Effect of Nicotinic Acid on Cerebroside Synthesis in Rat Brain

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Summary The effect of nicotinic acid on the synthesis of cerebrosides in the brain was studied during brain development. The concentration of cerebrosides in the brain was significantly lower in nicotinic acid-deficient animals than in those receiving a nicotinic acid-supplemented diet. The total lipid concentration in the brain of nicotinic acid-deficient rats was slightly lower than that of rats fed on the nicotinic acid-supplemented diet. Therefore, the ratio of cerebrosides to total lipids of nicotinic acid-deficient rats was markedly lower than that of nicotinic acid-supplemented rats. However, this low cerebroside level in nicotinic acid-deficient rats was restored by the administration of the nicotinic acid-supplemented diet. Synthesis of cerebrosides was followed in the brain of developing rats after intracerebral injection of L-[U-14C]serine. The total amount of radioactivity incorporated into the cerebroside fraction of nicotinic acid-deficient rat was smaller than that of nicotinic acid supplemented rats. These observations suggest that nicotinic acid affects cerebroside synthesis in the brain of rats.

Key Words cerebroside, nicotinic acid

Nicotinic acid has been shown to be a factor responsible for pellagra, which is frequently observed with disturbances of the central nervous system, leading to dementia. In addition, administration of antagonistic agents of nicotinic acid causes mental deficiency (1). In the brain, almost all the nicotinic acids are present as pyridine nucleotides (2, 3). Singal et al. have demonstrated that nicotinic acid in the brain is markedly decreased in nicotinic acid-deficient rats (4).

Previous studies in our laboratory have shown that rats on a nicotinic acid-deficient diet from the 12th day after birth for 3 weeks had a decreased concentration of cerebrosides in the brain compared with that of rats on a nicotinic acid-supplemented diet (5). In recent years it has been recognized that cerebroside is a valuable index of myelination of the central nervous system, being one of the major lipid components of myelin membranes (6–8). Cerebrosides are almost

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nonexistent before 10 days after birth but their level increases sharply from the second to the third postnatal week. This age of maximum biosynthesis correlates well with the period of most active myelination (9).

Long-chain fatty acids are essential constituents of sphingolipids and are precursors of \( \alpha \)-hydroxy fatty acids (10), characteristics of cerebrosides and sulfatides. Biosynthesis of long-chain fatty acids involves de novo synthesis by cytoplasmic enzymes that mainly produce palmitic acid (11, 12), and chain elongation by mitochondrial and microsomal enzymes (13, 14). Mitochondrial enzyme prefers acetyl-CoA as substrate and requires both NADH and NADPH (15–17), whereas microsomal enzyme is specific to malonyl-CoA and NADPH. The system of \( \alpha \)-hydroxylation requires reduced pyridine nucleotide in conjugation with molecular oxygen (18).

Therefore, nicotinic acid apparently affects the biosynthesis of cerebrosides in the brain. In this study, we have investigated the effect of nicotinic acid on the synthesis and quantitative changes of cerebrosides in the developing brain.

METHODS

Nicotinic acid-deficient diet. Nicotinic acid-deficient diet was prepared as described by Nakashima et al. (19). 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 was added and from which tryptophan was excluded (tryptophan-imbalanced diet) (20–22), since pyridine nucleotides are synthesized from tryptophan in rats (23). 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 rats each at birth. Offspring were weaned 12 days after birth. Weaned animals were placed on 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.

Measurement of the rate of cerebroside synthesis. Weanling rats (weaned 12 days after birth) were fed on the nicotinic acid-deficient or the nicotinic acid-supplemented diet for 10 days. The rats anesthetized with ether were given intraventricular injection of 1 \( \mu \)Ci of L-[\( U-{ }^{14}\)C]serine (0.1 \( \mu \)mol) in a 10 \( \mu \)l volume, as described by Hayes and Jungalwala (24). Forty-eight hours after the \(^{14}\)C-serine injection, the rats were decapitated and the whole brain was removed, weighed, and homogenized in 10 ml of ice-cold 0.32 M sucrose in a Potter-Elvehjem homogenizer. To 0.1 ml of the homogenate was added 0.2 ml of 50% trichloroacetic acid, and the mixture was centrifuged after 10 min. The specific radioactivity of the acid-soluble pool was measured. The homogenate was centrifuged at 105,000 \( \times \) g for 60 min and the supernatant fluid was carefully removed. The pellet was suspended in 2 ml of

