 0.3	4.3 ± 0.3	2.8 ± 0.6 *
Phosphatidylserine	12.8 ± 0.5	13.3 ± 0.2	12.5 ± 0.2	15.7 ± 0.5 *
Phosphatidylinositol	1.2 ± 0.1	2.0 ± 0.3	2.9 ± 0.1 *	2.3 ± 0.2 *
Lysophosphatidylethanolamine	tr	tr	tr	tr
Sphingomyelin	5.2 ± 0.3	2.8 ± 0.2 *	4.9 ± 0.2	4.6 ± 0.4
Material at TLC origin	3.3 ± 0.2	2.7 ± 0.2	3.2 ± 0.2	4.0 ± 0.3
Phosphorus recovery	98.7 ± 0.3	97.5 ± 0.5	97.8 ± 0.4	97.8 ± 0.5
Cholesterol	100.1 ± 0.8	112.8 ± 0.6 *	94.4 ± 0.6 *	93.3 ± 1.4 *
Cerebrosides	46.1 ± 0.9	46.8 ± 1.1	47.1 ± 0.8	46.8 ± 1.1

* Statistical significance was determined as before.

effects of diet and ethanol were the reverse of those seen in microsomes. Ethanol also produced an increase in myelin plasmenylethanolamine at the expense of phosphatidylethanolamine, as seen in whole brain lipid analyses. Phosphatidylinositol changes were similar to those seen in microsomes, while phosphatidylserine showed a significant change, an increase, only in the group combining both ethanol and poor-diet effects (EB). Poor diet alone also produced a relative decrease in sphingomyelin.

Discussion

The overall focus of this investigation was to initiate a study of effects of chronic ethanol consumption on membrane lipids in rat brain, and to identify these separately from those produced by malnutrition typical of human alcoholics. The results reported here indicate a variety of statistically significant compositional changes, the biological significance of which is difficult to assess.

The effect of chronic ethanol consumption in elevating the relative cholesterol content of microsomes is consistent with the increase reported by Chin et al. [17] in synaptosomal and erythrocyte membranes of ethanol-consuming mice. These workers suggested that the synaptosomal membrane cholesterol increase was probably an adaptive response to chronic ethanol treatment, providing an explanation for their earlier findings [28], that such membranes are resistant to the in vitro fluidizing effect of ethanol. If this indeed is an adaptive response, then myelin, shown here to decrease in relative cholesterol content, does not adapt in a likewise manner, and whatever changes ethanol may induce in the microsomal cholesterol biosynthetic system are utilized there and in the synaptic membranes, even at the expense of myelin. The cholesterol decrease in myelin is consistent with the white matter cholesterol decrease noted by Lesch in brains of human alcoholics [10], while the ethanol-induced decrease in total lipid phosphorus is also consistent with Lesch's findings [9—12].

The changes effected by ethanol on the composition of the ethanolamine family of phospholipids in myelin are the reverse of those recently reported in hibernating hamsters [29]. Here, chronic ethanol consumption resulted in an increase in the proportion of plasmenylethanolamine at the expense of phosphatidylethanolamine. If the hibernator changes contributed increased membrane fluidity as part of a 'homeoviscous' adaptation to lower temperature, it is possible that the opposite disproportionation caused by ethanol might represent the manner by which myelin increases its fluidity or its resistance to the fluidizing effect of ethanol. It should be noted, however, that the effect reported here, which is to a lesser degree reflected in our whole brain data, is inconsistent with the report by Dietrich [14] of decreased cerebellar plasmalogen in alcohol-consuming rats. It remains possible, however, that the apparent disagreement can be ascribed to a specific regional effect in cerebellum or on differing conditions in the two sets of experiments.

The striking whole-brain and microsomal increases in proportions of phosphatidylserine after alcohol consumption, versus the lack of such change in myelin, may reflect the intracellular location of $(Na^+ + K^+)$-ATPase. This microsomal and synaptosomal, lipid-dependent enzyme is particularly sensi-

tive to phosphatidylserine [30], and has been reported to increase in activity in brains of alcohol-consuming rats [31].

