Effect of Nicotinic Acid on Myelin Lipids in Brain of Developing Rat

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Summary Weanling rats (weaned 12 days after birth) were fed on nicotinic acid-deficient and on nicotinic acid-supplemented diet separately for 7 and 21 days. The yield of myelin in the brain of rat fed on the nicotinic acid-deficient diet was lower than that in the case of receipt of the nicotinic acid-supplemented diet at 19, 26 and 33 days of age. Despite the changing yield of myelin, the proportion of protein and total lipids, and the percentage composition of lipid were not changed between the groups fed on the nicotinic acid-deficient and the nicotinic acid-supplemented diet. Moreover, the ratio of long chain fatty acid (C_{20–23}/C_{14–18}) was markedly decreased in the nicotinic acid-deficient rats. These findings imply that nicotinic acid may play an important role in myelination associated with the synthesis of cerebrosides which contain high levels of long chain fatty acid.

Key Words nicotinic acid deficiency, myelin composition, fatty acid composition in myelin, cerebroside

Nicotinic acid deficiency is characterized by dermatitis, inflammation of mucous membranes and psychic disturbances (1). Mental symptoms with nicotinic acid deficiency are various, depression, irritability and anxiety being the most common ones.

Previous studies from our laboratory have shown that the concentration of cerebrosides in brain of rat fed on nicotinic acid-deficient diet is lower than that of rat fed on nicotinic acid-supplemented diet (2). Deposition of cerebrosides in the brain of nicotinic acid-deficient rat was delayed owing to the decrease of the biosynthesis of cerebrosides (3).

Cerebrosides are characteristic myelin lipids (4–7). They are almost non-existent until about 10 days after birth but increase sharply from the second to the third postnatal week. This age of maximum biosynthesis correlates well with the period of most active myelination (8). The causes of the lower cerebroside levels of nicotinic acid-deficient rat were considered as being due to: 1) low yield of myelin,
and 2) chemical abnormalities of myelin composition. In order to investigate these causes, we measured the quantity and composition of myelin in brain of rat respectively fed on nicotinic acid-deficient diet and nicotinic acid-supplemented diet.

METHODS

Nicotinic acid-deficient diet. The nicotinic acid-deficient diet was prepared as described by Nakashima et al. (9). In order to limit the amount of tryptophan in the nicotinic acid-deficient diet, we used a nicotinic acid-free low-casein diet to which a small amount of both methionine and threonine has been added and from which tryptophan has been excluded (tryptophan-imbalanced diet) (10–12), because pyridine nucleotides are synthesized from tryptophan in rat (13). To the control group, 10 mg of nicotinic acid were added per 100 g of this nicotinic acid-deficient diet.

Animals. Animals employed were rats of the Sprague-Dawley strain. Dams of suckling animals were fed on commercial diet. Litters were reduced to ten each at birth. Offspring were weaned 12 days after birth. The weaned animals were placed in either the nicotinic acid-deficient diet or the nicotinic acid-supplemented diet, given ad libitum or by pair-feeding according to the quantity consumed by the deficient group on the previous day. They were weighed at least twice weekly during the experimental period.

Preparation of myelin fraction. Weanling rats (weaned 12 days after birth) were fed on the nicotinic acid-deficient or the nicotinic acid-supplemented diet for 7 or 21 days. At 19 and 33 days of age, they were killed by decapitation, and the whole brains were removed. The brains were weighed, and from each brain, myelin was isolated and analyzed separately. The procedure of myelin isolation was that of Norton (14). For determination of dry weight, the myelin pellet was washed several times with distilled water, and freeze-dried in a vacuum. The purity of the myelin was checked for microsomal and mitochondrial contamination by marker-enzyme assays. NADPH-cytochrome c reductase was used as a mitochondrial marker (15). The lyophilized myelin was weighed and analyzed for protein and lipids.

