The Differences in Growth and Activity of the Tryptophan-NAD Pathway between Wistar and Sprague Dawley Strains of Rats Fed on Tryptophan-Limited Diet

Katsumi Shibata, Kazumi Motooka, and Kiku Murata

Food and Nutrition Laboratories, Faculty of Domestic Science, Teikoku Women's University, Moriguchi, Osaka 570, Japan
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Summary Three-week-old weanling rats of Wistar and Sprague Dawley strains were fed on tryptophan-limited and nicotinic acid-free diets for 46 days and the following was observed. (1) Gain in body weight of the Sprague Dawley strain was significantly higher than that of the Wistar strain from the 10th day of feeding. (2) Tryptophan oxygenase [EC 1.13.11.11] activity in the Wistar strain was constant but was significantly increased in the Sprague Dawley strain during the latter period of this experiment. (3) The total amount of nicotinic acid, quinolinic acid, N1-methylnicotinamide and tryptophan in liver and kidney of the Sprague Dawley strain was significantly higher than that of the Wistar strain. (4) Total urinary nicotinic acid, quinolinic acid and N1-methylnicotinamide levels were not very different between the two strains, but it was observed that at the 38th day the levels in the Sprague Dawley strain were significantly higher than those in the Wistar strain. From the above result, it was presumed that the Sprague Dawley strain of rat was more resistant to deficiency of nicotinic acid than the Wistar strain fed on a low tryptophan and nicotinic acid-free diet. Aminocarboxymuconate-semialdehyde decarboxylase [EC 4.1.1.45] activities in livers of both strains dropped to half the original value at the end of the experiment. This change may indicate metabolic control of increase in flow from tryptophan to NAD.

Key Words tryptophan-NAD pathway, difference in growth rate, tryptophan oxygenase, aminocarboxymuconate-semialdehyde decarboxylase, NAD, quinolinic acid, nicotinic acid, N1-methylnicotinamide

A difference in growth rate between Wistar and Sprague Dawley (SD) strains of rats was observed (1) when the rats were fed on a diet of 8.0% amino acid mixture

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simulating rice protein plus the limiting amino acids (2). The limiting nutrients of this diet are tryptophan and nicotinic acid. The activities of some enzymes in the tryptophan-NAD pathway were higher in the SD strain than in the Wistar strain. One of the reasons for this difference in the growth rate between the two strains was considered to be the difference in the efficiency of tryptophan utilization. The experiment and results are reported herein with some discussion.

MATERIALS AND METHODS

Chemicals. Amino acids were obtained from Kyowa Hakko Kogyo Co., Ltd. Vitamins and sucrose were acquired from Wako Pure Chemical Industries Ltd. Salt mixture was the product of Tanabe Amino Acids Research Foundation. Chocola A, Chocola D and d-α-tocopherol made at Eizai Co., Ltd., were used as vitamin A, D and E, respectively. α-Maize starch and maize oil were purchased respectively from Nichiden Kagaku Co., Ltd., and Nippon Shokuhin Kako Ltd. Other chemicals were of the highest purity available from commercial sources.

Animals and diet. Eleven male weanling rats of Wistar and SD strains respectively (3 weeks old, body weight 40–50 g) were obtained from J.C.L. Each rat was kept in an individual wire-bottomed cage and water supplied ad libitum. The

| Ingredient | (g/kg diet) | (I.U./kg diet) |
|------------|------------|----------------|
| Amino acid mixture | 80 |  |
| Lysine·HCl | 4 |  |
| Threonine | 2 |  |
| Valine | 1 |  |
| Isoleucine | 2 |  |
| Methionine | 1 |  |
| Histidine·HCl | 1 |  |
| α-Maize starch | 561 |  |
| Sucrose | 281 |  |
| Maize oil | 20 |  |
| Salt mixture | 40 |  |
| Vitamin mixture (nicotinic acid-free) | 2.5 |  |
| Choline·HCl | 2 |  |
| Vitamin A | 3,000 |  |
| Vitamin D | 300 |  |
| Vitamin E | 0.6 |  |

*The amino acid composition of rice protein used was based on "The Amino Acid Composition of Food in Japan, Resources Bureau, Science and Technology Agency (1966)." *b These compositions were based on Harper’s salt and vitamin mixture (5).

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composition of the diet is shown in Table 1. The amount of food intake and body weight were measured at 10 a.m.

Five rats of each strain were killed on the 3rd day, 3 rats on the 38th day and 3 rats on the 46th day. The activity of tryptophan oxygenase [EC 1.13.11.11] in liver and of aminocarboxymuconate-semialdehyde decarboxylase [EC 4.1.1.45] in liver and kidney, and the content of NAD, nicotinic acid, quinolinic acid, \( N^1 \)-methylnicotinamide and tryptophan in liver and kidney were determined as in the previous report (3).

