

Acta Medica Okayama

Volume 46, Issue 6

1992

Article 1

DECEMBER 1992

Sulfur Amino Acid Levels and Related Enzyme Activities in Various Brain Regions (and Other Tissues) in Normal Mice and Rolling Mice Nagoya

Kazuhiro Myojin*

Tadashi Hiroi†

Hisao Ikeda‡

Hiroyuki Kodama**

*Kochi Medical School,

†Kochi Medical School,

‡Kochi Medical School,

**Kochi Medical School,

Sulfur Amino Acid Levels and Related Enzyme Activities in Various Brain Regions (and Other Tissues) in Normal Mice and Rolling Mice Nagoya*

Kazuhiro Myojin, Tadashi Hiroi, Hisao Ikeda, and Hiroyuki Kodama

Abstract

The contents of the sulfur amino acids, and the activities of cystathionine beta-synthase and cystathionine gamma-lyase were measured in various regions of the brain and several other tissues in both normal mice and rolling mice Nagoya. The cystathionine content and cystathionine beta-synthase activity were found to be unevenly distributed in various regions of the brain in both normal mice and rolling mice Nagoya, being highest in the cerebellum. Except for the mesencephalon and thalamus plus hypothalamus, the cystathionine content and cystathionine beta-synthase activity in the brain regions of rolling mice Nagoya were much higher than those of the normal mice. The cystathionine content after D,L-propargylglycine treatment was also found to be unevenly distributed in various brain regions in both normal mice and rolling mice Nagoya. The concentrations of cystine and methionine were also higher in all regions of the brain of rolling mice Nagoya than those of normal mice, while the concentration of taurine in the various regions of the brain was almost the same in normal mice and rolling mice Nagoya. Cystathionine beta-synthase and cystathionine gamma-lyase activities in the liver, kidney, and pancreas were almost the same in both the normal mice and rolling mice Nagoya.

KEYWORDS: cystathionine, rolling mouse Nagoya, brain, propargylglycine

*PMID: 1485534 [PubMed - indexed for MEDLINE]

Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL

Sulfur Amino Acid Levels and Related Enzyme Activities in Various Brain Regions (and Other Tissues) in Normal Mice and Rolling Mice Nagoya

Kazuhiro Myojin*, Tadasu Hiroi, Hisao Ikeda and Hiroyuki Kodama^a

Departments of Neuropsychiatry and ^aChemistry, Kochi Medical School, Nankoku-shi, Kochi 783, Japan

The contents of the sulfur amino acids, and the activities of cystathionine β -synthase and cystathionine γ -lyase were measured in various regions of the brain and several other tissues in both normal mice and rolling mice Nagoya. The cystathionine content and cystathionine β -synthase activity were found to be unevenly distributed in various regions of the brain in both normal mice and rolling mice Nagoya, being highest in the cerebellum. Except for the mesencephalon and thalamus plus hypothalamus, the cystathionine content and cystathionine β -synthase activity in the brain regions of rolling mice Nagoya were much higher than those of the normal mice. The cystathionine content after D, L-propargylglycine treatment was also found to be unevenly distributed in various brain regions in both normal mice and rolling mice Nagoya. The concentrations of cystine and methionine were also higher in all regions of the brain of rolling mice Nagoya than those of normal mice, while the concentration of taurine in the various regions of the brain was almost the same in normal mice and rolling mice Nagoya. Cystathionine β -synthase and cystathionine γ -lyase activities in the liver, kidney, and pancreas were almost the same in both the normal mice and rolling mice Nagoya.

Key words : cystathionine, rolling mouse Nagoya, brain, propargylglycine

Rolling mouse Nagoya (RMN) is an ataxic mutant mouse discovered by Oda in Nagoya, Japan (1, 2). Despite its severe ataxia, little is known on the pathoanatomical and biochemical changes responsible for this motor disturbance. Nishimura reported the presence of atrophy in the cerebellum of this animal, localized in the anterior part of the lobulus centralis with a decreased number of granules and basket and stellate cells (3). This observation, however, has not been

confirmed by other authors (4). Determinations of free amino acids and some enzyme activities in various regions of the brain were carried out in these animals by Ando *et al.* (5) and Nagatsu *et al.* (6). They found differences between the contents of free amino acids in control mice and rolling mice Nagoya only in the cerebellum, where glutamate content was decreased, and taurine and glycine contents were increased in the latter mice.

