EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Peculiarities of Carnosine Metabolism in a Patient with Pronounced Homocarnosinemia

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The article describes a case of homocarnosinemia with increased liquor and plasma content of homocarnosine, increased urinary excretion of homocarnosine, and low activity of serum carnosinase. These metabolic disturbances were accompanied by moderate neurological disorders. Changes in carnosine metabolism in family members were less pronounced and not accompanied by neuropathological symptoms.

Key Words: *carnosine; homocarnosine; homocarnosinemia; homocarnosinosis, serum carnosinase*

Tissue level of carnosine (β-alanine-L-histidine) and its derivatives depends on the proportion and distribution of specific ligase (carnosine synthase EC 6.3.2.11) and tissue (TC) and serum (SC) carnosinases (EC 3.4.13.3 and EC 3.4.13.20, respectively), the key enzymes of carnosine metabolism. The concentrations of carnosine and related compounds (homocarnosine, anserine, *etc.*) are high in excitable tissues (brain, myocardium, and skeletal muscles), where TC is not expressed, and low in the kidney, liver, and plasma containing carnosinase, but lacking carnosine synthase [1,6,7]. SC present in the brain and plasma hydrolyzes not only carnosine and anserine, but also homocarnosine (γ-aminobutyryl-histidine), a brain-specific dipeptide.

Biological functions of carnosine remain unknown, but its role in cell protection from oxidative damage is beyond doubt [1]. Disturbances in carnosine metabolism are often accompanied by pathology of the nervous and muscle systems [1,3]. Case histories of patients with stable increased plasma and urinary contents of carnosine and typical neurological symptoms were reported [3-5,8,11,12]. These metabolic abnormalities were associated with reduced SC activity [4, 12,13] and high homocarnosine concentrations in the liquor and urine [8,10,11]. This phenomenon was not studied in detail, probably due to low incidence of this disease (only 16 cases of SC deficiency were reported before 1989) [9].

Here we describe a case history of homocarnosinemia in an adolescent; plasma and urinary contents of carnosine and its derivatives were measured in the patient and members of his family and the data were compared with neurological symptoms. This is the first case of homocarnosinemia described in Russian literature.

MATERIALS AND METHODS

Neurological symptoms were analyzed using standard neurological test for diagnosis of CNS and peripheral nervous disorders and evaluation of intellectual and mnestic functions. Electroencephalography, electroneuromyography, magnetic resonance imaging of the brain and spinal cord, echocardiography, and ultrasound examination of internal organs were also used.

Blood taken from the ulnar vein was stabilized with heparin (25 U/100 ml blood) and centrifuged at

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7000*g* for 1 min. Plasma proteins were precipitated with methanol (1:4). The presence of amino acids and dipeptides in biological fluids was detected by the reaction with o-phthalaldehyde [10]. The samples were frozen and stored for 14 days before the analysis.

The concentrations of amino acids and dipeptides in the serum, plasma, and urine were determined by reverse phase HPLC with fluorescence detection [10]. Samples were fractionated on an Ultrasphere-C18 column (Beckman) under isovolumic elution regimen (1 ml/min elution rate) using a mixture of 0.1 M acetate buffer pH 5.8 and methanol (70:30) as the mobile phase. The same procedure was used for carnosine assay in the liquor.

SC activity was determined as described previously [10] using carnosine, homocarnosine, or anserine as the substrates. SC activity was measured by the rate of accumulation of histidine (substrates carnosine or homocarnosine) or N^1-methylhistidine (substrate anserine). The concentrations of reaction products were measured by HPLC using o-phthalaldehyde as the fluorescent probe.

Carnosine load test was used for evaluation of carnosine excretion. After a 3-day meat-free diet, the patient and his mother, who showed neither neurological symptoms, nor metabolic disturbances, received carnosine (water solution, 68 mmol/kg) and accumulation of carnosine in the blood and its urinary excretion were studied for 24 h. L-carnosine (99.2% purity) for this test and for evaluation of enzyme activity was purchased from Samson medical plant. Homocarnosine and anserine were purchased from Sigma.