Protein and lipid analysis. Myelin protein was determined using the methods of Lowry et al. (16). Lipids were extracted with 20 vol chloroform–methanol (2:1, v/v) from the lyophilized myelin fraction in a nitrogen atmosphere using the methods of Folch et al. (17). The lipid extract was taken to dryness under nitrogen at 60°C, and placed in a vacuum desiccator at 4°C after lyophilization to measure total lipid weight. The total lipids were fractionated by silicic acid (Wakogel Q-23, 100–200 mesh) column chromatography and purified by thin layer chromatography (TLC) on Silica-gel (Merk) as described by Norton and Poduslo (7). The total lipids and the purified lipids were analyzed for galactose (2), phosphorus (18) and cholesterol (19). An average molecular weight for mixed galactolipids of 846, for phospholipids of 775 and for cholesterol of 387 is assumed.

Gas-liquid chromatographic analysis of fatty acids in myelin. For transmethy-
EFFECT OF NICOTINIC ACID ON MYELIN LIPID

The combined extracts were evaporated to dryness in vacuo, and the esters were redissolved in chloroform–hexane (1:1). Methyl esters of non-hydroxy fatty acids were separated by chromatography on silicic acid according to the methods of O'Brien and Rouser (21). Methyl esters of non-hydroxy fatty acids were analyzed by gas-liquid chromatography (Ohkura gas chromatograph, Model 103). Hydroxy fatty acid were acetylated and analyzed by gas-liquid chromatography according to O'Brien and Rouser (21). Identification of non-hydroxy and hydroxy fatty acids were separated by chromatography on silicic acid according to the Mix-H-C, Applied Science Laboratories, Inc.).

Assay of nicotinic acid. An aliquot of the brain homogenate in 0.32 M sucrose was used for the analysis of nicotinic acid. Nicotinic acid in tissue was extracted as described previously (9). The nicotinic acid content of the extract was determined microbiologically by the methods of Snell and Wright using Lactobacillus arabinosus strain 17-5 ATCC 8014 (22).

RESULTS

Effect of nicotinic acid on body and brain weight, and nicotinic acid concentration in brain and liver

Weanling rats (weaned 12 days after birth) were fed on either the nicotinic acid-deficient diet or the nicotinic acid-supplemented diet for 7 or 21 days. The final body weight of animals fed on the nicotinic acid-deficient diet was significantly reduced at both 19 and 33 days for the groups given the nicotinic acid-supplemented diet ad libitum (ad libitum control). In the pair-fed group given the nicotinic acid-supplemented diet (pair-fed control), body weight was remarkably lower than that for the ad libitum control group (Table 1). No remarkable difference in brain weight was observed between the nicotinic acid-deficient group and the nicotinic acid-supplemented group. At both 19 and 33 days of age, nicotinic acid concentration in the whole brain of rats fed on the nicotinic acid-deficient diet was significantly lower than with the animals fed on the nicotinic acid-supplemented diet (pair-fed group and ad libitum group).

Concentration of myelin in brain of rat fed on the nicotinic acid-deficient and the nicotinic acid-supplemented diet

The effects of nicotinic acid on myelin concentration as a function of age are shown in Fig. 1. Myelin is almost unrecognized until about 10 days after birth. However, the amount of myelin in brain increased sharply from the third to the fourth postnatal week. The concentration of myelin in the brain was significantly
Table 1. Effect of nicotinic acid on body and brain weight, and concentration of nicotinic acid in brain and liver.
Weanling rats (weaned 12 days after birth) were fed on the nicotinic acid-deficient diet or the nicotinic acid-supplemented diet for 7 or 21 days.

| Age (days) | Nicotinic acid in diet | Body weight (g) | Brain weight (g) | Concentration of nicotinic acid |
|------------|------------------------|-----------------|-----------------|-------------------------------|
|            |                        | M ± SE          | M ± SE          | Brain (μg/g tissue) | Liver (μg/g tissue) |
| 19         | –                      | 27 ± 2          | 1.1 ± 0.1       | 27.6 ± 2.1             | 115 ± 6.8             |
|            | + (P)                  | 29 ± 3          | 1.2 ± 0.1       | 36.2 ± 2.4             | 153 ± 9.6             |
|            | +                      | 33 ± 2          | 1.3 ± 0.1       | 35.1 ± 2.5             | 165 ± 8.8             |
| 33         | –                      | 30 ± 3          | 1.3 ± 0.1       | 30.8 ± 3.5             | 121 ± 4.7             |
|            | + (P)                  | 34 ± 2          | 1.3 ± 0.1       | 40.4 ± 3.2             | 175 ± 6.5             |
|            | +                      | 72 ± 5          | 1.6 ± 0.1       | 39.5 ± 2.9             | 184 ± 8.5             |

(P); The group was fed on the nicotinic acid-supplemented diet by pair feeding. The concentration of nicotinic acid in brain and liver was assayed as described under METHODS. All values represent the mean of 6 rats ± SE.

