Determination of Ascorbic Acid in ODS Rat Brain and Plasma

III. 13 Determination of Ascorbic Acid in ODS Rat Brain and Plasma

Abe K., Ido T., Kimura S.* and Ishiwata K.
Cyclotron and Radioisotope Center, Tohoku University
Laboratory of Nutrition, Faculty of Agriculture, Tohoku University*

Introduction

Ascorbic acid is synthesized exclusively in the liver of mammals except primates, guinea-pig and a few other animals. Ascorbic acid is the putative reductant for the enzyme dopamine β-hydroxylase which catalyzes the conversion of dopamine to norepinephrine. The enzyme is localized within the catecholamine storage vesicle both in adrenergic neurons and in adrenomedullary chromaffin cell.[1] Nonetheless, the role of ascorbic acid in adrenergic neurons has not been established. Recently, it has been reported that an osteogenic-disorder strain rat (ODS rat) has a hereditary defect in L-ascorbic acid synthesizing ability.[2] Lack of the ascorbic acid should cause imbalance of some brain functions.

In this paper, to confirm the significance of the ODS rat as an experimental animal model of allometabolic brain, we determined the contents of ascorbic acid in the brain and plasma of the ODS rats under dietary control.

Materials and Methods

ODS (OD/OD) rats, 4 weeks old and weighing 50-60 g were obtained from Shionogi, Co., Ltd., and were breeded with non-ascorbate-added diet (purified diet, combination A., Oriental Yeast Co., Ltd.), which contained 3 mg ascorbate per 100 g diet.

At the fixed time after giving non-ascorbate-added diet, rats were administrated 0.1 ml of heparin calcium, and 30 min after administration, whole blood was collected from the abdominal aorta in the tube containing 10 mg EDTA-2Na, and immediately the tube was centrifuged at 1000 × g for 5 min at 2 °C to obtain plasma. Then 500 µl of the plasma was transferred to a tube containing 30 µl of perchloric acid (60 %), agitated using a vortex mixer, and centrifuged. The supernatant fluid was passed through the membrane filter (0.45 µm) and the filtrate was applied to HPLC analysis.

The brain was removed, frozen on dry ice, and stored at -80°C until assay. Extraction of ascorbic acid from the brain was carried out according to the method of Thrivikraman et al.[3] The brain was homogenized in 10-times volume of in ice-cold 50 mM perchoric acid containing 2 mM thiourea and 1 mM EDTA-2Na with the use of a Potter Elvejhem conical glass tube and motor-driven Teflon pestel (4 strokes). The homogenate was centrifuged at 18000 × g for 20 min at 2 °C. The supernatant fluid was collected. The pellet was resuspended

in the same amount of extracting medium and centrifuged as described above. The same procedure was repeated one more time. All supernatant fluid was gathered.

The concentration of ascorbic acid in the brain extract and plasma was determined by HPLC coupled to an electrochemical detector.[4] Pulse dampering was accomplished with tubing between the pump and preclumn (Pre-column Gel, 37-53 Microns, Whatman). Ten µl aliquots of the supernatant fluid were applied to a Waters Radial-PAK C18 cartridge (10 cm × 8 mm I. D.). The mobile phase consisted of 0.1 M soldium acetate, 0.02 % EDTA-2Na (w/v), and 1 mM N-octylamine (pH 5.0). The flow rate was 1.0 ml/min. Ascorbic acid was oxidized at +0.60 V with reference to a Ag^{+}/AgCl electrode.

Results and Discussion

Table 1 shows the time changes of growth (body weight gain) of ODS rats and ascorbic acid content in brain and plasma. At the time of 6 weeks of age, the growth stopped, and the symptoms of scurvy was observed. After that time, the body weight was decreased gradually, and hematomas around the scapulae, femurs and humerus were also observed. In case of femal rats, the significant decrease was observed as in male rats (Table 1). The content of ascorbic acid in brain was decreased slowly, but the decrease in the ascorbic acid content of plasma was rapid. This suggests that the possibility of utilizing the level of ascorbic acid in plasma as an index of scurvy is low. It is considered that a considerable amount of ascorbic acid was maintained in brain, because of its significant role like a regulator of dopamine β-hydroxylase activity in the bovine chromaffin cell.[1]

Four weeks after the beginning of breeding, some of ODS rats went to die. Under our experiment conditions, ODS rats at 8 weeks of age were compelled to be terminal scurvy. In order to compare the level of ascorbic acid in ODS(OD/OD) rats 8 weeks age, the contents in Wistar rats were also measured (Table 2) as a control. At this stage, the level of ascorbic acid in ODS rat brain was 51.5 µg/g whole brain. The level in plasma was 0.11 µg/ml plasma. These values were 1/6, and 1/50, compared with those of Wistar rats.

In conclusion, the level of ascorbic acid in ODS rats brain had been remarkably decreased in comparison with Wistar rats. And it is possible to regulate the content of ascorbic acid in the ODS rats brain with a diet control. The ODS rat could be useful for studies of the brain with allometabolism.

References

1) Frank S., Menniti Jane., Knoth Emanauel J., Diliberto J., J. Biol. Chem. 261 (1986) 16901.

2) Mizushima Y., Harauchi T., Yoshizaki T., Makino S., Experimenta. 40 (1984) 359.

3) Thrivikraman K. V., Refshauge C., Adams R. N., Life Science. 15 (1974) 1335.
4) Baker J. K., Kapeghian J., Verlangieri A., J. Liquid Chromatography 6 (1983) 1319.

Table 1. Time change of ascorbic acid content in ODS rats brain and plasma. On the first day (24 h after obtainment), rats were sacrified for the determination of ascorbic acid in rats brain and plasma. This time, we defined as Number 0. Number refers to the week number after giving the non-ascorbate-added diet. Age were expressed as the weeks of age. The values are the mean ±S.E.M.3 rats were used in each group.

ODS(OD/OD)rats	Number	Age (week)	Weight (g)	Ascorbic acid content Whole brain (μg/g)	Plasma (μg/ml)
male	0	4	54.8 ±4.1	389.9 ±10.7	3.741 ±0.344
	1	5	75.7 ±1.4	211.9 ± 6.0	0.296 ±0.021
	2	6	90.5 ±1.5	137.4 ± 7.7	0.165 ±0.006
	3	7	87.2 ±5.0	73.9 ± 3.0	0.119 ±0.013
	4	8	67.0 ±3.8	51.5 ± 1.6	0.112 ±0.015
female	0	4	68.3 ±2.0	365.7 ±15.7	1.312 ±0.056
	1	5	76.3 ±2.0	225.4 ± 0.7	0.167 ±0.006
	2	6	91.3 ±2.4	123.3 ± 4.1	0.147 ±0.017
	3	7	80.7 ±3.3	82.9 ± 1.7	0.117 ±0.003
	4	8	65.7 ±2.7	61.9 ± 3.0	0.107 ±0.004

Table 2. Comparison of ascorbic acid content in brain and plasma of ODS(OD/OD), and Wistar rats.

male rats	Number	Age (week)	Weight (g)	Ascorbic acid content Whole brain (μg/g)	Plasma (μg/g)
ODS(OD/OD)	4	8	67.0 ± 3.8	51.5 ± 1.6	0.112± 0.015
Wistar	4	8	204.8 ± 1.1	309.0 ± 5.0	5.523± 0.786