RESULTS

A 15-year-old patient with carnosinemia was examined at the Institute of Neurology (Russian Academy of Medical Science) in March 2000. The boy was born in term from the third pregnancy without asphyxia. At the age of 6 months he showed retardation both in mental and motor activities: he began to sit with support by 8-9 months, to stand by 1 year, and to walk with support (unsteadily) by 1.5 years. At the age of 1 year he manifested general myasthenia. Clinical diagnoses were "myopathy", "floppy child", and "autonomic dysfunction with unclear metabolic disturbances". Skin desquamation and itching were observed since the age of 4 months. The patient was observed by a dermatologist and treated without positive effects. In 1988 disseminated neurodermatitis was diagnosed.

Disturbances in carnosine metabolism were revealed during examination (at the age of 3 years) at the Institute of Molecular Genetics (Academy of Sciences). The patient exhibited motor disturbances associated with decreased tone of skeletal muscles and mental and speech retardation. Homocarnosinemia was diagnosed on the basis of urine test revealing the presence of dipeptides homocarnosine and anserine, and anserine metabolite N^1-methylhistidine. SC activity was not measured and SC deficiency was suspected on the basis of high urinary concentration of carnosine and its metabolites. Since the age of 6 years the patient followed a special protein-free diet containing all necessary amino acids except histidine. The diet slightly improved patient's general state and alleviated myasthenia, but new symptoms such as difficulties in walking and moderate hand tremor appeared. After the patient quit the diet at the age of 9-10 years, myasthenia increased again and his growth accelerated. In 1994 the patient was examined at the Institute of Pediatrics. The diagnoses were ataxia, myopathy, and neuropathy (?). Intellectual development of the patient was also retarded (he received home education according to auxiliary school program).

In 2000 the patient was examined at the Institute of Neurology (Russian Academy of Medical Sciences). The examination revealed asthenic constitution with skeletal deformations: narrow shoulders, winged

TABLE 1. Serum Carnosinase Activity in Patient and Members of His Family (nmol/ml serum/h)

Examinee	Carnosine		Anserine		Homocarnosine	
	abs.	% of control	abs.	% of control	abs.	% of control
Patient	405±35	21	200±4	24	0	0
Father	890±26	47	421±11	50	5.1±2	28
Mother	3473±22	179	632±20	74	12.2±0.7	67
Brother	421±13	22	173±5	20	6.3±0.4	35
Sister	706±19	37	409±12	48	6.7±0.3	37
Brother	910±21	48	737±28	87	20.8±1.3	115
Control *M*±*SD* (*n*=7)	1000-3500		660-1130		14-2.1	
	1900±81		850±31		18±4.5	

TABLE 2. Carnosine Metabolites in the Blood of Patient and His Relatives after Common Diet

Examinee	N^1-methylhistidine	Histidine	Carnosine	Homocarnosine
Patient	++	++	++	+
Father	+	+	-	+
Mother	+	+	-	-
Brother	+	+	+	-
Sister	+	+	+	-
Brother	+	+	-	-

Note. (++) high concentration; (+) detectable concentration; (-) not detected.

scapuli, keeled chest, high height (190 cm). Neurological examination revealed diffuse hypotrophy, reduced strength in extremities, moderate ataxia, and arrhythmic tremor of extremities. Psychological tests revealed moderate disturbances of intellectual and mnestic activities and impaired verbal and auditory memory and attention. Otoneurological examination revealed involvement of the central vestibular structures. The analysis of evoked potentials (P300) showed disturbances of cognitive processes and impairment of short-term memory. Electroencephalography showed moderate diffuse changes in brain bioelectric activity: disorganization of middle-amplitude rhythms. Magnetic resonance imaging demonstrated the absence of focal changes in the brain and highly heterogeneous signal intensity in bodies of the lumbar and sacral vertebrae. Electroneuromyography excluded involvement of peripheral neuromotor structures. The ophtalmological examination found no pathological changes, *e.g.* retina pigmentation. Echocardiography showed the first degree prolapse of the mitral valve. Ultrasound examination of internal organs revealed signs of pancreas inflammation. Examined relatives (father, mother, two brothers and sister) were clinically healthy.