Fig. 1. Concentration of myelin in brain of rats fed on the nicotinic acid-deficient and the nicotinic acid-supplemented diet. Weanling rats (weaned 12 days after birth) were fed for 7, 14 or 21 days on the nicotinic acid-deficient diet (×) or the nicotinic acid-supplemented diet, given ad libitum (●) or by pair feeding (▲), equivalent to the quantity consumed by the deficient group on the previous day. Myelin was isolated as described under METHODS. Each plot represents the mean value for six animals.

lower in rats fed on the nicotinic acid-deficient diet than in those receiving the nicotinic acid-supplemented diet (pair-fed group and ad libitum group) at each age studies. However, there was no difference in the concentration of myelin between

J. Nutr. Sci. Vitaminol.
| Age (days) | Nicotinic acid in diet | Myelin composition $^b$ | Protein | Lipid composition $^b$ | Cholesterol | Galactoceroid | Phospholipid |
|-----------|------------------------|------------------------|---------|-----------------------|-------------|--------------|-------------|
| 19        | 7.8±0.8                | 2.1±0.2                | 1.5±0.1 | 1.0±0.1               | 3.0±0.2     | 13.8±1.1     |
|           | (mg) (mg) (mg) (mg) (mg) (mg) | (26.9) | (71.8) | (27.0) | (18.1) | (52.8) |
| 33        | 9.9±0.6                | 3.7±0.3                | 2.7±0.3 | 2.0±0.1               | 5.0±0.4     | 2.2±0.2      |
|           | (mg) (mg) (mg) (mg) (mg) (mg) | (71.7) | (26.5) | (20.0) | (31.6) | (52.4) |

Yield of myelin $^a$. All values represent the mean of 6 rats ± SE, mg brain $^b$, mg myelin. Isolation and analysis of myelin in brain was performed as described under METHODS.
the pair-fed group and the *ad libitum* group.

**Effect of nicotinic acid on myelin composition in brain of rat**

The data in Table 2 demonstrate the gross composition of myelin, the proportion of protein and total lipids, and the percentage composition of lipids. The quantity of myelin (mg dry wt/brain) in brain of rats fed on the nicotinic acid-supplemented diet at both 19 and 33 days of age. Despite the changing yield of myelin, the proportion of protein and total lipids was not changed between the groups fed on the nicotinic acid-deficient and the nicotinic acid-supplemented diet. This myelin contained about 70–73% of dry weight as lipids and 25–28% of dry weight as protein.

The total amount of lipid in myelin (mg dry wt/brain) of nicotinic acid-deficient rats was lower than that of rats fed on the nicotinic acid-supplemented diet at both 19 and 33 days of age. Despite the difference in the total amounts of lipid, the per-

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**Table 3. Effect of nicotinic acid on non-hydroxy fatty acid composition of myelin lipids.**

Treatment of groups is explained in the footnote to Table 1. The fatty acid composition of myelin was determined by gas-liquid chromatography, as described under METHODS. Values are percentages of total non-hydroxy fatty acids. The numbers of samples were five for the nicotinic acid-deficient rats and six for the nicotinic acid-supplemented rats (*ad libitum* control and pair-fed control).

