EFFECT OF NICOTINIC ACID ON THE CONCENTRATION OF CEREBROSIDE IN RAT BRAIN

Yoko NAKASHIMA and Ryokuero SUZUE

National Institute of Nutrition, Tokyo 162, Japan
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Summary The effect of nicotinic acid on the changes of rat brain cerebroside levels has been studied during brain development. There is a gradual increase in the concentration of cerebroside from 12 days of age to adult level at approximately 47 days of age. However, the concentration of cerebroside was significantly lower in brain of rat fed the nicotinic acid-deficient diet. Therefore, nicotinic acid may play an important role in cerebroside synthesis in brain of developing rat.

Keywords cerebroside, nicotinic acid

Nicotinic acid has been recognized as a factor responsible for pellagra, which was frequently observed with disturbances of the central nervous system, leading to dementia. In addition, the administration of antagonistic agents of nicotinic acid caused mental deficiency (1).

It has been well recognized that malnutrition during the vulnerable period of growth impairs brain maturation. Morphological studies have shown that migration of cells is retarded, proliferation of neuronal fibers decreased and formation of synapses and myelin reduced in undernourished rats. They are rendered undernourished from birth to 35 days of age by increasing the litter size and also by limiting the suckling time to 16 hr/day (2). Parallel biochemical investigations showed significantly lower levels of myelin lipid components in undernourished rat brains (3).

The cerebrosides are ceramide monosaccharides occurring most abundantly in the myelin sheath of nervous tissues. The concentration of the cerebroside in whole brain was barely measurable as early as 10 days of age, and then increased sharply, especially between 14 to about 23 days of age. The age of maximum biosynthesis of cerebroside correlates well with the period of most active myelination (4).

To assess the effect of nicotinic acid deficiency on myelin formation, we have measured the amount of cerebroside in brain of rat fed the nicotinic acid-deficient diet during the period of most active myelination.
1. **Nicotinic acid-deficient diet.** The nicotinic acid-deficient diet was prepared as described by Nakashima et al. (5). In order to limit the amount of tryptophan therein, we used a nicotinic acid-free low casein diet to which a small amount of methionine and threonine containing an amino acid mixture was added (tryptophan-imbalanced diet) (6), because pyridine nucleotides were synthesized from tryptophan in rat (7). The control diet (nicotinic acid-supplemented diet) had 10 mg of nicotinic acid added per 100 g of the nicotinic acid-deficient diet.

2. **Animals.** The Sprague-Dawley strain of rats was used in all the experiments. Gestational rats used in the experiments were commercially obtained (Clea, Japan, Inc.). They were fed commercial rat chow throughout the gestational and lactational periods. Litters were reduced to ten rats each at birth and offspring were weaned 12 days after birth. Weaned animals, weighing 25–30 g, were divided into three groups; nicotinic acid-deficient group, ad libitum control and pair-fed control. The nicotinic acid-deficient group was fed the nicotinic acid-deficient diet for 3 weeks. The nicotinic acid-deficient-supplemented groups received the nicotinic acid-supplemented diet, which was fed ad libitum (ad libitum control) or by pair-feeding, equivalent to the quantity consumed by the deficient group on the previous day (pair-fed control). They were weighed at least twice weekly during the experimental periods. Rats were killed by decapitation, and whole brains were removed.

3. **Measurement of total lipid and cerebrosides content in brain.** Brain lipid was extracted according to the methods of Folch et al. (8). Total lipid was determined gravimetrically. Cerebrosides were separated from the extract by Florisil column chromatography according to the methods of Kishimoto and Radin (9). Forty-five volumes of NaOH (40 g per 45 ml water) was added to the crude cerebroside. After stirring and warming at 37–39°C for 3 hr, concentrated HCl was added until the orange end point was reached, using phenol red as indicator. The mixture was evaporated to dryness, the residue was extracted with warm chloroform–methanol (2:1), salt was filtered off, and the filtrate was washed with water. The ionic lipids in the filtrate were removed by passing the solution through a column of mixed ion-exchange resins. The galactose content of these crude cerebrosides was determined by the phosphoric acid-anthrone methods (10).