Plasma SC activity in the patient (carnosine and anserine substrates) was 5 times below the normal. When homocarnosine was used as the substrate, no enzyme activity was detected (Table 1). Carnosinase deficiency was found in all family members (except mother). In 19 year-old brother, SC activity with respect to carnosine and anserine were reduced to the same extent as in the patient, but homocarnosine was hydrolyzed, although more slowly than in the norm; his plasma contained carnosine, although in a lower concentration than in the patient. At the same time, plasma of other brother contained no carnosine (Table 2).By peculiarities of carnosine metabolism patient's sister was closer to the younger, rather than to the older brother (Tables 1 and 2). They were not so far from the norm in these parameters. The absence of clinical symptoms in patient's relatives can be explained by mild metabolic disturbances.

TABLE 3. Concentrations of Amino Acids and Dipeptides in Urine (mg/ml) after 3-Day Meat-Free Diet

Index	Normal	Patient	Mother
Carnosine+arginine	1-10	27.1	9.7
N^1-methylhistidine	2-40	161.8	75.7
β-alanine+taurine	0…60+2…5	151.7	29.9

In additions to SC deficit, patient's plasma contained homocarnosine and increased concentration of carnosine, histidine and N^1-methylhistidine (Table 2). Homocarnosine in high concentration (12.3 nmol/liter *vs.*<5 nmol/liter in the normal [5]) was also found in patient's liquor. These findings suggest that SC deficiency is not the only the molecular and genetic mechanism of this pathology.

Patient's mother had normal SC activity and no carnosinemia. Therefore, carnosine excretion in the patient can be evaluated by comparing the corresponding parameters with those in his mother. The urinary contents of carnosine, N^1-methylhistidine, and the total content of taurine and β-alanine (they were not separated under present conditions) in the patient were considerably higher, than in his mother (Table 3).

After single carnosine load, its accumulation in the patient's serum and urine was much more intensive and carnosine clearance was one order of magnitude higher than in his mother (Fig. 1). Normally, carnosine clearance varies from 2 to 10 ml/min [13]. Accumulation in blood and increased excretion of carnosine are characteristic of SC deficiency and homocarnosinosis [6,13].

The diagnosis of homocarnosinosis was made on the basis of these findings and the presence of homocarnosine in patient's plasma and liquor, although other typical signs of homocarnosinosis (pigmented retina or homocarnosinuria), which are usually applied for differential diagnosis of this disease and SC deficiency, were not observed.

Carnosinase deficiency is a rare autosomal-recessive disease with large phenotypic polymorphism (from

