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Incorporation of ^{14}C-acetate into Myelin Lipids in Rats treated with Aminopterin during Suckling period

Haruko HIRONO

Department of Nursing, College of Medical Sciences, Tohoku University

アミノプテリン投与哺乳期ラットの脳ミエリン脂質への ^{14}C-アセテートのとりこみ

廣 野 治 子

東北大学医療技術短期大学部看護学科

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Folate deficiency during the suckling period was induced by intraperitoneal injections of aminopterin to both lactating dams and offsprings. On the day 18 of life, the rats were given intraperitoneal injections of 10 μC of 1-^{14}C acetate. At the age of 6 weeks, the rats thus treated were killed and radioactivity elicited from each lipid fraction of brain myelin was determined. Incorporation of 1-^{14}C acetate into cholesterol, cerebrosides and phospholipids was decreased in the rats with the aminopterin-treatment. The incorporation into ethanolamine phosphoglycerides was further investigated in detail. There was a decrease of 1-^{14}C acetate incorporation into both aldehydogenic moiety and monoacyl group in phosphatidal ethanolamine as well as phosphatidyl ethanolamine in the myelin fraction of the aminopterin- treated rats. However, there was no difference in the ratio of myelin phosphatidyl ethanolamine to phosphatidal ethanolamine between control and aminopterin - treated rats. The implications of these results are that the effect of aminopterin on acetate incorporation seems to interfere in one of the earliest levels of acetate metabolism.

In our previous study[1], it was found that dietary folate deficiency caused significant decreases in myelin yield and desaturation of fatty acid in developing rat brain, despite no changes in protein content, lipid distribution and hydroxy fatty acid composition of myelin. These comparisons between the folate deficient and control groups were made in animals that had marked different body weight and brain weight from the controls. In the present report, incorporation of 1-^{14}C acetate into myelin lipid fractions of 6-week-old rats treated with aminopterin during suckling period are compared with controls of similar body and brain weights.

Materials and Methods

The Wistar strain rats were used throughout this study and were placed on a standard diet[1]. The litter size was made so as to be 8 in number

of rats nursing from one lactating dam. Newborn rats were divided into 2 groups (Group 1 and 2). Group 1 consisted of rats which were not treated with aminopterin and served as the controls. Group 2 consisted of rats which were treated with aminopterin during the suckling period in the same way as described by Arakawa et al.[2]. Aminopterin, 0.038 mg/kg, 0.038 mg/kg and 0.076 mg/kg, was given intraperitoneally to lactating dams on the 4th, 10th and 18th day after delivery, respectively, and 0.038 mg/kg of aminopterin was injected intraperitoneally to offsprings on only the day 18 of life.

1-^{14}C acetate injection : In Groups 1 and 2, on the day 18 after the birth, 2 male rats of each litter were injected intraperitoneally with 10 μC of sodium 1-^{14}C acetate per rat, dissolved in 0.2 ml of physiological saline solution.

Isolation of myelin fraction : Myelin fraction was prepared according to Laatsch et al.[3] with the minor modifications[4]. The brains weighing 1.266 to 1.574 g were used as a starting sample for the myelin fractionation. The dissected samples, except those used for myelin preparation, were frozen and stored in a freezer at -20°C. We however, found that there is no difference of phospholipid content in myelin between fresh and frozen brain[5]. After killing by exsanguination, the brains were removed quickly and washed with cold physiological saline, then homogenized with 9 volumes of 0.25 M sucrose solution in a smooth glass homogenizer with a teflon pestle rotating at 600 rpm at 4°. The homogenate were subjected to ultracentrifugation at 13,000×g in a preparative ultracentrifuge[2]. First density gradient centrifugation with 0.88 M sucrose was done at 100,000×g. Second density gradient centrifugation was done after osmotic shock at 100,000×g.

Lipid analysis : Details of lipid analysis have previously been reported[6].

Determination of plasmalogens : Ethanolamine phosphoglycerides were hydrolyzed by exposure to HCl fumes according to method by Horrocks[7]. Development in the second dimension quantitatively separates acid-labile (plasmalogens) and acid-stable ethanolamine phosphoglyceride.

Determination of radioactivity : Spots on TLC plates were scraped off into counting vials

Table 1. Body and brain weights in 6-week-old control (Group 1) and aminopterin-treated (Group 2) rats

	Group 1 Control rats (4)	Group 2 Aminopterin-treated rats (6)
Body wt. (g)	121±14	127±4
Brain wt. (g)	1.41±0.06	1.40±0.01

The numbers of animals used are given in brackets in the heading.
Values are given as means±S.E.M.