4. **Assay of nicotinic acid.** The tissue extract was prepared for the estimation of nicotinic acid as described previously (5). The nicotinic acid content of the extract was determined microbiologically by the method of Snell and Wright (11) using a *Lactobacillus arabinosus* strain, 17-5 ATCC 8014.

**RESULTS AND DISCUSSIONS**

1. **Effect of nicotinic acid on growth of rats fed the nicotinic acid-deficient and the nicotinic acid-supplemented diets**

   The growth response curves of weanling rats fed the experimental diets are...
NICOTINIC ACID AND CEREBROSIDE

Fig. 1. Effect of nicotinic acid on growth of rat. Weanling rats (weaned 12 days after birth) were separated into three groups. The nicotinic acid-deficient group was fed the nicotinic acid-deficient diet (○). The nicotinic acid supplemented groups received the nicotinic acid supplemented diet, fed *ad libitum* (×) or by pair-feeding (P) (▲) for 3 weeks. Each plot represents M±SE for 9 animals in each group.

shown in Fig. 1. The body weight gain of animals fed the nicotinic acid-deficient diet was much less than that of the group given the nicotinic acid-supplemented diet which was fed *ad libitum* (*ad libitum* control). In the pair-fed group given the nicotinic acid-supplemented diet (pair-fed control), body weight gains were significantly reduced compared with the *ad libitum* control. This significant weight reduction in the pair-fed control compared with the *ad libitum* control demonstrated that body weights were affected more by undernourishment from restricted food intake than by nicotinic acid deficiency.

2. *Nicotinic acid content of brain and liver of rat fed the nicotinic acid-deficient and the nicotinic acid-supplemented diet*

Nicotinic acid levels in whole brain and liver of rats fed the nicotinic acid-deficient and the nicotinic acid-supplemented diet for 21 days were measured (Table 1). In both brain and liver, the concentration of nicotinic acid of the nicotinic acid-deficient rats was significantly lower than in animals fed the nicotinic acid-supplemented diet (pair-fed and *ad libitum* controls) (Table 1). However, no significant difference of nicotinic acid concentration was observed between the pair-fed and the *ad libitum* control groups.

The rats fed the nicotinic acid-deficient diet for 3 weeks developed nicotinic acid deficiency symptoms characterized by roughness and unkempt appearance of fur, and alopecia over the nose and neck.

3. *Cerebroside and total lipid levels in brain of rat fed the nicotinic acid-deficient and the nicotinic acid-supplemented diet*

As shown in Table 2, no significant difference of brain weight was observed
Table 1. Concentration of nicotinic acid in brain and liver of rats fed the nicotinic acid-deficient and the nicotinic acid-supplemented diet. On the day of birth each litter was reduced to 10 offspring. Offspring were weaned 12 days after birth and separated into three groups. The nicotinic acid-deficient group was fed the nicotinic acid-deficient diet for 3 weeks. The nicotinic acid-supplemented groups received the nicotinic acid-supplemented diet, fed ad libitum or by pair-feeding (P) for 3 weeks. Rats were killed by decapitation and brains were removed and homogenized with 2 volumes of 0.14 M KCl. The tissue specimens were prepared for analysis by autoclaving 0.1 ml of the tissue homogenates with 1.0 ml of 1.0 N H$_2$SO$_4$ for 30 min at 120–130°C, and assayed for nicotinic acid as described under METHODS. All values represent the mean of 9 rats ± SE.

| Nicotinic acid in diet | Nicotinic acid content |                  | Brain (µg/g tissue) |
|-----------------------|------------------------|------------------|---------------------|
|                       | Liver (µg/g tissue)    |                  |                     |
|                       | 121 ± 4.7              | 30.3 ± 4.0       |
| + (P)                 | 165 ± 6.5              | 40.2 ± 4.3       |
| +                     | 184 ± 8.5              | 42.7 ± 4.8       |