Urine samples were collected in a flask containing 1 ml of 0.1 N HCl and 0.5 ml of toluene using a metabolic cage for days 0–3, 19–22, 36–38 and 44–46. Nicotinic acid, quinolinic acid and \( N^1 \)-methylnicotinamide in urine were determined as previously reported (3).

**Statistical analysis.** Statistical analysis was carried out using analysis of variance and Student’s t-test.

**RESULTS**

*The difference in growth rate*

The difference in growth rate between the Wistar and the SD strains fed on the tryptophan-limited, nicotinic acid-free diet is shown in Fig. 1. Body weight gains of both strains were the same for the first 5 days. From this time on, the weight gain of the SD strain was greater than that of the Wistar strain. A statistically significant difference was observed from the 10th day of feeding (\( p < 0.05 \)). Weight gains of the SD and the Wistar strains were 68.7 \( \pm \) 11.6 g and 40.0 \( \pm \) 7.5 g respectively on the 46th day (\( p < 0.05 \)). The food intake of the SD strain for 46 days (386.0 \( \pm \) 28.1 g) was also much higher than that of the Wistar strain (309.3 \( \pm \) 29.1 g) (\( p < 0.05 \)). Food efficiency ratios of the SD and the Wistar strains were 0.179 \( \pm \) 0.032 and 0.128 \( \pm \) 0.013, respectively. The difference was not statistically significant.

*Tryptophan oxygenase activity in liver*

Changes in activity of tryptophan oxygenase in liver are shown in Fig. 2. The enzyme activity in the Wistar strain was constant during the experiment. On the other hand, the activity in the SD strain was significantly increased on the 38th day compared with the value on the 3rd day (\( p < 0.05 \)).

*Aminocarboxymuconate-semialdehyde decarboxylase activity in liver and kidney*

As shown in Fig. 3, the difference in the enzyme activity between the two strains was not statistically significant. The activity in the liver of both strains decreased by 50% on the 46th day compared with the value on the 3rd day (\( p < 0.01 \), SD strain: \( p < 0.05 \), Wistar strain). It is presumed that the flow from tryptophan to NAD might be increased during the latter part of the experiment. On the other hand, the activity in kidney increased on the 46th day, but the difference between the two strains was not statistically significant.
Fig. 1. The difference in weight gains between the rats of the Wistar and Sprague Dawley strains. ○, SD strain; ●, Wistar strain. Vertical lines indicate the standard deviations. Statistically significant difference was observed at $p < 0.05$ from the 10th day.

Fig. 2. Changes in activities of liver tryptophan oxygenase of the rats of the Wistar and Sprague Dawley strains. ○, SD strain; ●, Wistar strain. Values of the 3rd, 38th and 46th day are the averages of 5, 3 and 3 rats, respectively. Common superscript letters mean statistically significant difference at $p < 0.05$.

Tryptophan metabolites in liver and kidney

The NAD content of both strains is shown in Fig. 4. The amount in liver of the Wistar strain remained constant at about 0.23 μmol/g liver. However, the NAD
Fig. 3. Changes in activities of aminocarboxymuconate-semialdehyde decarboxylase in liver (A) and kidney (B) of the rats of the Wistar and Sprague Dawley strains. The explanation is the same as that for Fig. 2.

Fig. 4. Changes in NAD content in liver (A) and kidney (B) of the rats of the Wistar and Sprague Dawley strains. The explanation is the same as that for Fig. 2.

content in liver of the SD strain fell to about half on the 38th day compared with the value on the 3rd day ($p<0.01$ at the 38th and the 46th day). A statistically significant difference of NAD content between the SD and the Wistar strains was seen on the 46th day ($p<0.01$). However, the total amount of NAD per liver in the two strains was almost the same. A significant difference of NAD level in kidney between the two strains was not observed throughout the experiment, and the values of both strains dropped on the 38th day ($p<0.05$) compared with the value on the 3rd day and increased on the 46th day in the SD strain only compared with the value on the 38th day ($p<0.01$). The meaning of the increase is obscure.