Table 2. Lipid content of brain myelin from 6-week-old rats (Group 1 and 2)
(mg/whole brain myelin)

	Group 1 Control rats (4)	Group 2 Aminopterin-treated rats (6)
Cholesterol	6.32±0.51	5.70±0.25
Cerebrosides	4.27±0.61	3.98±0.13
Sulfatides	1.03±0.05	0.90±0.04
Sphingomyelin	0.78±0.07	0.72±0.05
Choline phosphoglycerides	3.36±0.13	3.02±0.12
Inositol and Serine phosphoglycerides	1.79±0.01	1.50±0.13
Ethanolamine phosphoglycerides	4.64±0.40	4.22±0.31
Phosphatidal ethanolamine	2.99±0.30	2.66±0.20
Phosphatidyl ethanolamine	1.65±0.11	1.56±0.10

The numbers of animals used are given in brackets in the heading.
Values are given as means±S.E.M.

Table 3. Plasmalogen content and per cent of ethanolamine phosphoglycerides of brain myelin from 6-week-old rats (Group 1 and 2)

	Group 1 Control rats	Group 2 Aminopterin-treated rats
	(mg/whole brain myelin)	
No. of materials analysed	4	6
Ethanolamine phosphoglycerides	4.64±0.40	4.22±0.31
	(per cent of ethanolamine phosphoglycerides)	
Phosphatidyl ethanolamine	35.6 ±1.9	36.8 ±1.4
Phosphatidal ethanolamine	64.3 ±1.9	63.1 ±1.4

Values are given as means±S.E.M.

Table 4. Incorporation of 1-^{14}C acetate into myelin lipid fractions and specific activity of the brain from 6-week-old rats (Group 1 and 2)

	Total radioactivity (cpm/whole brain myelin)		Specific activity (cpm/mg of lipid fraction)	
	Group 1 Control rats	Group 2 Aminopterin-treated rats	Group 1 Control rats	Group 2 Aminopterin-treated rats
No. of materials analysed	4	6	4	6
Cholesterol	5150±130	4220± 69	816± 36	740±46
Cerebrosides	2130± 77	1610± 52	501± 31	404±55
Sulfatides	459± 58	375± 49	445± 92	417±80
Sphingomyelin	550± 10	510± 11	721± 59	711±40
Choline phosphoglycerides	2720± 86	2020± 63	815± 35	682±77
Inositol and Serine phosphoglycerides	1930±124	1360± 58	1074± 51	906±23
Ethanolamine phosphoglycerides	3870±276	3080±324	869± 56	669±39
Phosphatidal ethanolamine	3180±230	2576±248	969±119	813±67
Phosphatidyl ethanolamine	694± 55	504± 78	590± 43	401±79

Values are given as means±S.E.M.

containing 4% Cab-O-Sil toluene scintillator according to Snyder and Stephens[8] and radioactivity was determined using liquid scintillation counter[3].

Results

Body and brain weight in aminopterin-treatment: As shown in Table 1, the values for body and brain weight of the control and aminopterin-treated group were almost the same.

Myelin lipid content: The lipid content in myelin fraction from the brain of 6-week-old rats with or without the aminopterin-treatment during the suckling period, are given in Table 2. The content in each lipid fractions of the brain myelin were decreased in the aminopterin-treated rats as compared to those in the control rats. There was a reduction of phosphatidal ethanolamine content as low as ethanolamine phosphoglycerides in the rats with the aminopterin-treatment during suckling period compared with the control rats. The ratio of

Table 5. Radioactive distribution of phosphatidal ethanolamine after hydrochloric acid hydrolysis (Group 1 and 2)

	Group 1 Control rats (4)	Group 2 Aminopterin-treated rats (6)	% relative to control
	(cpm/whole brain myelin)		
Ethanolamine phosphoglycerides	3870±276	3080±324	79
Phosphatidyl ethanolamine	694± 55	504± 78	81
Phosphatidal ethanolamine	3178±230	2576±248	81
(2-acyl) lysophosphatidyl ethanolamine	1074± 83	834± 81	78
(Alk-1-enyl group) aldehydes released from acid labile ethanolamine phosphoglycerides	2102±149	1742±163	83

The numbers of animals used are given in brackets in the heading.
Values are given as means±S.E.M.

Table 6. Relative incorporation of each ethanolamine phosphoglyceride fraction (Group 1 and 2)

	Group 1 Control rats (4)	Group 2 Aminopterin-treated rats (6)
Phosphatidyl ethanolamine	19±2.6%	16±1.7%
Aldehydogenic moiety	58±3.0	61±2.2
Monoacyl group	22±0.9	22±2.4

The numbers of animals used are given in brackets in the heading.
Values are given as means±S.E.M.

plasmalogen content to ethanolamine phosphoglyceride was not changed between the groups with and without aminopterin-treatment (Table 3).