In the present paper, we report the levels of sulfur amino acids and activities of some related

*To whom correspondence should be addressed.

enzymes in the various brain regions and several other tissues in normal mice and rolling mice Nagoya.

Materials and Methods

Chemicals. D, L-Propargylglycine was purchased from Sigma Chem. Co., St. Louis, MO, USA. Other reagents were obtained from Wako Pure Chemical Ind., Ltd., Osaka, Japan.

Animals. Rolling mice Nagoya used in this experiment were kindly supplied by Dr. Oda (Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan). Rolling mice Nagoya and normal mice (C3H) were housed separately in metabolic cages. Mice (6 animals in each group) were injected intraperitoneally with 0.5, 1.0, or 2.0 mg of D, L-propargylglycine per 10 g of body weight, respectively.

Analysis of amino acids. Normal mice and rolling mice Nagoya, and D, L-propargylglycine-treated mice (at 20 h after the administration of D, L-propargylglycine) were killed by decapitation. The brain, liver, kidney, and pancreas were quickly removed and blotted. The brain was separated into six regions (mesencephalon, cerebellum, hippocampus, thalamus plus hypothalamus, cerebral cortex and caudatum) at 0°C. Each sample was homogenized in 4 volumes of 2% sulfosalicylic acid and centrifuged at $3,000 \times g$ for 15 min. The resulting supernatant was applied to a column containing 5 ml of Diaion SA (OH-form, anion exchanger, mesh 100, Mitsubishi Chemical Ind., Ltd, Tokyo, Japan). The column was washed with 50 ml of water and eluted with 50 ml of 2 M HCl. The eluate was dried under reduced pressure and the residue was dissolved in 1 ml of 0.02 M HCl. Aliquots of the solution were analyzed with an amino acid analyzer (Hitachi Model 835 liquid chromatograph). As cysteine was oxidized to cystine during the sample preparation, it was analyzed as cystine and its data were presented as cystine.

Enzyme assays. Normal mice and rolling mice Nagoya were killed by decapitation. The liver, kidney, pancreas, and brain were removed and homogenized in 3 volumes of cold 0.01 M potassium phosphate buffer (pH 7.5) containing 0.15 M KCl, 0.1 mM pyridoxal phosphate and 0.5 mM EDTA. The homogenates were centrifuged at $10,000 \times g$ for 10 min, and the resulting supernatants were used for the enzyme assay. All these procedures were performed at 0 to 4°C.

Cystathionine β -synthase (EC 4. 2. 1. 1) activity was determined by the method of Kashiwamata and Greenberg (7). Cystathionine γ -lyase (EC 4. 4. 1. 1) activity was determined by measuring the α -ketobutyric acid produced from homoserine according to the method of Greenberg (8).

Results and Discussion

The six brain regions studied in this study were the mesencephalon, cerebellum, thalamus plus hypothalamus, caudatum, hippocampus, and cerebral cortex. Table 1 shows that cystathionine was unevenly distributed in these various regions in both types of mice, which was in accordance with previous reports (9-11). The concentration of cystathionine was greatest in the cerebellum in both types of mice. In rolling mice Nagoya, the content of cystathionine in all brain regions were greater than those in all brain regions of normal mice, except for the mesencephalon and thalamus plus hypothalamus.

Cystathionine β -synthase activity was measured to investigate the differences in the cystathionine synthesis in various brain regions of normal mice and rolling mice Nagoya. This activity was highest in the cerebellum, corresponding with the cystathionine content, in both types of mice as shown in Table 2. Cystathionine β -synthase activity was also higher in all brain regions of rolling mice Nagoya than that in those

Table 1 Concentration of cystathionine in various regions of the brain in normal and rolling mice Nagoya^a

| Region of brain | Normal mice | Rolling mice |
|----------------------------|-------------|--------------|
| Mesencephalon | 20.2 ± 4.9 | 24.5 ± 3.4 |
| Cerebellum | 33.2 ± 4.6 | 39.9 ± 6.7 |
| Hippocampus | 9.8 ± 4.2 | 16.2 ± 3.1 |
| Thalamus plus hypothalamus | 13.7 ± 3.1 | 14.4 ± 5.5 |
| Cerebral cortex | 9.7 ± 4.2 | 14.5 ± 6.8 |
| Caudatum | 7.4 ± 0.5 | 23.2 ± 3.3 |