| Age (days) | 19     | 33     |
|-----------|--------|--------|
| Nicotinic acid in diet | (%) | (%) | (%) | (%) | (%) | (%) |
| Fatty acid | + | + (P) | − | + | + (P) | − |
| 14:0       | 3.1 | 3.1 | 4.3 | 1.4 | 2.1 | 2.6 |
| 16:0       | 16.7 | 15.8 | 21.5 | 7.6 | 7.9 | 11.5 |
| 18:0       | 19.0 | 20.4 | 24.6 | 10.8 | 11.8 | 22.3 |
| 18:1       | 26.6 | 28.4 | 26.9 | 33.2 | 31.8 | 30.9 |
| 20:1       | 6.8 | 4.2 | 1.2 | 13.5 | 11.6 | 9.2 |
| 20:4       | 5.4 | 5.8 | 6.2 | 5.3 | 5.3 | 5.1 |
| 22:1       | 1.1 | 1.0 | — | 1.6 | 1.5 | 1.5 |
| 24:0       | 4.5 | 4.6 | 2.8 | 9.2 | 8.6 | 4.8 |
| 24:1       | 8.7 | 7.0 | 3.2 | 11.8 | 10.9 | 3.6 |
| Unidentified | 8.1 | 9.7 | 9.3 | 5.6 | 9.5 | 8.5 |
| 14–18      | 65.4 | 67.7 | 77.3 | 53.0 | 53.6 | 67.3 |
| 20–24      | 26.5 | 22.6 | 13.4 | 41.4 | 36.9 | 24.2 |
| Ratio      | 20–24 | 14–18 | | 0.41 | 0.33 | 0.18 | 0.78 | 0.69 | 0.36 |

*J. Nutr. Sci. Vitaminol.*
percentage composition of cholesterol, galactolipid and phospholipid in myelin was not changed between the groups fed on the nicotinic acid-deficient and the nicotinic acid-supplemented diet. From this data, it is assumed that nicotinic acid-deficiency leads to a retardation of myelin formation but does not induce an abnormal lipid pattern of myelin.

**Effect of nicotinic acid on the composition of fatty acids of myelin lipids**

The non-hydroxy fatty acid composition of myelin from rats fed on either the nicotinic acid-deficient diet or the nicotinic acid-supplemented diet is shown in Table 3. There was a decrease of 20:1, 20:0 and 24:1 in the rats fed on the nicotinic acid-deficient diet, while 14:0, 16:0 and 18:0 increase. Then, the ratio (20-24/14-18) was calculated to be 0.18 in rats fed on the nicotinic acid-deficient diet, 0.14 in the ad libitum control and 0.33 in the pair-fed control at 19 days of age. The ratio was 0.36 in nicotinic acid-deficient rats, 0.76 in the ad libitum control and 0.96 in the pair-fed control at 33 days of age. Therefore, the ratio of long chain fatty acid was markedly decreased in nicotinic acid-deficient rats at both 19 and 33 days of age. Moreover, in both groups fed on the nicotinic acid-deficient and the nicotinic acid-supplemented diet, there was an increase of long chain fatty acid composition during development.

The \( \alpha \)-hydroxy fatty acid composition of myelin is given in Table 4. The hydroxy fatty acid composition of myelin increased with age, in accordance with the findings of Bauman et al. (23). The percentages of 24h:0 and 24h:1, which are typical characteristic fatty acids in brain myelin, increased in the group fed on the nicotinic acid-supplemented diet, whereas the percentage of 22h:0 decreased.

Table 4. Effect of nicotinic acid on hydroxy fatty acid composition of myelin lipids.

| Treatment of groups is explained in the footnote to Table 1. The fatty acid composition of myelin was determined by gas-liquid chromatography as described under METHODS. Values are percentages of total hydroxy fatty acids. The numbers of samples were five for the nicotinic acid-deficient rats and six for the nicotinic acid-supplemented rats. |
|---|---|---|---|---|---|---|
| Age (days) | 19 | | | 33 | | |
| | + (P) | (%) | (%) | (%) | (%) | (%) |
| Fatty acid | + | + (P) | - | + | + (P) | - |
| Nicotinic acid in diet | | | | | | |
| 18h:0 | 2.0 | 1.9 | 2.2 | 0.6 | 0.5 | 1.6 |
| 20h:0 | 1.5 | 1.5 | 2.9 | 0.9 | 1.0 | 1.2 |
| 22h:0 | 26.6 | 27.4 | 34.3 | 20.7 | 19.9 | 27.8 |
| 23h:0 | 3.2 | 3.8 | 3.4 | 4.1 | 3.6 | 4.2 |
| 24h:0 | 56.2 | 55.4 | 50.3 | 59.4 | 59.3 | 54.7 |
| 24h:1 | 5.3 | 5.0 | 3.8 | 9.6 | 8.9 | 6.2 |
| 26h:0 | 2.2 | 2.2 | 1.9 | 3.6 | 4.0 | 3.3 |
| Unidentified | 3.0 | 2.8 | 2.1 | 0.1 | 2.8 | 1.0 |