Amounts of nicotinic acid, quinolinic acid, $N^1$-methylnicotinamide and tryptophan in liver and kidney are shown in Table 2 and 3, respectively. Quinolinic acid contents in liver on the 46th day and kidney on the 38th day of the SD strain were significantly higher than those of the Wistar strain ($p<0.05$). The $N^1$-methyl-
Table 2. Contents of nicotinic acid, quinolinic acid, N\textsuperscript{1}-methyl nicotinamide and tryptophan in liver. Values of the 3rd, 38th and 46th days are the average of 5, 3 and 3 rats, respectively and expressed as μmol/g liver wet weight.

|                          | 3rd day  | 38th day | 46th day |
|--------------------------|----------|----------|----------|
| Nicotinic acid (SD)      | 0.15 ± 0.08 | 0.24 ± 0.18 | 0.19 ± 0.07 |
| Nicotinic acid (Wistar)  | 0.19 ± 0.04\textsuperscript{a} | 0.09 ± 0.01\textsuperscript{a,b} | 0.17 ± 0.04\textsuperscript{b} |
| Quinolinic acid (SD)     | 0.75 ± 0.52 | 0.54 ± 0.33 | 0.53 ± 0.09\textsuperscript{c} |
| Quinolinic acid (Wistar) | 0.57 ± 0.25 | 0.25 ± 0.10 | 0.22 ± 0.03\textsuperscript{c} |
| N\textsuperscript{1}-Methyl nicotinamide (SD) | 0.17 ± 0.05 | 0.23 ± 0.02\textsuperscript{d} | 0.23 ± 0.04 |
| N\textsuperscript{1}-Methyl nicotinamide (Wistar) | 0.18 ± 0.03 | 0.15 ± 0.02\textsuperscript{d} | 0.20 ± 0.05 |
| Tryptophan (SD)          | 0.10 ± 0.02 | 0.09 ± 0.06 | 0.15 ± 0.03 |
| Tryptophan (Wistar)      | 0.08 ± 0.02 | 0.06 ± 0.01 | 0.12 ± 0.02 |

Common superscript letters mean statistically significant difference at $p < 0.05$.

Table 3. Contents of nicotinic acid, quinolinic acid, N\textsuperscript{1}-methyl nicotinamide and tryptophan in kidney. Values of the 3rd, 38th and 46th days are the average of 5, 3 and 3 rats, respectively and expressed as μmol/g kidney wet weight.

|                          | 3rd day  | 38th day | 46th day |
|--------------------------|----------|----------|----------|
| Nicotinic acid (SD)      | 0.21 ± 0.09 | 0.11 ± 0.06 | 0.13 ± 0.04 |
| Nicotinic acid (Wistar)  | 0.24 ± 0.02\textsuperscript{a,b} | 0.11 ± 0.06\textsuperscript{a} | 0.08 ± 0.01\textsuperscript{b} |
| Quinolinic acid (SD)     | 0.89 ± 0.32\textsuperscript{c} | 0.87 ± 0.32\textsuperscript{d,e} | 0.29 ± 0.11\textsuperscript{c,e} |
| Quinolinic acid (Wistar) | 0.65 ± 0.46 | 0.30 ± 0.13\textsuperscript{d} | 0.32 ± 0.15 |
| N\textsuperscript{1}-Methyl nicotinamide (SD) | 0.16 ± 0.03 | 0.15 ± 0.04 | 0.20 ± 0.08 |
| N\textsuperscript{1}-Methyl nicotinamide (Wistar) | 0.12 ± 0.02 | 0.13 ± 0.03 | 0.17 ± 0.02 |
| Tryptophan (SD)          | 0.11 ± 0.04\textsuperscript{f,g,h} | 0.25 ± 0.11\textsuperscript{g,i} | 0.66 ± 0.05\textsuperscript{h,i} |
| Tryptophan (Wistar)      | 0.06 ± 0.01\textsuperscript{f,j,k} | 0.19 ± 0.01\textsuperscript{j,l} | 0.63 ± 0.04\textsuperscript{k,l} |

Common superscript letters mean statistically significant difference at $p < 0.05$.

nicotinamide content on the 38th day in the liver of the SD strain was also greater than that of the SD strain. The tryptophan content of livers of both strains was almost the same and remained at about the same level throughout the experiment, but the level in the kidneys of the two strains varied widely during the experiment. The tryptophan content in kidney of the SD strain on the 3rd day was significantly higher than that of the Wistar strain and the level in both strains gradually increased by the 38th and then up to the 46th day. The content on the 46th day in kidneys of both strains was much higher than on the 3rd and the 38th days. The biological meaning will be discussed later.

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Fig. 5. Changes in nicotinic acid, quinolinic acid, \( N^1 \)-methylnicotinamidine and tryptophan contents in liver (A) and kidney (B) of rats of the Wistar and Sprague Dawley strains. The explanation is the same as that for Fig. 2. QA, quinolinic acid; NA, nicotinic acid; N1MN, \( N^1 \)-methylnicotinamide.

