Myelination in chronically-alcoholic mice[1]

P.A. Sedmak, D. Sedmak, H.I. Fritz and G.R. Peterson[2]

Departments of Pharmacology and Biological Chemistry, Wright State University, School of Medicine and College of Science and Engineering, Dayton (Ohio 45431, USA), 1 November 1977

Summary. Chronically-administered ethanol, not impaired nutrition, exerts a specific effect to decrease myelination as measured by the activity of the marker enzyme for myelin, 2′, 3′-cyclic nucleotide 3′-phosphohydrolase.

A general retardation of CNS myelination occurs at birth in the newborn of chronically alcoholic guinea-pigs[3]. Litters show abnormally poor locomotion, suckling and feeding difficulties, and incoordination. There is an increase in total lipid content in brains of mice chronically-fed ethanol[4]. Chronically administered alcohol does not effect the rate of lipogenesis but may decrease fatty acid oxidation.

Studies have demonstrated that undernutrition decreases myelinogenesis. There is a reduced recovery of CNS myelin in undernourished infant rats[5,6], and a significant depression of myelin-specific components[7]. The incorporation of sulfatide into myelin of rat brain is reduced both in vivo and in vitro by undernutrition during the first 3 weeks of life. In addition, reduction in the activity of the enzyme responsible for this incorporation, galactocerebroside sulfokinase[8], is observed. A significant reduction in total brain cholesterol and a lowering of cholesterol concentration is also reported[9-11].

Chronic alcoholism promotes malnutrition[12]. The high caloric content of ethanol (7.1 cal/g), combined with a high daily ingestion of ethanol, may reduce appetite. In addition, ethanol calories lack any nutritional value beyond the energy they provide. Chronically-administered alcohol causes inflammation of the digestive tract, impairing digestion of food and absorption of nutrients. Moreover, ethanol and its oxidation product, acetaldehyde, interfere with activation of vitamins by the liver. Consequently, any study attempting to elucidate an effect of ethanol must take into account the nutritional state of the intoxicated animal.

The aim of the present study was to study the effects of chronic ingestion of alcohol on myelinogenesis, as assessed by the activity of a marker enzyme, in adult mice.

Methods. 50-day-old female ICR Swiss mice were administered Lieber-DeCarli control diet[13] for 5 days with ethanol isocalorically replacing 12.5% of carbohydrates. After this period of adjustment, 25% of carbohydrate calories were replaced by ethanol for 113 days. These mice were fed ad libitum. 50-day-old pair-fed controls were administered control diet 1 week later and were maintained on the diet for 123 days.

Myelination was measured using the marker enzyme 2′,3′-cyclic nucleotide 3′-phosphohydrolase (CNPase) by the method of Prohaska, Clark and Wells[14]. Myelination is most rapid in mice from 15 to 30 days postnatally, and it continues at a decreasing rate up to 300 days[15]. Inorganic phosphate was assayed using the method of Trudinger[16]. Protein was determined by the method of Lowry et al.[17] using bovine serum albumin as a standard.

To examine the in vitro effects of ethanol on CNPase activity in untreated mouse brain, ethanol concentrations ranging from 0.02 to 1.0 M were reacted with the enzyme for 10 min prior to the addition of 2′,3′-cAMP substrate. No in vitro effects were noted.

1 unit of enzymatic activity is that amount which produces 1 μmole of 2′-AMP from 2′,3′-cAMP/min. Specific activity is expressed as units (U)/mg protein.

Results and discussion. The results of CNPase determination in the brains of mice ingesting ethanol for 123 days and their pair-fed controls are displayed in the table. The initial and final mouse weights and brain weights were not significantly different; however, percentage of total brain protein in the pair-fed controls was significantly less. Since total activity per brain (U) did not differ, specific activity of CNPase (U/mg protein) was reduced in the ethanol-treated group.

Although a group fed control diet ad libitum was not included in mice examined for CNPase activity, we have found that mice fed ad libitum ingest approximately 20 ml daily[18]. Mean daily dietary intake for experimental animals was 30% less than this, suggesting calorie-malnutrition would occur over the prolonged period of time of this study. Malnutrition has been shown to decrease the total amount of brain protein[19], while chronic treatment with ethanol has been shown to have no significant effect on brain protein synthesis[20]. Since normal mouse brain consists of 12% protein[21], the decreased percentage of total brain protein in pair-fed controls might be a consequence of impaired nutrition.

