Alterations in Acetyl Coenzyme A Carboxylase Activities in Voles and Mice Treated with Monosodium Aspartate

Toshiro ARAI, Minoru SASAKI[1)], Mie SHIOMI, Tetsu NONAKA, Kensuke OCHIAI[1)], and Yoshio OKI

Department of Veterinary Biochemistry, Nippon Veterinary and Animal Science University, Musashino, Tokyo 180 and [1)]Development Research Laboratory, Banyu Pharmaceutical Co., Ltd., Menuma, Saitama 360-02, Japan

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ABSTRACT. Changes of body weights and hepatic acetyl-CoA carboxylase activities were measured in voles and mice treated with monosodium-L-aspartate (MSA). MSA was administrated subcutaneously to neonates at 4 mg/g. The MSA-treated mice showed remarkable obesity, associated with the increase in the plasma insulin concentrations and acetyl-CoA carboxylase activities. The activity of acetyl-CoA carboxylase of control voles was very low; under half that of mice. In the MSA-treated voles, although the plasma insulin concentrations also increased, acetyl-CoA carboxylase activities were not elevated and signs of obesity were not observed.—KEY WORDS: acetyl CoA carboxylase, MSA, vole.

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Herbivorous voles, *Microtus arvalis* Pallas, have a pregastric fermentation digestive system to use volatile fatty acids and other organic acids produced in the forestomach as energy. They have been used as a model for herbivora in some experiments [1, 11], and they have different biochemical characteristics in glucose or lipid metabolism from mice [2, 3]. On the other hand, administration of monosodium glutamate (MSG), monosodium aspartate (MSA) or related substances to neonatal rodents has been reported to induce neuronal necrosis in several brain areas including the arcuate hypothalamic nucleus, and to result in a syndrome of obesity [6, 15, 16], that is, massive accumulation of body fat, hyperinsulinemia and increased hepatic lipogenesis. In voles, however, neonatal administration of MSA induced a severe glycosuria without a sign of obesity after 30 weeks of age [19]. Thus, voles showed a quite different response to neonatal administration of MSA from mice. To investigate the metabolic and endocrine basis for the different response to MSA administration, in the present study, we measured the activities of hepatic lipogenic enzyme, acetyl coenzyme A carboxylase, and concentrations of blood glucose and plasma insulin in voles and mice treated with MSA.

MATERIALS AND METHODS

M. arvalis and C57BL/6J mice were maintained in our laboratory. Vole were fed on commercial pellets for herbivora (ZC; Oriental Yeast Co., Tokyo, Japan) and cubed hay. Mice were fed on commercial pellet (CMF; Oriental Yeast Co.). In the experimental groups, at 0 (day of birth) or at 1 day of age, the single administration of monosodium-L-aspartate (Wako Pure Chemical Industries Ltd., Osaka, Japan) was given subcutaneously to the neonates at 4 mg/g of body weight. The untreated animals were used as controls. The body weights and naso-anal length of the animals were measured every week after 4 weeks of age. The animals were killed at 20 weeks of age by decapitation under ether anaesthesia.

Blood samples were collected from the jugular veins into heparinized microfuge tubes and the livers were removed immediately after decapitation. The glucose concentration in whole blood was measured by the glucose-oxidase method [8].

The plasma insulin concentration was measured by the micro ELISA sandwich method described previously [3]. The livers were weighed and homogenized in a glass tube with two volumes of buffer containing 50 mM tris-hydroxymethyl-aminomethane, Tris-HCl, 1 mM ethylene diamine-tetra acetic acid (EDTA) and 5 mM 2-mercaptoethanol (pH 7.4). The homogenate was centrifuged at 100,000 g for 1 hr at 4°C and the resulting supernatant was used for the assay of acetyl Coenzyme A carboxylase (EC 6. 4. 1. 2). Acetyl-CoA carboxylase activity was determined by the $H^{14}CO_3$-fixation assay [14]. An aliquot of the supernatant fluid was first preincubated at 37°C for 30 min in a mixture (final volume 1.0 m*l*) containing 50 mM Tris-HCl, pH 7.5, 10 mM potassium citrate, 10 mM $MgCl_2$, 3.75 mM GSH and 0.75 mg/m*l*