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0.32 M sucrose and extracted with 20 vol of chloroform–methanol (2:1, v/v) by the methods of Folch et al. (25). The lipid extract was washed three times with 0.2 vol of 0.9% NaCl. The lower phase was then washed three times with 0.2 vol of chloroform–methanol–water (1:15:15, v/v) containing 0.1% DL-serine and three times with the same mixture but without serine. The lipid extract was evaporated to dryness and then chromatographed on a column of florisil and ion-exchange resin as described previously (5). The cerebroside fraction was evaporated to dryness and dried further in a desiccator. The cerebroside fraction was dissolved in 0.5 ml of chloroform–methanol (2:1, v/v) and chromatographed on a silica-gel G thin-layer plate with chloroform–methanol–water (24:7:1, v/v) as solvent. Lipids were detected by brief exposure to I2 vapors. Bands corresponding to cerebroside standards were scraped from the plate, and radioactivity was determined. The remaining material was used for quantitative determination of cerebrosides as previously described (5).

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 (19). The nicotinic acid content of the extracts was determined microbiologically by the methods of Snell and Wright using Lactobacillus arabinosus strain 17-5, ATCC 8014 (26).

RESULTS

Effect of nicotinic acid on the growth of rats

Weanling rats (12 days after birth) were fed on either the nicotinic acid-deficient diet or the nicotinic acid-supplemented diet (10 mg per 100 g diet) for 17 days. The growth response curves of weanling rats are shown in Fig. 1. The body weight gain

Fig. 1. Effect of nicotinic acid on the growth of rats. Weanling rats (weaned 12 days after birth) were fed for 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. Each plot represents mean value ± S.E. for nine animals in each group.

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of animals fed on the nicotinic acid-deficient diet was far inferior than that of the group 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 gain was remarkably smaller than that of the *ad libitum* control group. This suggests that body weight was affected more by undernourishment from restricted food intake than by nicotinic acid deficiency.

*Amount of nicotinic acid, cerebrosides and total lipids in the brain of the nicotinic acid-deficient rats and the nicotinic acid-supplemented rats*

Weanling rats (weaned 12 days after birth) were given either the nicotinic acid-deficient diet or the nicotinic acid-supplemented diet *ad libitum* or by pair feeding for 10 days. As shown in Table 1, no remarkable difference in brain weight was observed between the nicotinic acid-deficient group and the nicotinic acid-supplemented group. Concentration of nicotinic acid in the whole brain of rats fed on the nicotinic acid-deficient diet was significantly lower than the animals fed on the nicotinic acid-supplemented diet (pair-fed group and *ad libitum* group).

The total lipid concentration in the brain of nicotinic acid-deficient rats was lower than that of the rats fed on the nicotinic acid-supplemented diet *ad libitum*. However, the total lipid concentration did not differ between the group fed on the nicotinic acid-deficient diet and that given the nicotinic acid-supplemented diet by pair feeding.

The concentration of cerebrosides in the brain was significantly lower in nicotinic acid-deficient animals than those receiving the nicotinic acid-supplemented diet (pair-fed group and *ad libitum* group). However, cerebroside concentration showed no difference between the pair-fed group and the *ad libitum* group. Therefore, the ratio of cerebrosides to total lipids of nicotinic acid-deficient rats was

<table>
<thead>
<tr>
<th>Nicotinic acid in diet</th>
<th>Body weight (g)</th>
<th>Brain weight (g)</th>
<th>Nicotinic acid in brain (μg/g brain)</th>
<th>Total lipids (mg/g brain)</th>
<th>Cerebrosides (mg/g brain)</th>
<th>Cerebrosides Total lipids</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>23 ± 2</td>
<td>1.1 ± 0.1</td>
<td>29.7 ± 4.0</td>
<td>39.8 ± 3.8</td>
<td>2.7 ± 0.3</td>
<td>0.068</td>
</tr>
<tr>
<td>+ (P)</td>
<td>25 ± 1</td>
<td>1.1 ± 0.1</td>
<td>36.8 ± 4.2</td>
<td>40.2 ± 2.3</td>
<td>4.2 ± 0.3</td>
<td>0.104</td>
</tr>
<tr>
<td>+</td>
<td>39 ± 2</td>
<td>1.2 ± 0.1</td>
<td>37.5 ± 4.5</td>
<td>44.7 ± 2.6</td>
<td>4.8 ± 0.5</td>
<td>0.107</td>
</tr>
</tbody>
</table>

markedly lower than that of nicotinic acid-supplemented rats (pair-fed group and ad libitum group). There was no difference in the ratio of cerebrosides to total lipid between pair-fed group and ad libitum group.

Nicotinic acid and cerebrosides concentration in the brain after administration of nicotinic acid to nicotinic acid-deficient rats

Weanling rats placed on the nicotinic acid-deficient diet for 10 days were fed on the same diet (Group I) or transferred to the nicotinic acid-supplemented diet for 7 days (Group II). Group III received the nicotinic acid-supplemented diet for 17 days.