Vol. 28, No. 5, 1982
DISCUSSION

The present study demonstrates that the amount of myelin in whole brain of nicotinic acid-deficient rats was significantly lower than that with animals fed on the nicotinic acid-supplemented diet. However, no significant difference was observed regarding the percentages of the gross composition of myelin; total lipids, protein and the ratio of cholesterol, galactolipids and phospholipids. The formation of myelin is a genetically defined process which is accompanied by dramatic changes in the content and composition of brain lipids. The concentration of cerebrosides is higher in myelin than in any other structure of the brain (24). A myelin synthesis-deficiency mutant, such as quaking and Jimpy mouse, involves a deficiency in cerebroside production (25, 26). Therefore, it was suggested that the rate of myelin synthesis is involved in cerebroside production. Moreover, it was considered that the marked reduction of myelin in the nicotinic acid-deficient rat is due to the decrease of the synthesis of cerebrosides.

Cerebrosides of the nervous system are characterized by a relatively high content of long chain fatty acids (27). The most abundant fatty acids in cerebrosides are those with 24 carbon atoms such as nervonic acid, cerebronic acid and lignoceric acid. The synthesis of these long chain fatty acids dramatically increases at the onset of myelination (25–28). Biosynthesis of long chain fatty acids involves de novo synthesis by cytoplasmic enzyme producing mainly palmitic acid, and chain elongation by microsomal and mitochondrial enzyme (29–32). The mitochondrial elongation system requires both NADH and NADPH, and acyl-CoA as substrate (33, 34). The microsomal elongation system requires NADPH, and utilized malonyl-CoA as a source of C2 units (35). Therefore, since pyridine nucleotide is the preferred cofactor for fatty acid elongation, it is supposed that the elongation of fatty acid decreases in the brain of rats fed on nicotinic acid-deficient diet.

As shown in this paper, comparison of the fatty acid pattern in myelin lipids of rats fed on nicotinic acid-deficient diet with that in those of rats fed on nicotinic acid-supplemented diet confirmed the decrease of the proportion of long chain fatty acids in nicotinic acid-deficient rat. It seemed therefore that in the normal maturation process, the appearance of fatty acid follows the appearance of cerebrosides. A deficiency of cerebrosides, which contain long chain fatty acid, as observed in the nicotinic acid-deficient rat may lead to cessation of myelination. The key step may be the formation of these long chain fatty acids of their association with cerebroside precursor through the action of chain-length-specific enzyme.

Moreover, pyridine nucleotide is the preferred cofactor for fatty acid synthetase (36) and fatty acid \(\alpha\)-hydroxylating enzyme (28, 34, 36). Although fatty acid synthesis in \(^{14}\)C-acetate was high in the brain but low in the liver of weanling rats, the reverse was observed in adult animals (37). \(\alpha\)-Hydroxylating enzyme activity in microsomes rose from a low point in the immature brain to a maximum at 21 days of age and then declined to low levels in the mature brain (38). The microsomal enzyme activity was reduced in myelin-deficient mutants compared to their

J. Nutr. Sci. Vitaminol.
EFFECT OF NICOTINIC ACID ON MYELIN LIPID

controls (28, 33). Although our results differ from those with mutant mice, the data presented in this paper clearly show that nicotinic acid can influence the proportion of long chain fatty acid of myelin in the developing rat brain. Because metabolism of cerebrosides which contain high levels of long chain fatty acid is associated with myelination, further study is necessary to clarify the role of nicotinic acid in myelin synthesis in the developing rat brain.

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*J. Nutr. Sci. Vitaminol.*