^a: Each value (nmoles/g wet wt.) represents mean ± SD obtained from normal mice (n = 18) and rolling mice (n = 18)

of normal mice, except for the mesencephalon and thalamus plus hypothalamus. However, the positive relationship between cystathionine content and cystathionine β -synthase activity was observed in some but not in all regions of both brains, suggesting that cystathionine γ -lyase activity, which degrades cystathionine, is also involved in the regulation of the cystathionine concentration in these brain regions.

Cystathionine γ -lyase activity in all brain regions examined was much lower than cystathionine β -synthase activity. Since the activity of this enzyme in the various regions of the mouse brain was much lower than those in the liver, kidney and pancreas. It was difficult to measure this activity separately in the brain regions by Greenberg's method (8), which was used for the liver, kidney, and pancreas in this experiment.

Table 2 Activity of cystathionine β -synthase in various regions of the brain in normal mice and rolling mice Nagoya^a

| Region of brain | Control mice | Rolling mice |
|----------------------------|-------------------|-------------------|
| Mesencephalon | 0.021 \pm 0.006 | 0.024 \pm 0.012 |
| Cerebellum | 0.034 \pm 0.011 | 0.046 \pm 0.010 |
| Hippocampus | 0.007 \pm 0.009 | 0.016 \pm 0.012 |
| Thalamus plus hypothalamus | 0.017 \pm 0.010 | 0.014 \pm 0.009 |
| Cerebral cortex | 0.007 \pm 0.009 | 0.022 \pm 0.012 |
| Caudatum | 0.014 \pm 0.009 | 0.025 \pm 0.010 |

a: Each value represents mean \pm SD (μ moles/min/g; n = 18)

In a previous paper (12), we reported that in the rat, cystathionine γ -lyase activity in the liver was almost completely inhibited at 2h after the administration of D, L-propargylglycine, while cystathionine β -synthase activity was not inhibited at all, and that cystathionine accumulated in the liver, kidney, pancreas and various brain regions. Therefore, in this study, we measured the concentrations of cystathionine in various regions of the brain in normal mice and rolling mice Nagoya after the treatment with D, L-propargylglycine, and results are shown in Table 3. There was a relationship between cystathionine β -synthase activities and cystathionine contents in various regions of the brain, except for those in the mesencephalon and thalamus plus hypothalamus, in normal and rolling mice Nagoya which were treated with D, L-propargylglycine. These findings suggest that, in the cerebellum, hippocampus, cerebral cortex and caudatum of the mouse brain, cystathionine β -synthase activity is predominantly involved in the regulation of cystathionine concentration, whereas in the mesencephalon and thalamus plus hypothalamus, both cystathionine β -synthase and cystathionine γ -lyase activities are involved in the regulation of the concentration of cystathionine.

The contents of methionine and cystine were greater in all regions of the brain in rolling mice Nagoya than those in normal mice. The meth-

Table 3 Concentration of cystathionine in various regions of the brain in normal mice and rolling mice Nagoya treated with D, L-propargylglycine^a

| Region of brain | Cystathionine concentration (nmoles/g wet weight) | | | |
|----------------------------|---|-------------|--------------|-------------|
| | Normal mice | | Rolling mice | |
| | (1 mg/10 g) | (2 mg/10 g) | (1 mg/10 g) | (2 mg/10 g) |
| Mesencephalon | 225.4 | 300.5 | 234.5 | 294.5 |
| Cerebellum | 227.6 | 261.6 | 249.5 | 313.9 |
| Hippocampus | 67.5 | 88.3 | 92.2 | 108.0 |
| Thalamus plus hypothalamus | 182.3 | 155.4 | 166.4 | 196.5 |
| Cerebral cortex | 108.7 | 115.4 | 97.6 | 87.4 |
| Caudatum | 137.3 | 81.1 | 94.5 | 131.3 |

a: Each value was obtained using pooled brain regions from 6 normal mice and 6 rolling mice, which were treated with 1 or 2mg of D, L-propargylglycine per 10g of body weight.

ionine content in the cerebellum of rolling mice Nagoya was three times that in the cerebellum of normal mice, while the content of cystine in this area was twice that in normal mice (Table 4). In mammals, taurine is synthesized from methionine via cysteine, cysteine sulfinic acid, and hypotaurine, and it functions as a neurotransmitter (13). The taurine contents in the cerebellum and hippocampus of rolling mice Nagoya were much lower than those in these regions of normal mice (Table 4).