Table 2. Concentration of cerebroside and total lipid in brain of rats fed the nicotinic acid-deficient and the nicotinic acid-supplemented diet. Treatment of groups is explained in the footnote to Table 1. The rats were killed by decapitation, their brains being removed and homogenized with 2 volumes of 0.14 M KCl. Lipid in the brain homogenate were extracted according to the methods of Folch et al. and assayed for total lipid and cerebroside as described under METHODS. All values represent the mean of 9 rats ± SE.

| Nicotinic acid in diet | Body weight (g) | Brain weight (g) | Total lipid (mg/g brain) | Cerebroside (mg/g brain) | Cerebroside Total lipid |
|-----------------------|-----------------|-----------------|--------------------------|--------------------------|-------------------------|
|                       | 30.1 ± 2.3      | 1.22 ± 0.04     | 57.2 ± 3.0               | 3.7 ± 0.1                | 0.065                   |
| + (P)                 | 34.3 ± 3.5      | 1.24 ± 0.04     | 58.4 ± 4.2               | 6.5 ± 0.6                | 0.112                   |
| +                     | 69.8 ± 5.0      | 1.33 ± 0.03     | 66.7 ± 1.2               | 6.6 ± 0.3                | 0.097                   |

between the ad libitum control, the pair-fed control and the nicotinic acid-deficient groups. Total lipid concentration in brain of the nicotinic acid-deficient rat was significantly lower than that in rats fed the nicotinic acid-supplemented diet ad libitum. However, no significant difference of total lipid concentration was observed between the nicotinic acid-deficient and the pair-fed control groups. The concentration of cerebroside in brain of the nicotinic acid-deficient rat was
significantly lower in rats fed the nicotinic acid-supplemented diet (pair-fed and *ad libitum* controls). However, there was no difference of cerebroside concentration between the pair-fed and the *ad libitum* controls. The cerebroside:total lipid ratio of the nicotinic acid-deficient rats was markedly lower than that of the nicotinic acid-supplemented groups (pair-fed and *ad libitum* controls). There was no difference in the cerebroside:total lipid ratio between pair-fed and *ad libitum* controls. This clearly indicated that nicotinic acid deficiency has a greater influence on cerebroside concentration at this early stage of development.

4. Developmental changes of the nicotinic acid and cerebroside levels in brain of rat fed the nicotinic acid-deficient and the nicotinic acid-supplemented diets

The changes of nicotinic acid concentration were measured during brain development in rats fed the nicotinic acid-deficient and the nicotinic acid-supplemented diets (Fig. 2). The concentration of nicotinic acid in whole brain of suckling rats 24 hr after birth was lower than that of the rats of 12 days of age. In the rats fed the nicotinic acid-supplemented diet (pair-fed and *ad libitum* controls), the concentration of nicotinic acid in brain gradually increased from 12 days through 30 days of age. However, the nicotinic acid concentration in brain of the nicotinic acid-deficient rats was not increased during development. Therefore, 30 days after birth, the concentration of nicotinic acid in the rats receiving the nicotinic acid-deficient diet was significantly lower than that in those receiving the nicotinic acid-supplemented diet.

Fig. 2. Developmental changes of nicotinic acid concentration in brain of rat fed the nicotinic acid-deficient and the nicotinic acid-supplemented diet. Weanling rats (weaned 12 days after birth) were separated into three groups. The nicotinic acid-deficient group was fed the nicotinic acid-deficient diet (*•*). The nicotinic acid-supplemented groups received the nicotinic acid-supplemented diet, fed *ad libitum* (×) or by pair-feeding (P) (▲) for 3 weeks. ○, concentration of nicotinic acid in whole brain of suckling rats 24 hr after birth. Each plot represents M ± SE for 4 animals in each group.
Fig. 3. Effect of nicotinic acid on the concentration of cerebroside in developing rat brain. Weanling rats (weaned 12 days after birth) were separated into three groups. The nicotinic acid-deficient group was fed the nicotinic acid-deficient diet (○). The nicotinic acid-supplemented groups received the nicotinic acid-supplemented diet, fed ad libitum (×) or by pair-feeding (P) (△) for 3 weeks. Each plot represents the mean of 3 animals.

