EFFECT OF NIACIN DEFICIENCY ON THE METABOLISM OF BRAIN AMINES IN RATS

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Summary The effect of niacin deficiency on the activities of tyrosine hydroxylase and tryptophan-5-monooxygenase was studied in relation to the contents of catecholamines and serotonin in brain to clarify the role of the vitamin in brain function. Male rats 4 weeks of age were fed a niacin-free, low-protein diet for 3 weeks. Tests for the avoidance learning behavior were started at 14th day in experimental period and were repeated 3 times every other day. After sacrifice on the 21st day, the activities of tyrosine hydroxylase and tryptophan-5-monooxygenase in the brain homogenates were assayed. In the other experiments, contents of dopamine, norepinephrine and serotonin in brain were determined in rats fed on the same niacin-deficient, low-protein diet for 3 weeks. The activity of crude tyrosine hydroxylase in brain was found to be increased significantly in niacin deficiency and was reduced below that of the control one week after niacin supplementation to the deficient rats. On the contrary, contents of dopamine and norepinephrine in whole brain of the niacin-deficient rats showed a significant decrease. No significant difference in brain tryptophan-5-monooxygenase activities was observed among niacin-deficient, low-protein control and normal commercial diet groups. Brain serotonin contents of the niacin-deficient animals were almost the same as that of the low-protein control, but were less than that of the rats fed on niacin-supplemented 20% casein diet. Though, no significant difference was observed between the learning abilities of niacin-deficient and control groups, these results suggested that the change of catecholamine and serotonin metabolism in brain was one of the causes for some malfunction of the central nervous system induced by niacin deficiency.

Pellagra which has been known as a disease caused by niacin deficiency is characterized by dermatitis, diarrhea and dementia. Though the major role of

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the niacin derivatives, NAD, NADP and their reduced forms, has been found to participate in a variety of metabolic reactions as cofactors of many kinds of redox enzymes, the disturbance of the central nervous system caused by niacin deficiency has not yet been elucidated. Dopamine, norepinephrine and serotonin are thought to be the synaptic neurotransmitters present mainly in the basal ganglia region of brain and have an important role in the brain function. These catecholamines and serotonin have been shown to be synthesized from tyrosine and tryptophan, respectively, in neurons by enzymatic hydroxylation for which a pteridine cofactor, pteridine reductase, and NAD(P)H are necessary in vivo. In our previous experiments, ascorbic acid deficiency in guinea pig was recognized to cause a decrease of tyrosine hydroxylase activities in brain or adrenals (1-3) and resulted in the low concentration of catecholamines in the adrenals (3). WURTMAN et al. reported a decreased amount of catecholamines in brain of infant rats fed a low-protein diet during the brain developing stage (4, 5). Serotonin concentration in brain was also shown to be decreased by feeding a low-protein diet and the amount of tryptophan intake was reported to affect serotonin biosynthesis (6). In the meantime, many pharmacologists showed the interrelation between catecholamines or serotonin metabolism and brain function using many kinds of drugs (7-9). In this report, we describe the effect of niacin deficiency on the metabolism of catecholamines and serotonin to clarify causal factors of dysfunction of central nervous system. In addition, the avoidance learning behaviors of niacin-deficient animals were tested.

MATERIALS AND METHODS

Male Wistar rats, 4 weeks of age, were given free access for 3 weeks to water and one of the following diets: 1. niacin-deficient, low-protein diet, 2. niacin-supplemented control diet, 3. commercial stock rations. The composition of the experimental diets are shown in Table 1. They were housed individually in a temperature-controlled room and were exposed to light from 7 am. to 7 pm. Tests for the avoidance of conditioning learning behavior were started at 14 th day after their feeding of experimental diet. The tests, of 30 trials each time (totally 90 trials per rat), were repeated 3 times every other day. The apparatus used was a shuttle box with buzzer and grid floors for delivering an electric shock (11). Conditioning and unconditioning stimuli were 3 seconds of buzzing and 3 seconds of electric shock, respectively. The rats were all killed on the 21st day between 10 am. and noon by decapitation. Their brains and livers were removed quickly and chilled on ice. Brain tryptophan-5-monooxygenase [EC 1.14.16.4] was assayed by the methods of ICHIYAMA et al. (12) and tyrosine hydroxylase [EC 1.14.16.2] by that of NAGATSU et al. (13) as modified by NAKASHIMA et al. (1). Niacin contents in brain and liver were microbiologically assayed using L. arabinosus (14). In the second experiments, male Sprague-Dawley rats, 3 weeks of age,
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Table 1. Composition of experimental diets.