The effect of aminopterin on ^{14}C-acetate incorporation into myelin lipids : As shown in Table 4, there was a decrease of 1-^{14}C acetate incorporation into each lipid fraction except sphingomyelin of brain myelin in the aminopterin-treated rats comparing with that in the controls. The reductions in radioactivity in all lipid fractions with aminopterin-treatment were about 20% of the controls. Incorporation of 1-^{14}C acetate into ethanolamine phosphoglycerides of the myelin are given in Table 5. Radioactivity of aldehydes released from acid labile ethanolamine phosphoglycerides (Alk-1-enyl group) and lysophosphatidyl ethanolamine (2-acyl group) with aminopterin treatment, were as low as about 80% of those in controls. However, among each ethanolamine phosphoglyceride fractions which are consisted of acid-stable and acid labile groups (aldehydes and monoacyl group), there was almost the same proportion of incorporation of 1-^{14}C acetate into three moiety between with and without aminopterin-treatment (Table 6).

Discussion

Galli et al.[9] reported that the brain was almost as sensitive as other organs to nutritional deficiency provided that the deficient condition was induced from the beginning of postnatal life and that lipid deposition was affected both quantitatively and qualitatively under these circumstances. Davison and Dobbing[10] pointed out there was the apparent sparing of the adult brain to the effects of starvation, while even minor dietary restrictions

applied to suckling rats could produce significant changes in the cholesterol content and wet weight of the brain. In the present study, brain and body weights of rats with or without aminopterin-treatment were similar. The incorporation of 1-^{14}C acetate into lipid fractions except sphingomyelin, however, was reduced in the aminopterin-treated animals. It was reported that inhibition of lipid and protein biosynthesis as a result of nutritional restriction in developing animals might lead to incomplete or faulty of myelination[11]. Smith[12] stated that sphingomyelin turns over very slowly with a half-life of 10 months. In present experiment, the very slow turn over might have resulted in no change of incorporation in sphingomyelin between with and without aminopterin-treatment.

Davison[13] stated in a review "Biochemistry and the myelin sheath" that there are morphological changes during the early stages of myelination until 25 days after birth, at this time myelination is well established as seen by histological methods and that the myelin from developing rat brain contained less cerebrosides and more phospholipids than adult myelin. Myelin phospholipids from the central nervous systems of animals contain 32-36% phosphatidal ethanolamine which is in turn the largest part of myelin phospholipids, whereas phosphatidyl ethanolamine occurs in smaller amounts of about 8-14%. Choline and serine plasmalogens accounted less than 1% of the myelin phospholipids. Thus, phosphatidal ethanolamine forms about 70% of ethanolamine phosphoglycerides, while phosphatidal choline and serine are only 4 and 3% of total myelin choline and serine phosphoglycerides. Table 5 shows that aminopterin-treatment reduced incorporation of radioactivity in both phosphatidyl and phosphatidal ethanolamine. This suggested that both the vinyl ether linked moiety (which is usually composed of saturated type) and the acyl ester linked moiety are affected. It means that folate deficiency induced by the aminopterin-treatment interfered not only desaturation of fatty acids but also all myelin lipid synthesis.

It has been shown that there was some relationship between the blood-brain barrier and malnutrition, e.g. deficiency of thiamin was shown to affect transport of pyruvic acid from blood to the brain[14]. Referring to the previous study[1] and evidence presented in this study it seems that both steroids and fatty acids decreased about 20% with folated deficiency, the effect of aminopterin on acetate incorporation seems to interfere in one of the earliest levels of acetate metabolism. Since the reduction of 20% was rather small, it does not seem likely that folate deficiency affected the blood-brain barrier. Kidder and Dewey reported that the biosynthesis of sterols and unsaturated fatty acids in Crithidia fasciculata requires unconjugated pteridine[15,16]. Since steroid synthesis, fatty acid synthesis and desaturation require NADPH[4] and NADH[5], it seems likely that folate deficiency may cause the reduced production of NADPH and NADH.

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Footnotes

[1] Purchased from Oriental Co. Tokyo, Japan.

[2] Type 55 p-2, Hitachi, Tokyo, Japan.

[3] Aloka Liquid Scintillation Counter, Type LSC-600, Japan Radiation Medical Electronics, Inc. Tokyo, Japan.

[4] Dihydronicotinamide adenine dinucleotide phosphate.

[5] Dihydronicotinamide adenine dinucleotide.