The ethanol group ingested an equal amount of diet, but no reduction in total brain protein was observed. A possible explanation is that ethanol metabolism depresses the oxidative metabolism of the brain[12]. A decreased oxidation of fatty acids in brain[4], a decrease in potassium-stimulated respiration[22] and a fall in the respiratory quotient of cerebral cortex[23] have been reported. Ethanol thus seems to prevent the effects of malnutrition on protein synthesis by lowering the energy requirements of brain tissue.

Since total protein was significantly reduced in the pair-fed mice, total CNPase activity per brain (U) would also be excepted to be less. However, there was no significant difference in total activity between the 2 groups (table). The greater specific activity of CNPase (U/mg protein) in the pair-fed controls suggests that a reduction in myelination occurred in the ethanol-treated group. Since nutrition was controlled, any difference between the groups is due to an effect of alcohol; thus, ethanol metabolism, not impaired nutrition, exerts a specific effect to decrease myelination.

Effects of chronic ethanol treatment on activity of CNPase in mouse brains

Diet	Sample size	Mouse weight initial (g)	Mouse weight final (g)	Brain weight (mg)	Protein as % of brain weight	Spec. activity (U/mg of protein)	Total activity (U/brain)
Ethanol	6	24.6 ± 0.3	30.5 ± 1.1	450 ± 14	11.0 ± 0.4*	5.1 ± 0.6*	250 ± 30
Control	6	24.6 ± 0.3	30.4 ± 0.8	471 ± 14	7.6 ± 1.1	7.7 ± 0.8	274 ± 20

Mice were fed Lieber-DeCarli diet for 123 days with ethanol isocalorically replacing carbohydrates. Controls were pair-fed (see methods for details). Values indicate mean ± SEM. * Significantly different from controls, $p < 0.05$.

1 Acknowledgment. This work was supported in part by N.I.H. Biomedical Research Support Grant Program, Grant No. 1-507-RR07155-01.
2 To whom reprint requests should be addressed.
3 D. Papara-Nicholson and I.R. Telford, Anat. Rec. *127*, 438 (1957).
4 A.K. Rawat, Res. Commun. Chem. Path. Pharmac. *8*, 461 (1974).
5 N.H. Bass, M.C. Netsky and E. Young, Arch. Neurol. *23*, 303 (1970).
6 M.A. Fishman, P. Madyastha and A.L. Prensky, Lipids *6*, 458 (1971).
7 P. Morell, M.B. Bornstein and C.S. Raine, in: Basic Neurochemistry, p.581. Ed. G.J. Siegel, R.W. Albers, R. Katzman and B.W. Agranoff. Little, Brown and Co., Boston 1976.
8 H.P. Chase, J. Dorsey and G.M. McKhann, Pediatrics *40*, 551 (1967).
9 A.N. Davison and J. Dobbing, Br. med. Bull. *22*, 40 (1966).
10 A.N. Davison and J. Dobbing, in: Applied Neurochemistry, p.254. Ed. A.N. Davison and J. Dobbing. Blackwell Scientific Publications, Oxford 1968.
11 W.T. Culley and R. Lineberger, J. Nutr. *96*, 370 (1968).
12 C.S. Lieber, Scient. Am. *234*, 25 (1976).
13 C.S. Lieber and L.M. DeCarli, Fedn Proc. *35*, 1232 (1976).
14 J.R. Prohaska, D.A. Clark and W.W. Wells, Analyt. Biochem. *56*, 283 (1973).
15 P. Morell, S. Greenfield, E. Constantino-Ceccarini and H. Wisniewsky, J. Neurochem. *19*, 2545 (1972).
16 P.A. Trudinger, Analyt. Biochem. *36*, 225 (1970).
17 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
18 D. Sedmak, unpublished research.
19 M. Winick, J. Brasel and P. Rosso, Curr. Concepts Nutr. *1*, 49 (1972).
20 M. Renis, A. Giovine and A. Bertolino, Life Sci. *16*, 1447 (1975).
21 T. Kurihara, J.L. Nussbaum and P. Mandel, Life Sci. *10* (II), 421 (1971).
22 C.T. Beer and J.H. Quastel, Can. J. Biochem. Physiol. *36*, 543 (1958).
23 H.E. Himwich, L.H. Nahum, N. Rakieten, J.F. Fazekas, D. Dubois and E.F. Gilden, J. Am. med. Ass. *100*, 651 (1933).