| Component                        | Niacin-deficient low-protein diet (g/100 g diet) | Low-protein control diet (g/100 g diet) | 20% casein diet (g/100 g diet) |
|----------------------------------|-----------------------------------------------|-----------------------------------------|-------------------------------|
| Casein (vitamin free)           | 7.0                                           | 7.0                                     | 20.0                          |
| Amino acid mixture               | 1.0                                           | 1.0                                     | 0                             |
| Soybean oil                     | 4.0                                           | 4.0                                     | 4.0                           |
| Cod liver oil                    | 1.0                                           | 1.0                                     | 1.0                           |
| Sucrose                          | 80.0                                          | 80.0                                    | 68.0                          |
| Mineral mixture                  | 4.0                                           | 4.0                                     | 4.0                           |
| Cellulose                        | 2.0                                           | 2.0                                     | 2.0                           |
| Vitamin mixture (niacin free)    | 0.85                                          | 0.85                                    | 0.85                          |
| Choline chloride                 | 0.15                                          | 0.15                                    | 0.15                          |
| Niacin                           | 0                                             | 2.5                                     | 2.5                           |

* One gram of amino acid mixture contains DL-methionine 0.30, DL-threonine 0.36, L-lysineHCl 0.162, DL-phenylalanine 0.14, and L-histidine 0.038g. b Composition of mineral mixture was described by HARPER (10). c 0.85 gram of vitamin mixture contains thiamine HCl 1.0, riboflavin 1.0, pyridoxine HCl 1.0, Ca-pantothenate 2.0, inositol 10.0, biotin 0.02, folic acid 0.2, vitamin K₃ 0.1, vitamin B₁₂ 0.02, ascorbic acid 10.0 and lactose 825 mg.

were fed on one of the following diets: 1. niacin-deficient, low-protein diet, 2. niacin-supplemented control diet, 3. 20% casein diet. The composition of these diets is shown in Table 1. After 3 weeks, the rats were decapitated and their whole brains were removed. The catecholamines in the brains were extracted by the methods of ANTON and SAYRE (15). Then norepinephrine and dopamine were assayed by the methods of VON EULER (16) and CARLSSON (17), respectively. Serotonin was extracted and assayed by the methods of BOGDANSKI et al. (18).

RESULTS

The body weight of the niacin-deficient group had begun to decrease about 2 weeks after feeding of experimental diet. Growth was recovered when their diet was changed to the niacin-supplemented one and they were pair-fed with the niacin-deficient group thereafter (recovered group) (Fig. 1). As shown in Table 2, liver niacin concentration was found to be reduced markedly in the niacin-deficient group. While, niacin supplementation for the last 7 days caused a significant increase in liver niacin concentration, higher than that of any other groups. These phenomena may suggest that niacin deficiency would give rise to the increase of the niacin retaining capacity of the liver. On the other hand, no significant difference appeared in niacin concentration in the brain of niacin-deficient, control and recovered groups. Only a weak reducing tendency was observed in that of the niacin-deficient group.

The activities of tryptophan-5-monooxygenase, the enzyme catalyzing the first step of serotonin biosynthesis, were almost similar for the niacin-deficient and
Fig. 1. Growth of experimental animals. The rats were fed on niacin-deficient (×—×), low-protein control (○—○) or normal commercial diet (●—●) for 3 weeks. One group was fed on the niacin-deficient diet for 2 weeks, and then on the low-protein diet for next one week (△—△). The composition of the diets were shown in Table 1. The time when diet was changed in recovered group is indicated by an arrow. Duration of avoidance tests is indicated by black bar. Each point expresses the mean of the body weight in 8 animals.