The effects of D, L-propargylglycine administration on the accumulation of cystathionine in

several other tissues of the normal mouse and rolling mouse Nagoya were also investigated. In the liver and pancreas of these mice, the accumulation of cystathionine was maximal at a dose of 2 mg of D, L-propargylglycine per 10 g of body weight, while, in the kidney, it was maximal at a dose of 1 mg/10 g of body weight (Table 5). The differences in cystathionine content in these tissues after the administration of D, L-propargylglycine agreed well with the differences in cystathionine β -synthase activities in these tissues (Table 6). There were no differences in the cystathionine contents and cystathionine

Table 4 Concentrations of methionine and cystine in various regions of the brain in normal mice and rolling mice Nagoya^a

| Region of brain | Concentrations (nmoles/g wet weight) | | | | | |
|----------------------------|--------------------------------------|-------------|------------------------|--------------|-------------|------------------------|
| | Normal mice | | | Rolling mice | | |
| | Methionine | Cystine | Taurine | Methionine | Cystine | Taurine |
| Mesencephalon | 36.5 ± 15.3 | 37.4 ± 7.9 | 3.11 × 10 ³ | 50.6 ± 0.8 | 76.5 ± 16.8 | 3.83 × 10 ³ |
| Cerebellum | 14.9 ± 3.4 | 31.4 ± 11.8 | 5.73 × 10 ³ | 54.1 ± 2.4 | 71.3 ± 2.7 | 2.92 × 10 ³ |
| Hippocampus | 28.1 ± 6.6 | 54.2 ± 4.4 | 7.03 × 10 ³ | 64.3 ± 5.8 | 71.8 ± 26.3 | 4.79 × 10 ³ |
| Thalamus plus hypothalamus | 26.9 ± 19.2 | 38.0 ± 12.7 | 3.46 × 10 ³ | 38.3 ± 16.7 | 73.5 ± 34.5 | 3.73 × 10 ³ |
| Cerebral cortex | 26.9 ± 15.9 | 50.6 ± 14.0 | 4.65 × 10 ³ | 42.5 ± 12.2 | 63.6 ± 26.8 | 5.67 × 10 ³ |
| Caudatum | 29.3 ± 4.9 | 61.6 ± 26.8 | 6.12 × 10 ³ | 34.2 ± 9.2 | 93.4 ± 13.8 | 6.57 × 10 ³ |

^a: Each value of methionine and cystine concentrations represents mean ± SD of 18 mice. Taurine concentration was obtained from pooled brain regions of 6 animals.

Table 5 Concentration of cystathionine in several tissues from normal mice (A) and rolling mice Nagoya (B) treated with D, L-propargylglycine^a

| Tissue | Cystathionine concentration (nmoles/g of fresh tissue) | | | |
|----------|--|---------------------------------|----------------|----------------|
| | Control | D, L-propargylglycine treatment | | |
| | | 0.5 mg/10 g | 1 mg/10 g | 2 mg/10 g |
| (A) | (16) | (3) | (3) | (3) |
| Liver | ND | 736.8 ± 56.1 | 1333.3 ± 86.5 | 3283.9 ± 173.1 |
| Kidney | ND | 396.8 ± 31.5 | 694.6 ± 45.1 | 482.5 ± 37.5 |
| Pancreas | 41.1 ± 8.9 | 1531.7 ± 96.3 | 2991.7 ± 172.1 | 4329.4 ± 291.6 |
| (B) | (6) | (1) | (1) | (1) |
| Liver | ND | 946.0 | 1054.6 | 3379.4 |
| Kidney | ND | 174.2 | 757.3 | 635.6 |
| Pancreas | 81.7 ± 27.0 | 1545.4 | 3168.5 | 5112.5 |

^a: Mice were injected with 0.5, 1.0 or 2.0 mg of D, L-propargylglycine per 10 g of body weight. Each value represents mean ± SD. Members in parentheses show numbers of animals.