Table 2. Nicotinic acid content of the brain of rats after the administration of the nicotinic acid-supplemented diet to nicotinic acid-deficient rats for 7 days.

<table>
<thead>
<tr>
<th>Nicotinic acid in diet</th>
<th>Body weight (g)</th>
<th>Brain weight (g)</th>
<th>Nicotinic acid (µg/g brain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (−)</td>
<td>31 ± 3</td>
<td>1.2 ± 0.1</td>
<td>30.1 ± 3.2</td>
</tr>
<tr>
<td>Group II (− → +)</td>
<td>48 ± 3</td>
<td>1.3 ± 0.1</td>
<td>39.4 ± 3.8</td>
</tr>
<tr>
<td>Group III (+)</td>
<td>63 ± 4</td>
<td>1.4 ± 0.1</td>
<td>39.9 ± 4.1</td>
</tr>
</tbody>
</table>

Fig. 2. Cerebroside content of the brain of rats after the administration of nicotinic acid to the nicotinic acid-deficient rats. Weanling rats (weaned 12 days after birth), fed on the nicotinic acid-deficient diet for 10 days, were separated into three groups. Group I (●) received the same diet for 7 days. Group II (△) was transferred to the nicotinic acid-supplemented diet for 7 days. Group III (×) received the nicotinic acid-supplemented diet for 17 days. Concentration of cerebrosides in the brain was assayed as described under METHODS. All values represent the mean of 3 rats ± S.E.
days. Concentrations of nicotinic acid and cerebrosides in the brain of these groups were determined (Table 2 and Fig. 2). The growth of all rats fed on the nicotinic acid-deficient diet for 17 days was significantly slower than that of those fed the nicotinic acid-supplemented diet. However, the body weight of rats fed on the nicotinic acid-deficient diet for 10 days increased with the administration of the nicotinic acid-supplemented diet. No significant difference in brain weight was observed between the nicotinic acid-deficient group and the nicotinic acid-supplemented group. Concentration of nicotinic acid in the brain of nicotinic acid-deficient rats was significantly lower than that of animals fed on the nicotinic acid-supplemented diet. Nicotinic acid concentration in the brain was restored when nicotinic acid-deficient rats were given the nicotinic acid-supplemented diet (Table 2).

Concentration of cerebrosides in the brain of nicotinic acid-deficient rats was significantly lower than that of animals fed on the nicotinic acid-supplemented diet (Fig. 1). However, this low cerebroside level in the brain of nicotinic acid-deficient rats was restored by the administration of the nicotinic acid-supplemented diet. These observations suggest that nicotinic acid affects cerebroside synthesis in the brain of rats.

Effect of nicotinic acid on incorporation of radioactivity of $^{14}$C-serine into cerebroside fraction

Experiment A. Weanling rats (weaned 12 days after birth) were separated into three groups. Group I received the nicotinic acid-deficient diet for 10 days. Group II was given the nicotinic acid-supplemented diet by pair feeding, equivalent to the quantity consumed by the deficient group on the previous day. Group III was given the nicotinic acid-supplemented diet ad libitum. Ten days after weaning, these rats were anesthetized with ether and were given intraventricular injection of 1 μCi of L-$[^{14}$C]serine. Forty-eight hours after the administration of $^{14}$C-serine, the radioactivity incorporated into the cerebroside fraction was determined.

As shown in Table 3, A, the total amount of radioactivity incorporated into the cerebroside fraction of nicotinic acid-deficient rats was smaller than that of nicotinic acid-supplemented rats. Concentration of cerebrosides in the brain of nicotinic acid-deficient rats was lower than that of rats fed on the nicotinic acid-supplemented diet. The specific radioactivity of the cerebroside fraction showed no difference between the nicotinic acid-deficient group and the nicotinic acid-supplemented group.

Experiment B. Weanling rats (weaned 12 days after birth) fed the nicotinic acid-deficient diet for 10 days were separated into two groups. One group was given 1 mg of nicotinic acid per rat in a volume of 2 ml saline solution by intraperitoneal injection (Group II). The other group was given the same volume of 0.9% NaCl solution. Immediately after the nicotinic acid administration, these rats were anesthetized with ether and given intraventricular injection of 1 μCi of L-$[^{14}$C]-serine. Forty-eight hours after the $^{14}$C-serine injection, the animals were killed by decapitation, the brain was removed immediately and radioactivity in the

Table 3. Effect of nicotinic acid on cerebroside synthesis in the brain of rats fed on the nicotinic acid-deficient diet. Treatment groups explained in the method and the text section. All values represent the mean of 3 rats ± S.E.