Table 2. Total niacin contents in liver and brain of the experimental animal.a

| Group           | Content of nicotinic acid (μg/g tissue) |
|-----------------|----------------------------------------|
|                 | Liver                                  | Brain                                |
| Niacin deficient| 117.5±6.4*                             | 32.0±1.8                             |
| Control         | 140.6±6.1                              | 35.0±2.0                             |
| Recovered       | 209.5±3.9**                            | 34.5±0.9                             |
| Normal          | 169.0±1.2**                            | 41.0±1.4*                            |

The rats were fed on niacin-deficient low-protein diet (niacin-deficient group), low-protein control diet (control group) or normal commercial stock rations (normal group) for 3 weeks. Recovered group was fed on niacin-deficient low-protein diet for 2 weeks followed by low-protein control diet for one week. The composition of each diet is shown in Table 1. Data are presented as the mean±s.e.m. Four rats are used in each group.

* p<0.05, **p<0.005 (compared with control group).

control groups (Table 3). Its elevation in the recovered group might be caused by the starved state of the animal during the pair feeding with the niacin-deficient group. The activity of brain crude tyrosine hydroxylase, the rate limiting enzyme of catecholamine biosynthesis, was found to be clearly higher in normal commercial diet group than that of the niacin-supplemented control group (Table 3). As the total protein concentration of the control diet was only 8%, the low protein level of the control diet might have reduced the crude enzyme activity. Niacin deprivation, on the contrary, resulted in the elevation of the enzyme activity, even though the diet contained only 8% of a protein-amino acid mixture. Niacin
### Table 3. The activities of tyrosine hydroxylase and tryptophan-5-monooxygenase in brain of the experimental animal.

| Group         | Tyrosine hydroxylase (10^6 cpm/g tissue) | Tryptophan-5-monooxygenase |
|---------------|------------------------------------------|---------------------------|
| Niacin-deficient | 30.5±1.1*                               | 21.1±1.4                  |
| Control       | 13.5±1.6                                 | 21.4±2.0                  |
| Recovered     | 8.2±2.1                                  | 27.8±0.8**                |
| Normal        | 26.3±1.2*                                | 22.4±1.7                  |

* Treatment of each group is described in legend to Table 2. Data are presented as the mean±s.e.m. Three rats were used for tyrosine hydroxylase assay and 4 rats for tryptophan-5-monooxygenase, respectively, in each group.

supplementation to the niacin-deficient rats (recovered group) lowered the enzyme activity below that of the rats fed the niacin-containing diet for the entire experimental duration. These results indicated that the activity of the brain crude tyrosine hydroxylase was affected not only by dietary protein but also by niacin supply.

Niacin deficiency has been known to cause a disturbance in the central nervous system. So, the avoidance test was carried out to obtain some knowledge about the relationship between niacin deficiency and learning behavior with regard to catecholamine and serotonin metabolism. However, no significant difference was recognized among the results of the tests for avoidance of learning behavior in these groups in cases of over 30 trials (Fig. 2). Therefore, it was suggested that

![Fig. 2. Effect of niacin deficiency on avoidance learning behavior. Feeding treatment of the groups were described in the legend to Table 2. Eight rats were used for the tests in each group. Each bar represents the average and s.e.m. of the number of success per rat in 30 trials.](image)
the neuronal dysfunction in rats caused by niacin deficiency would not be evaluated by avoidance learning behavior.

In the second experiments, brain catecholamine and serotonin contents were measured in niacin-deficient, low-protein, niacin-supplemented control and 20% casein diet groups. As shown in Table 4, brain dopamine and norepinephrine

Table 4. Catecholamine and serotonin contents in brain of the experimental animal.*

| Group      | Brain weight (g) | Norepinephrine (µg/g tissue) | Dopamine (µg/g tissue) | Serotonin (µg/g tissue) |
|------------|------------------|-----------------------------|------------------------|------------------------|
| Niacin-deficient | 1.52±0.03       | 0.076±0.003                | 0.372±0.032            | 0.528±0.038            |
| Control    | 1.59±0.03       | 0.078±0.003                | 0.416±0.008            | 0.519±0.031            |
| 20% casein | 1.67±0.02*      | 0.080±0.004                | 0.381±0.026            | 0.640±0.025**          |