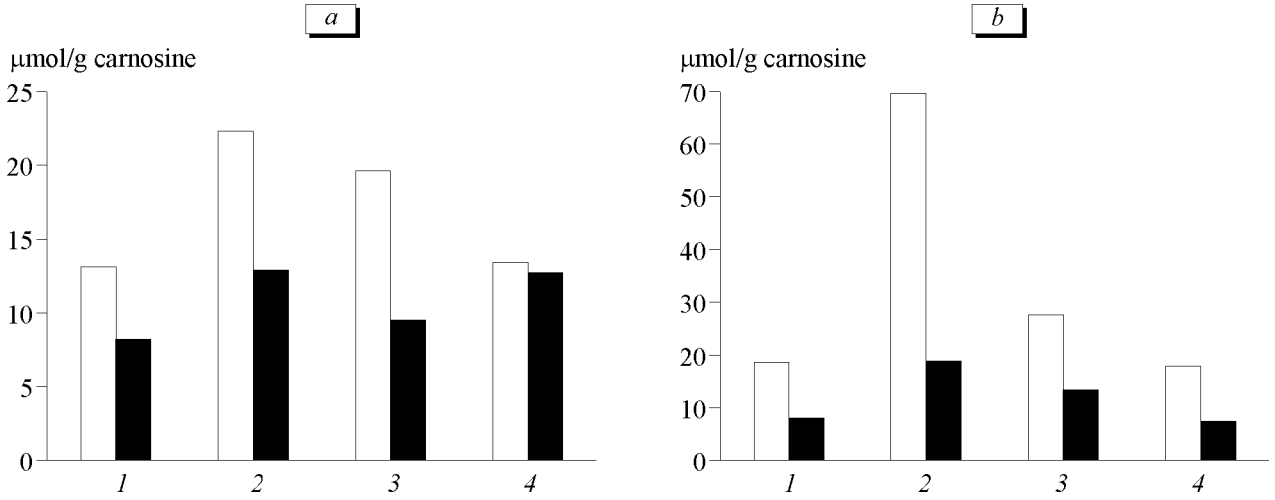

Fig. 1. Carnosine concentration in the serum (*a*) and urine (*b*) of the patient (open bars) and his mother (filled bars) after carnosine load (68 mmol/kg). *1*) before load; *2*) 0-2 h after load; *3*) 2-7 h; *4*) 7-24 h

severe neurological and intellectual disorders to clinically negative cases) [4,8]. It can be assumed that in this type of inheritance both parents can be heterozygous carriers with partially reduced enzyme activity, but without other metabolic disturbances or clinical manifestations of the disease. This situation explains why all researchers have noted the absence of a clear correlation between the severity of neurological symptoms and SC activity in patients. However, SC deficiency in children is accompanied by mental retardation in 82% cases, which confirms interrelations between these parameters [2]. Examination of a patient with SC deficiency revealed deletion in the q21 region of the long arm of chromosome 18 [15]. We hope, that further achievements of molecular genetics will help to identify some other factors responsible for disturbances in carnosine metabolic and leading to the appearance of homocarnosine in the liquor and plasma and neurological symptoms typical of homocarnosinosis.

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REFERENCES

1. A. A. Boldyrev, *Carnosine* [in Russian], Moscow (1998), 119-231.
2. M. Cohen, P.L. Hartlage, A. G. Krawiecki, *et al., J. Ment. Def. Res.,* **23,** 383-389 (1985).
3. P. Duane and T. J. Peters, *Clin. Sci.,* **75**, 185-190 (1988).
4. L. R. Gjessing, H. A. Lunde, L. Morkrid, *et al., J. Neural Transmission*, **29**, Suppl., 91-106 (1990).
5. L. R. Gjessing and O. Sjaastad, *Lancet,* **26,** 1028 (1974).
6. S. J. Kish, T. L. Perry, and S. Hansen, *J. Neurochem.,* **32,** 1629-1636 (1979).
7. J. F. Lenney, *Biochem. Biophys. Acta*, **429,** 214-219 (1976).
8. J. F. J. Lenney, *Oslo City Hosp.,* **35,** 27-40 (1985).
9. J. F. Lenney, S. C. Peppers, and C. M. Kucera, *Biochem. J.,* **228,** 653-660 (1985).
10. J. F. Lenney, S. C. Peppers, C. M. Kucera, *et al., Clin. Chim. Acta,* **132,** 157-165 (1983).
11. H. Lunde, O. Sjaastad, and L. R. Gjessing, *J. Neurochem.,* **38,** 242-245 (1982).
12. W. H. Murfey, D. G. Lindmark, L. I. Patchen, *et al., Pediatr. Res.,* **7,** 601-606 (1973).
13. O. Sjaastad, J. Berstad, P. Gjesdahl, and L. Gjessing, *Acta Neurol. Scand,* **53,** 275-290 (1976).
14. C. R. Scriver and T. L. Perry, *The Metabolic Basis of Inherited Disease,* Eds. C. R. Scriver *et al*., New York (1989), 755-771.
15. S. M. Will, Y. Zhang, J. B. Hill, *et al., Pediatr. Res.,* **41,** No. 2210-2213 (1997).