The combined effects of ethanol consumption and of low-thiamin, low-protein diets on lipid composition are generally similar to those produced by ethanol itself, except in the case of microsomal cholesterol. Here, the cholesterol-decreasing effect of the poor diet apparently outweighs the cholesterol-increasing effect of ethanol. This suggests that the poor diet may deny the membrane the ability to adapt to the fluidizing effect of ethanol, which could contribute to the significantly greater neuropathology produced by both stresses than by ethanol alone. It is interesting to note that the poor diet alone produces an increase in myelin cholesterol which, however, does not occur in the presence of ethanol. The increase is perhaps at the expense of microsomal cholesterol, but no ready explanation suggests itself for these phenomena.

References

1 Freund, G. (1973) Annu. Rev. Pharmacol. 13, 217—227
2 Dreyfus, P.M. (1974) in the Biology of Alcoholism (Kissin, G. and Begleiter, H., eds.), Vol. 3, pp. 265—290, Plenum Press, New York
3 Vogel, F.S. (1977) Alcoholism 1, 67—72
4 Marchiafava, E. and Bignami, A. (1903) Riv. Patol. Nerv. Ment. 8, 544—549
5 Allsop, J. and Turner, B. (1966) J. Neurol. Sci. 3, 238—245
6 Tarter, R.E. (1975) Dis. Nerv. Syst. 36, 185—187
7 Victor, M. and Adams, R.D. (1961) Am. J. Clin. Nutr. 9, 379—397
8 Neville, J.N., Eagles, J.A., Samson, G. and Olson, R.E. (1968) Am. J. Chem. Nutr. 21, 1329—1340
9 Lesch, P. and Remberg, G. (1973) Klin. Wochenschr. 51, 132—134
10 Lesch, P., Schmidt, E. and Schmidt, F.W. (1972) Z. Klin. Chem. Klin. Biochem. 10, 410—415
11 Lesch, P. (1972) Klin. Wochenschr. 50, 269—270
12 Lesch, P. (1972) Klin. Wochenschr. 50, 663—665
13 Dietrich, R.A. (1977) Alcoholism: Clin. Exp. Res. 1, 57—59
14 Dietrich, R.A. (1976) Annu. Conf. Natl. Counc. Alcohol., May, 1976
15 Sun, G.Y., Creech, D.M., Corbin, D.R. and Sun, A.Y. (1977) Res. Commun. Chem. Path. Pharmacol. 16, 753—756
16 Littleton, J.M., John, G.R. and Grieve, S.J. (1979) Alcoholism: Clin. Exp. Res. 3, 50—56
17 Chin, J.H., Parsons, L.M. and Goldstein, D.B. (1978) Biochim. Biophys. Acta 513, 358—365
18 Moscatelli, E.A., Switzer, M.K. and Blaker, W.D. (1978) Res. Commun. Physiol. Psychiatr. Behav. 3, 267—283
19 Liang, C.C. (1962) Biochem. J. 83, 429—434
20 Sun. G.Y. and Horrocks, L.A. (1973) J. Lipid Res. 14, 206—214
21 Norton, W.T. and Poduslo, S.W. (1973) J. Neurochem. 21, 749—757
22 Rouser, G., Fleischer, S. and Yamamoto, A. (1970) Lipids 5, 494—496
23 Horrocks, L.A. and Sun, G.Y. (1972) in Research Methods in Neurochemistry (Rodnight, R. and Marks, M., eds.), pp. 223—231, Plenum Press, New York
24 Yamamoto, A. and Rouser, G. (1970) Lipids 5, 442—444
25 Zlatkis, A. and Zak, B. (1969) Anal. Biochem. 29, 143—148
26 Saville, P.D. and Lieber, C.S. (1965) J. Nutr. 87, 477—485
27 Patek, A., Jr., Bowry, S.C. and Savesin, S.M. (1976) Arch. Pathol. Lab. Med. 100, 19—24
28 Chin, J.H. and Goldstein, D.B. (1977) Science 196, 684—685
29 Blaker, W.D. and Moscatelli, E.A. (1978) J. Neurochem. 31, 1513—1518
30 Fenster, L.J. and Copenhaver, J.H. (1967) Biochim. Biophys. Acta 137, 406—408
31 Rangaraj, N. and Kalant, H. (1978) Biochem. Pharmacol. 27, 1139—1144