Table 6 Activity of cystathionine β -synthase and cystathionine γ -lyase in liver, kidney and pancreas of normal mice and rolling mice Nagoya^a

| Tissue | Normal mice | Rolling mice |
|----------|--|-----------------|
| | Cystathionine β -synthase (μ moles/min/g) | |
| Liver | 1.32 \pm 0.42 | 1.73 \pm 0.40 |
| Kidney | 0.56 \pm 0.14 | 0.51 \pm 0.11 |
| Pancreas | 1.83 \pm 0.38 | 1.94 \pm 0.51 |
| | Cystathionine γ -lyase (μ moles/min/g) | |
| Liver | 1.94 \pm 0.57 | 2.60 \pm 0.46 |
| Kidney | 0.24 \pm 0.05 | 0.32 \pm 0.03 |
| Pancreas | 0.31 \pm 0.07 | 0.37 \pm 0.07 |

a: Each value represents mean \pm SD of 18 animals.

β -synthase and cystathionine γ -lyase activities in the tissues between normal and rolling mice Nagoya. These results suggest that the metabolism of sulfur amino acids in the liver, kidney and pancreas of rolling mouse Nagoya is normal, but that the metabolism of these amino acids in the cerebellum is disturbed. It may also be suggested that rolling mice Nagoya can be used for the study of the physiological role of sulfur amino acids in the brain.

References

- Oda S: The observation of rolling mouse Nagoya, a new neurological mutant and its maintenance. *Exp Anim* (Tokyo) (1973) **22**, 281-288 (in Japanese).
- Oda S: A new allele of the tottering locus, rolling mouse Nagoya, on chromosome no. 8 in the mouse. *Jpn J Genet* (1981) **56**, 295-299.
- Nishimura Y: The cerebellum of rolling mouse Nagoya. *Adv Neurol Sci* (1975) **19**, 670-672 (in Japanese).
- Mukaiyama M and Mizuno K: Studies on the cerebellum of rolling mouse Nagoya by the optical and electron microscope. *N Med* (1976) **31**, 233-238 (in Japanese).
- Ando K and Muramoto O: Neurotransmitters in the brain of rolling mouse Nagoya; in Annual Report of the Ministry of Health and Welfare Spinocerebellar Degenerations Research Committee Japan, Vol 54 (1981) pp 121-129 (in Japanese).
- Nagatsu I, Kondo Y, Inagaki S, Oda S and Nagatsu T: Dopamine- α -hydroxylase and tyrosine hydroxylase activities in brain regions of rolling mouse Nagoya. *Biomed Res* (1980) **1**, 88-90.
- Kashiwamata S and Greenberg DM: Studies on cystathionine β -synthase of rat liver properties the highly purified enzyme. *Biochem Biophys Acta* (1970) **212**, 488-500.
- Matsuo Y and Greenberg DM: A crystalline enzyme that cleaves homoserine and cystathionine. *J Biol Chem* (1985) **230**, 545-559.
- Hope DB: Cystathionine accumulation in the brains of pyridoxine deficient rats. *J Neurochem* (1964) **11**, 327-332.
- Shaw RK and Heine JD: Ninhydrin positive substances present indifferent areas of normal rat brain. *J Neurochem* (1965) **12**, 151-155.
- Kodama H, Ikeda H, Awata S and Nakayama K: Cystathionine accumulation in various regions of brain of D,L-propargylglycine-treated rats. *J Neurochem* (1984) **44**, 1207-1209.
- Kodama H, Mikasa H, Sasaki K, Awata S and Nakayama K: Unusual metabolism of sulfur-containing amino acids in rats treated with D,L-propargylglycine. *Arch Biochem Biophys* (1983) **225**, 25-32.
- Kaczmarek LK: A comparison of the evidence for taurine and GABA as neurotransmitters; in Taurine, Huxtable and Barbeau eds, Raven Press, New York (1976) p 283.

Received February 10, 1992; accepted June 16, 1992.