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

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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

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

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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 cytathionine 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 anino acids and activities of some related

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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 × *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 × *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 ± 0.006	0.024 ± 0.012
Cerebellum	0.034 ± 0.011	0.046 ± 0.010
Hippocampus	0.007 ± 0.009	0.016 ± 0.012
Thalamus plus hypothalamus	0.017 ± 0.010	0.014 ± 0.009
Cerebral cortex	0.007 ± 0.009	0.022 ± 0.012
Caudatum	0.014 ± 0.009	0.025 ± 0.010

a: Each value represents mean ± SD (μmoles/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 2 mg of D, L-propargylglycine per 10 g 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 (μmoles/min/g)	
Liver	1.32 ± 0.42	1.73 ± 0.40
Kidney	0.56 ± 0.14	0.51 ± 0.11
Pancreas	1.83 ± 0.38	1.94 ± 0.51
	Cystathionine γ-lyase (μmoles/min/g)	
Liver	1.94 ± 0.57	2.60 ± 0.46
Kidney	0.24 ± 0.05	0.32 ± 0.03
Pancreas	0.31 ± 0.07	0.37 ± 0.07

a: Each value represents mean ± 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.

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