Table 4. Contents of nicotinic acid, quinolinic acid and \( N^1 \)-methylnicotinamidine in urine. Each value is the average of 3 rat urines and expressed as nmol/day urine.

|                  | 0–3 days | 19–21 days | 36–38 days | 44–46 days |
|------------------|----------|------------|------------|------------|
| Nicotinic acid (SD) | 73 ± 35  | 50 ± 17 | 30 ± 10 | 33 ± 15 |
| Nicotinic acid (Wistar) | 37 ± 6  | 60 ± 17 | 30 ± 0  | 23 ± 6   |
| Quinolinic acid (SD) | 80 ± 70  | 75 ± 14 | 100 ± 20 | 37 ± 15  |
| Quinolinic acid (Wistar) | 60 ± 10\(^a\) | 93 ± 51 | 60 ± 20 | 20 ± 10\(^a\) |
| \( N^1 \)-Methylnicotinamidine (SD) | 41 ± 9\(^b,c,d\) | 107 ± 10\(^e,e\) | 48 ± 6\(^e,f\) | 106 ± 34\(^d,f\) |
| \( N^1 \)-Methylnicotinamidine (Wistar) | 62 ± 10\(^b,g,h,i\) | 122 ± 29\(^e,j\) | 35 ± 8\(^b,j,k\) | 108 ± 28\(^i,k\) |

Common superscript letters mean statistically significant difference at \( p < 0.05 \).

The total amount of these tryptophan metabolites in liver of the SD strain was higher than in the Wistar strain on the 38th and 46th days as shown in Fig. 5A (\( p < 0.01 \) at the 38th day: \( p < 0.05 \) at the 46th day). The same was true in kidney, as shown in Fig. 5B. The total amount of tryptophan metabolites in liver and kidney of Vol. 28, No. 1, 1982
the SD strain was maintained at a constant level during the experiment, but those values on the 38th day for the Wistar strain were lowered compared with the values on the 3rd day ($p < 0.02$).

**Urinary excretion of tryptophan metabolites**

As shown in Table 4, urinary excretion of nicotinic acid and quinolinic acid did not differ between the two strains. However, the quinolinic acid content for days 44–46 in the Wistar strain decreased significantly compared with that for days 0–3. Urinary $N^1$-methylnicotinamide of both strains was extremely varied. Total amount of nicotinic acid, quinolinic acid and $N^1$-methylnicotinamide in urine for days 36–38 was significantly higher for the SD strain than for the Wistar strain ($p < 0.05$), as shown in Fig. 6.

**DISCUSSION**

Growth rates of rats of the SD and Wistar strains were about the same, if rats were fed on a complete diet (4). However, growth rates of rats of SD and Wistar strains were significantly different when the tryptophan-limited and nicotinic acid-free diet was fed (1).

Although the NAD content per g liver of the SD strain on the 46th day was significantly lower than that of the Wistar strain, the appearance of the nicotinic acid deficiency was more severe in the Wistar strain than in the SD strain. The NAD content per liver was the same. We did not determine total NAD content in the whole body; however, the content per rat might be higher in the SD strain than in the Wistar strain. The amount of quinolinic acid in liver from tryptophan was higher in the SD strain than in the Wistar strain. The total amount of nicotinic acid, quinolinic acid, $N^1$-methylnicotinamide and tryptophan in liver and kidney of the
Wistar strain was significantly lowered on the 38th day, but not in the SD strain (Fig. 5). However, tryptophan in kidney of both strains on the 46th day was much higher than on the 3rd and 38th days. The phenomenon may indicate that tryptophan utilization is decreased at that point of the nutritionally imbalanced condition. The increase in activity of tryptophan oxygenase of the SD strain on the 38th day may be attributed to efficient metabolic oxidation of tryptophan into NAD, but such change was not observed in the Wistar strain. In this experiment, aminocarboxymuconate-semialdehyde decarboxylase activity in kidney of both strains was much lower than that reported by Ikeda et al. (6), although the activity of liver was similar to the reported value. This low value for kidney and the occurrence of no significant change through the experimental period might be attributed to the difference in ages of rats and also in diets given to rats; the rats used in this experiment were 3 weeks old and were fed on the nicotinic acid-free and tryptophan-limited diets. The reasons for the value of the aminocarboxymuconate-semialdehyde decarboxylase activity of kidney is not clear, but it seems to indicate an efficient utilization of the limiting tryptophan to NAD pathway. The significant decrease of this enzyme activity in liver after the 38th day up to the 46th day for both strains may also indicate metabolic control for maintaining the minimum level of NAD in the body.

These results indicate that the tryptophan-quinolinic acid pathway of the SD strain is more active than that of the Wistar strain when the tryptophan-limited diet was fed. It was presumed that the SD strain of rat has greater resistance than the Wistar strain to a low tryptophan and nicotinic acid-free diet.

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