bovine serum albumin. The assay mixture (final volume 0.8 m*l*) contains 50 mM Tris-HCl, pH 7.5, 20 mM potassium citrate, 10 mM $MgCl_2$, 3.75 mM ATP, 0.125 mM acetyl-CoA, 3.75 mM GSH, 0.75 mg/m*l* bovine serum albumin and 12.5 mM $KH^{14}CO_3$ (specific activity 0.24 GBq/mmol, Du Pont Co., DE, U.S.A.). The reaction was started by adding an aliquot of the preincubated sample solution, followed by incubation at 37°C for 10 min, and terminated with 0.2 m*l* of 5 N HCl. After centrifugation, an aliquot of the supernatant fluid was taken to dryness in a scintillation counting vial at 80°C for 45 to 60 min to expel unreacted CO_2. The residue was dissolved in 0.5 m*l* of distilled water and counted by a liquid scintillation spectrometer. The acetyl-CoA carboxylase activity was expressed as n moles of malonyl-CoA formed at 37°C per min per mg protein. The protein concentration was determined by the method of Bradford [5] using bovine serum albumin as the standard. Statistical analysis was performed by Student's *t* tests.

RESULTS

Changes in morphological characteristics of voles and mice are shown in Tables 1 and 2, respectively. In voles, the body weight increased similarly in both sexes of both groups until 20 weeks of age. There was no significant difference in naso-anal length between the MSA-treated and the control groups, so the 'Lee index', which is a measure of obesity [4], was similar in both groups of voles up to 20 weeks of age, and a sign of obesity was not observed. In mice, the body weights increased similarly in both groups until about 4 weeks of age. Thereafter the rate of increase was more marked in the MSA-treated mice than in the controls. The naso-anal length of MSA-treated mice was significantly shorter ($P<0.05$) than that of the controls after 8 weeks of age. The 'Lee index' was significantly higher ($P<0.05$) in the MSA-treated mice than in the controls even at 4 weeks of age, and thereafter the difference between both groups became greater. The MSA-treated mice showed marked obesity. Changes of food intake in voles and mice after 8

Table 1. Morphological characteristics of the MSA-treated and control voles at respective age

Age		Weight (g)	Naso-anal length (cm)	Lee index[a)]
Male				
4 weeks	MSA (6)	16.9±0.7[b)]	8.0±0.2	322±3
	Control (6)	15.6±1.0	7.8±0.1	317±4
8 weeks	MSA	31.2±1.2[c)]	9.7±0.1[c)]	323±2
	Control	27.7±1.0	9.2±0.2	330±5
12 weeks	MSA	32.0±1.9	9.9±0.2	320±5
	Control	30.1±1.1	9.9±0.1	315±4
16 weeks	MSA	34.8±1.9	10.0±0.2	326±3
	Control	31.7±0.9	9.9±0.2	323±7
20 weeks	MSA	38.1±2.7	10.0±0.2	335±3
	Control	33.2±2.3	10.1±0.3	319±6
Female				
4 weeks	MSA (6)	16.7±1.8	8.2±0.2	314±7
	Control (6)	15.5±0.7	7.9±0.1	317±5
8 weeks	MSA	21.4±1.2	9.0±0.1	306±3
	Control	21.7±0.1	8.8±0.1	317±4
12 weeks	MSA	24.1±1.8	9.1±0.1	316±4
	Control	23.3±0.8	9.1±0.3	314±8
16 weeks	MSA	27.7±2.1	9.4±0.3	305±11
	Control	26.2±0.2	9.4±0.2	317±6
20 weeks	MSA	29.8±1.2	9.8±0.2	318±5
	Control	28.4±1.5	9.4±0.2	324±5

a) $\sqrt[3]{\text{body weight/naso-anal length}} \times 1000$
b) Values are expressed as the means±S.E.
c) Significantly high ($P<0.05$) against the controls. The number in parenthesis indicates the number of animals examined.