The data in Fig. 3 demonstrate the developmental changes of the brain cerebroside content. Twelve days after birth, the brain cerebroside content was low. There was a gradual increase thereof from 12 days of age to adult levels at approximately 47 days of age. The brain cerebroside content of rats fed the nicotinic acid-deficient diet from 12 to 47 days of age was lower than that of the rats fed the nicotinic acid-supplemented diet.

Cerebroside is one of the major lipids components of myelin membranes, and is also present in low concentration in other subcellular membranes of brain. In recent years, it has been recognized that cerebroside is a valuable index of central nervous system myelination. This becomes readily apparent if the rate of deposition of cerebroside is compared with that of other lipids in myelinating rat brain (12). Also, the data of O'Brien and Sampson on the age-lipid composition relationship of human brain gray matter, white matter and myelin indicate the importance of cerebroside compared to other brain lipids in assessing the degree of myelination (13).

In the present study, total lipid concentration was slightly reduced in rats fed the nicotinic acid-deficient diet for 3 weeks from 12 days of age, while the brain cerebroside concentration was more markedly reduced than that of total lipids. In the rat fed the nicotinic acid-supplemented diet by pair-feeding, total lipid concentration was unaltered compared with rats fed the nicotinic acid-supplemented diet ad libitum; the cerebroside concentration was not reduced such as was found in the nicotinic acid-deficient rats. Therefore, it was considered that there was a decrease in the amount of myelin deposited in brains of rats fed the
nicotinic acid-deficient diet from 12 days after birth.

In the rats fed the nicotinic acid-supplemented diet by pair-feeding for 3 weeks, body weight, brain weight and total brain lipid concentration were significantly reduced compared with the ad libitum control. These significant reductions in the pair-fed control demonstrated that the body weight, brain weight and the brain total lipid concentration were affected more by undernourishment from restricted food intake than by nicotinic acid deficiency. However, the occurrence of no significant difference of cerebroside concentration between pair-fed control and ad libitum control indicated that nicotinic acid deficiency had more influence on cerebroside synthesis than undernourishment from restricted food intake.

The known role of nicotinic acid is in the formation of pyridine nucleotides. In the brain, almost all the nicotinic acid is present as pyridine nucleotides (14, 15). Singal et al. demonstrated that brain nicotinic acid levels markedly decreased in the nicotinic acid-deficient rat (16).

Long-chain fatty acids are essential constituents of sphingolipids and are also precursors of \( \alpha \)-hydroxy fatty acids (17), characteristic of cerebroside and sulfatide. Biosynthesis of long-chain fatty acids involves de novo synthesis by cytoplasmic enzymes, mainly producing palmitic acid (18, 19) and chain elongation by mitochondrial enzyme prefers acetyl-CoA and requires both NADH and NADPH (22–24). The \( \alpha \)-hydroxylating enzyme requires molecular oxygen and pyridine nucleotides (25). Cerebrosides containing long-chain fatty acids are characteristic myelin lipids, and their maturational accumulation coincides with myelination (26). Therefore, nicotinic acid seems to affect the biosynthesis of cerebroside in brain.

Although the data presented in this paper clearly show that nicotinic acid can influence the developing brain cerebroside concentration of rat, the precise role of nicotinic acid in the process of cerebroside synthesis is unknown. Because cerebroside metabolism in rat brain is associated with myelination, further study is necessary to clarify the effect of nicotinic acid on the cerebroside synthesis in the periods of active myelination.

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