Monoamine oxidase activity in tissues of spontaneously hypertensive rats

F.M. Lai[1] and S. Spector

Department of Physiological Chemistry and Pharmacology, Roche Institute of Molecular Biology, Nutley (New Jersey 07110, USA), 30 January 1978

Summary. Monoamine oxidase (MAO) activity was assayed both in central and peripheral blood vessels of spontaneously hypertensive rats (SHR) and age-matched normotensive Wistar Kyoto rats (WKR). The activity of MAO in the brain and peripheral vasculature was essentially the same in both SHR and WKR. It can therefore be concluded that central and peripheral vascular MAO activity is not altered in the genetically hypertensive animals.

We have reported that neither central nor peripheral vascular MAO is involved in the maintenance of blood pressure in rats made hypertensive with deoxycorticosterone acetate (DOCA)-salt treatment[2]. Recently, it has been reported that the activity of the monoamine biosynthetic enzymes of SHR, namely, tyrosine hydroxylase and dopamine-β-hydroxylase, were not different from WKR controls[3]. It is not clear whether this is also true of the degradative enzyme, MAO. In the present study, we are reporting on the acitivty of MAO in the brain and vasculature from SHR at the advanced hypertensive stage.

Methods. Male SHR (15 weeks old) and age-matched normotensive WKR in this study were bred at Hoffmann-La Roche & Co. Ltd, Nutley, New Jersey. Systolic blood pressure of these animals were measured in conscious state by the tail cuff method. All rats were sacrificed by decapitation. Brain, mesenteric artery, mesenteric vein and aorta were removed. Brain microvessels were prepared by a modification of the method of Brendel et al.[4] as previously described[2]. The vascular tissues were cleared of fat and blood and kept on dry ice until analyzed. The MAO activity in the tissue was measured by using tyramine as substrate[2]. Tissue protein was determined by the method of Lowry et al.[5] with bovine serum albumin as standard.

Results. SHR of 15 weeks old had a mean systolic blood pressure of 191.2±3.7 (5) mmHg, a value significantly ($p < 0.001$) greater than WKR, 120.4±3.6 (5) mmHg. MAO activity of central and peripheral vasculature was determined and summarized in the table. There was no difference in enzyme activity of either the mesenteric vasculature or in the aorta between SHR and WKR.

Discussion. The present study demonstrates no difference in MAO activity in the central or the peripheral blood vessels between SHR and WKR. MAO has been ascribed to play an important role in the regulation of the intraneuronal norepinephrine level in the sympathetic nerve terminals[6]. However, it is not known whether there is a correlation between MAO activity and hypertension. Or, how this catecholamine degradative enzyme would affect the development and maintenance of hypertension in rats. Based on the results of the present study and our previous study with DOCA-salt hypertensive rats[2], we do not find that any correlation exists between the level of tissue MAO and hypertension. It is therefore, concluded that neither central nor peripheral MAO would be involved in the maintenance of hypertension in genetically hypertensive rats.

MAO activity in the brain and peripheral vascular vessels of SHR and WKR

Tissues	MAO activity (nmole/mg protein/h) WKR	SHR
Brain microvessels	366.8*	354.3*
Mesenteric artery	42.2±3.7 (5)	48.2±3.1 (5)
Mesenteric vein	89.6±7.2 (4)	92.0±9.8 (5)
Aorta	46.4±2.9 (5)	48.5±2.4 (5)

* Brain microvessels MAO activity was an average of duplicate determination based on a pool of 5 brains. Mesenteric artery, mesenteric vein and aorta were assayed individually. Figures represent mean±SE. The number in the parenthese denotes the number of experiments.

1 Present address: Cardiovascular-CNS Disease Research Section Lederle Labs, Division of American Cyanamid Co. Pearl River (New York 10965, USA).
2 F.M. Lai and S. Spector, Brit. J. Pharmac. *59*, 393 (1977).
3 A. Nagaoka and W. Lovenberg, Eur. J. Pharmac. *43*, 297 (1977).
4 K. Brendel, E. Meezan and E.C. Carlson, Science *185*, 953 (1974).
5 O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
6 I.J. Kopin, Pharmac. Rev. *16*, 179 (1964).