Table 2. Morphological characteristics of the MSA-treated and control C57BL/6J mice at respective age

Age		Weight (g)	Naso-anal length (cm)	Lee index[a)]
Male				
4 weeks	MSA (6)	16.7±1.7[b)]	7.0±0.2	364±7[c)]
	Control (6)	14.6±0.5	7.1±0.1	343±2
8 weeks	MSA	33.7±1.7[c)]	8.3±0.2[d)]	390±7[c)]
	Control	26.8±0.8	9.1±0.1	327±5
12 weeks	MSA	41.5±1.9[c)]	8.7±0.1[d)]	400±8[c)]
	Control	32.8±0.4	9.4±0.1	339±3
16 weeks	MSA	47.5±2.4[c)]	8.7±0.1[d)]	414±10[c)]
	Control	34.1±0.6	9.5±0.1	342±3
20 weeks	MSA	52.7±2.3[c)]	8.8±0.2[d)]	425±11[c)]
	Control	34.3±0.7	9.8±0.1	333±3
Female				
4 weeks	MSA (6)	15.2±0.8	6.9±0.1	359±7[c)]
	Control (6)	13.6±0.5	7.0±0.1	340±3
8 weeks	MSA	28.1±0.9[c)]	8.0±0.1[d)]	378±6[c)]
	Control	20.9±0.5	8.3±0.1	331±2
12 weeks	MSA	34.4±1.5[c)]	8.3±0.1[d)]	394±16[c)]
	Control	27.8±1.5	8.9±0.2	339±4
16 weeks	MSA	38.5±1.1[c)]	8.4±0.1[d)]	403±5[c)]
	Control	30.7±1.4	9.3±0.1	335±4
20 weeks	MSA	42.9±1.5[c)]	8.5±0.1[d)]	414±6[c)]
	Control	33.1±0.7	9.6±0.1	334±4

a) $\sqrt[3]{\text{body weight/naso-anal length}} \times 1000$
b) Values are expressed as the means±S.E.
c) Significantly high ($P<0.05$) against the controls.
d) Significantly low ($P<0.05$) against the controls.
The number in parenthesis indicates the number of animals examined.

Table 3. Comparison of food intake between control and the MSA-treated voles or C57BL/6J mice at respective age

Age		Voles				C57BL/6J mice	
		Control		MSA-treated		Control	MSA-treated
		ZC	Ch	ZC	Ch		
8 weeks	M	2.6±0.6	0.6±0.2	2.9±0.5	0.8±0.4	2.3±0.6	2.6±0.7
	F	2.2±0.5	0.5±0.3	2.3±0.3	0.7±0.3	2.1±0.4	2.2±0.5
12 weeks	M	3.0±0.7	0.8±0.3	3.1±0.6	0.9±0.3	2.7±0.7	2.7±0.6
	F	2.4±0.5	0.7±0.3	2.5±0.5	0.8±0.4	2.2±0.6	2.4±0.7
16 weeks	M	3.3±0.9	1.1±0.5	3.4±0.6	1.1±0.3	2.9±0.9	2.9±0.8
	F	2.7±0.6	0.9±0.4	2.7±0.7	1.0±0.4	2.6±0.6	2.8±0.7
20 weeks	M	3.4±0.8	1.0±0.4	3.7±0.9	1.2±0.4	3.0±0.8	3.2±0.9
	F	2.8±0.6	1.0±0.3	2.9±0.8	1.1±0.3	2.8±0.7	3.0±0.9

Values (means±S.E.) are expressed as amount of food (g) taken by each animal in a day.
ZC: pellets for herbivore, Ch: cubed hay
M: male (n=6), F: female (n=6)

weeks of age are shown in Table 3. No remarkable difference of food intake was observed between the MSA-treated and the control groups of both in voles and mice.

The concentrations of blood glucose and plasma insulin and the hepatic acetyl-CoA carboxylase activities in voles and mice are shown in Table 4. There was no difference between the sexes in these

Table 4. The mean (±S.E.) blood glucose and plasma insulin concentrations and hepatic acetyl-CoA carboxylase activities in 20-week-old voles and C57BL/6J mice

		Voles		C57BL/6J mice	
		Control	MSA-treated	Control	MSA-treated
Blood glucose (mg/d*l*)	M	70.2±5.5	93.4±7.2[a)]	131.0±7.0	144.8±17.0
	F	68.4±4.0	91.6±6.0[a)]	127.0±6.2	147.6±20.0
Plasma insulin (μU/m*l*)	M	20.9±3.0	38.2±4.4[a)]	21.0±2.5	39.8±5.4[a)]
	F	23.9±3.4	39.0±6.2[a)]	20.8±3.0	40.6±6.0[a)]
Acetyl-CoA carboxylase (nmoles/min/mg)	M	0.8±0.2	0.9±0.3	1.7±0.5	3.3±0.4[a)]
	F	0.8±0.2	1.1±0.4	1.9±0.4	3.7±0.6[a)]

a) Significantly high ($P<0.01$) against the controls.
M: male (n=6), F: female (n=6).

concentrations or activities. The mean blood glucose concentration (69.3 mg/d*l*) and acetyl-CoA carboxylase activities (0.8 nmoles/min/mg) of the control voles were very low compared to those of control mice. In the MSA-treated voles, the blood glucose and plasma insulin concentrations increased remarkably, but acetyl-CoA carboxylase activities were not elevated. The MSA-treated mice showed significantly higher concentrations of insulin and acetyl-CoA carboxylase activities than the controls. However, the blood glucose concentration was not significantly changed in the MSA-treated mice.