* Treatment of niacin-deficient and control groups is the same as that described in legend to Table 2. The rats in 20% casein group were fed on 20% casein diet for 3 weeks. The composition of the diet was shown in Table 1. In each group, 4 rats were used for the assay of each amine. Data are presented as mean±s.e.m. * p<0.01, **p<0.05 (compared with control group).

contents in the niacin-deficient group were found to be slightly lower than that of 20% casein or niacin-supplemented control groups. By comparing the results of the niacin-deficient group and the control, it was inferred that brain serotonin concentration was not affected significantly by niacin deficiency. Only the amount of tryptophan intake was thought to affect the brain serotonin concentration.

**DISCUSSION**

Though niacin is an essential nutrient, it is well known to be produced from tryptophan in humans, rat and many other kinds of animals. Pellagra, therefore, is caused by the diet not only deficient in niacin but also low in tryptophan content. One of the main symptoms of pellagra is dementia. The mechanism of such kind of neural dysfunction has not been clarified yet. In the present report, we described the change of brain catecholamine and serotonin contents together with the activities of their producing enzyme, according to the feeding of niacin-deficient, low-protein diet. The tyrosine hydroxylase activity of the whole brain homogenate was significantly elevated by niacin deficiency. On the contrary, dopamine and norepinephrine contents were rather decreased. As tyrosine hydroxylase was shown to be a rate limiting enzyme of catecholamine biosynthesis from tyrosine (19), these results seems to be inconsistent with the increased activity of brain crude tyrosine hydroxylase in the niacin-deficient group. To explain these contradictory observations, more precise examination as to the amount of enzyme protein, the presence of the enzyme inhibitor, the concentration of the cofactor...
and catecholamine turnover are required in the future. It was also probable that the addition of cofactor system to the enzyme assay medium might cover up the insufficiency of cofactor system in vivo in niacin-deficient animals. The niacin content of the whole brain of the niacin-deficient rat was not significantly different from that of the control animals. Therefore, the effect of niacin deficiency on the brain amine metabolism was expected to be caused indirectly by the secondary effect evoked by the vitamin deficiency or directly by a partial niacin deficiency in some place of the brain such as basal ganglia or hypothalamus. WURTMAN et al. reported the decrease of catecholamine content in brain of the infant rat fed a low-protein diet during the suckling or brain-developing stage (4). In their report, partially purified tyrosine hydroxylase activity was found to be rather higher in low protein group. Though their results seems to be inconsistent with the present data, the activity of tyrosine hydroxylase in the brain whole homogenate was likely to be different from that of the partial purified enzyme because the crude homogenate has been known to contain both of particle bound and free enzymes with small amount of unknown enzyme inhibiting factor (19). In addition, the effect of low-protein supply on the brain amine metabolism in the brain-developing stage might be different from that of the post-weanling rats. These may be the reason why our results are inconsistent with theirs. In some pharmacological studies using reserpine, tyrosine hydroxylase activity of adrenal or brain was found to be elevated (20). But the concentration of catecholamines in those tissues were decreased as a result of catecholamine releasing action of the β-blocking reagent. These findings suggested that the elevation of tyrosine hydroxylase activity was not always accompanied with the increase of the catecholamine content in tissue.

The decrease of the serotonin contents in the niacin-deficient and low-protein control groups was reasonable because brain serotonin concentration was shown to be changed according to the concentration of its precursor, tryptophan, in brain, which was varied proportionally to its level in blood (21, 22). The serotonin concentration in brain seems to be unaffected by niacin deficiency.

The relationship between the metabolism of neurotransmitters containing catecholamines and serotonin and behavior was reported by many pharmacologists or psychologists. For instance, such drugs as reserpine, p-chlorophenylalanine and some kinds of monoamine oxidase inhibitors, which have been known to affect the metabolism of those amines, cause the behavioral change in animals (7-9). In our experiments, deviations among the individual data for learning behavior were found to be very large, resulting no significant difference among the groups. Therefore, the relation between behavior and niacin deficiency was not elucidated in the present experiments.

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