<table>
<thead>
<tr>
<th>Experiment A</th>
<th>Total lipids (mg/brain)</th>
<th>Cerebrosides</th>
<th>Total lipids (mg/brain)</th>
<th>Cerebrosides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotinic acid in diet</td>
<td>Content (mg/brain)</td>
<td>Radioactivity (dpm/brain)</td>
<td>Content (mg/brain)</td>
<td>Radioactivity (dpm/brain)</td>
</tr>
<tr>
<td>Group I (−)</td>
<td>38.9 ± 3.2</td>
<td>2.4 ± 0.2</td>
<td>2,800 ± 320</td>
<td></td>
</tr>
<tr>
<td>Group II (+)→(P)</td>
<td>43.4 ± 3.6</td>
<td>3.7 ± 0.4</td>
<td>4,300 ± 310</td>
<td></td>
</tr>
<tr>
<td>Group III (+)</td>
<td>45.5 ± 4.0</td>
<td>4.0 ± 0.3</td>
<td>5,200 ± 460</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment B</th>
<th>Total lipids (mg/brain)</th>
<th>Cerebrosides</th>
<th>Total lipids (mg/brain)</th>
<th>Cerebrosides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicotinic acid injection (1 mg/rat)</td>
<td>Content (mg/brain)</td>
<td>Radioactivity (dpm/brain)</td>
<td>Content (mg/brain)</td>
<td>Radioactivity (dpm/brain)</td>
</tr>
<tr>
<td>Group I (−)</td>
<td>39.0 ± 3.3</td>
<td>2.8 ± 0.3</td>
<td>3,200 ± 380</td>
<td></td>
</tr>
<tr>
<td>Group II (+)</td>
<td>42.3 ± 3.8</td>
<td>3.7 ± 0.2</td>
<td>7,500 ± 620</td>
<td></td>
</tr>
</tbody>
</table>

cerebroside fraction was estimated.

As shown in Table 3, B, the total amount of radioactivity incorporated into the cerebroside fraction of the nicotinic acid-deficient group was smaller than that of the group administered nicotinic acid for 48 hr.

DISCUSSION

The results of the present study show that the total amount of radioactivity of $^{14}$C-serine incorporated into the cerebroside fraction of nicotinic acid-deficient rats was relatively low compared with nicotinic acid-supplemented rats. Our previous study has shown that the concentration of cerebrosides in the whole brain was barely measurable before 12 days after birth (5). There was a gradual increase in cerebroside concentration in the brain from 12 days after birth to the adult level at approximately 47 days after birth. The concentration of cerebrosides in the brain of rats fed on the nicotinic acid-deficient diet from the 12th day to the 47th day after birth, however, was lower than that of rats fed on the nicotinic acid-supplemented diet. Deposition of cerebrosides in the brain of nicotinic acid-deficient rats was delayed due to decreased biosynthesis of cerebrosides.

Long chain fatty acids ($C_{22}$–$C_{26}$) are essential constituents of cerebrosides.
Infant brain cerebrosides have a lower percentage of C_{22}–C_{26} fatty acids than those of child and adult (27). The importance of these long-chain fatty acids in myelinogenesis is evident by the dramatic increase in their formation at the onset of myelination in the mouse and rat (28).

Biosynthesis of long chain fatty acids involves \textit{de novo} synthesis by cytoplasmic enzyme producing mainly palmitic acid, and chain elongation by microsomal and mitochondrial enzymes. Pyridine nucleotide is the preferred cofactor for the formation of palmitoic-CoA from long chain fatty acids (28). The microsomal fatty acid elongating activity in the brain rose to the maximum level on the 21st day after birth and then declined to low levels in the mature brain. The developmental changes in brain microsomal fatty acid elongation activity are consistent with the maturational changes in the composition and content of sphingolipid fatty acids. Therefore, since pyridine nucleotide is the preferred cofactor for fatty acid elongation, it is likely that a small amount of long chain fatty acids in the central nervous system of nicotinic acid-deficient rats is responsible for reduced synthesis by the microsomal enzyme, which is directly related to myelination.

Myelination is a complex biological process by which oligodendroglial cells in the central nervous system and Schwann cells in the peripheral nervous system produce a multilayer membrane around nerve fibers. The onset of active myelination is abrupt, and the period of active myelination is relatively short. The biological factors responsible for this rather specific timing of myelination are yet unknown. Although the data presented in this paper clearly show that nicotinic acid can influence the cerebroside concentration in the developing rat brain, the precise role of nicotinic acid in the process of cerebroside synthesis is unknown. Because cerebroside metabolism in the rat brain is associated with myelination, further studies must be made to clarify the effect of nicotinic acid on cerebroside synthesis during the period of active myelination.

REFERENCES