DISCUSSION

Neonatal administration of acidic amino acids, such as MSG or MSA, to rodents has been reported to cause lesions in the arcuate nuclei of the hypothalamus and to result in a syndrome of obesity without hyperphagia [6, 13, 15]. In the present study, hyperphagia was not observed in the MSA-treated animals. This result may suggest that neonatal administration of MSA induces ventromedial hypothalamic (VMH) injury but does not destroy a 'satiety center' in voles and mice. Lesions in the ventromedial nuclei of rodents induced a hormonal disorder including elevation of insulin [6] and growth hormone deficiency [7, 13], which results in impaired lipolysis. The hypothesis that neural mediation of the rise in insulin is the primary factor in the development of hypothalamic obesity has been supported [9, 10]. Activated secretion of insulin may cause development of obesity through the induction of hepatic enzymes involved in glycolysis, pentose phosphate pathway and lipogenesis, which may result in active lipogenesis from glucose [21].

In the MSA-treated mice, a remarkable elevation of plasma insulin concentration and obesity were observed. It is considered that the increase of 'Lee index' is due to the significant decrease of naso-anal length which may indicate growth hormone deficiency. Moreover, activities of the hepatic lipogenic enzyme, acetyl-CoA carboxylase, increased to two times higher than those of the control mice. The increase of acetyl-CoA carboxylase activities are considered to be one of the important factors to cause obesity in mice.

On the other hand, the voles have the characteristics as a herbivore that the blood glucose concentration, renal threshold for glucose and hepatic glycolytic ability are considerably lower than those of mice [2, 3]. In the MSA-treated voles, neuronal necrosis in several brain areas including the arcuate nucleus and VMH, was also observed [19].

However, in the MSA-treated voles, although the plasma insulin concentration increased as observed in the MSA-treated mice, the acetyl-CoA carboxylase activities were not elevated and a sign of obesity was not observed. It has been reported that hepatic lipogenic enzyme activities in ruminant are significantly lower than those in monogastric animals [17, 18]. It is considered that the low activity of hepatic acetyl-CoA carboxylase is one of characteristics of voles as a herbivore and that the enzyme shows almost full activity at normal insulin concentration without the response to the increased insulin concentration. There was no difference in the naso-anal length between the MSA-treated and control voles, which indicates that growth hormone secretion is not changed in the MSA-treated voles. Moreover, in the MSA-treated voles, glucagon secretion in-

creased remarkably, the hepatic fructose-1,6-bisphosphatase activity was not suppressed and hyperglycemia was observed in the hyperinsulinemic condition [20]. It was pronounced that the activities of the hepatic glycolytic and lipogenic enzymes in voles were considerably lower than those in mice. Moreover, an increase in glucagon secretion and no decrease in growth hormone secretion were preferably observed in the MSA-treated voles. Hyperglycemia observed at 20 weeks of age was maintained and seemed to be heavy stress to voles with low glycolytic ability. The continuous hyperglycemia changed to severely diabetic condition in the MSA-treated voles after 30 weeks of age [20].

Hyperphagia is considered to be one of important factors to cause obesity, and remarkable hyperphagia has been reported in obese mice treated with goldthioglucose [12]. Such hyperphagia was not observed in the MSA-treated voles and mice in the present study. Obesity in the MSA-treated mice or hyperglycemia in the MSA-treated voles was considered to be due to hormonal disorder as hyperinsulinemia induced by VMH injury rather than hyperphagia.

A great difference in response to MSA administration between voles and mice seemed to be associated with the difference in hepatic glycolytic and lipogenic enzymes activities. To further clarify such different response to MSA administration between voles and mice, the secretory changes of hormones and the alterations in the lipolytic enzyme activity should be studied.

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