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The Mechanism of Hyperammonaemia and Hyperornithinaemia in the Syndrome of Hyperornithinaemia, Hyperammonaemia with Homocitrullinuria

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Hyperornithinaemia associated with hyperammonaemia and homocitrullinuria, which is a well-defined biochemical entity, was first reported by Shih *et al.* (1969) and additional cases have been described by several authors (Wright and Pollitt, 1973; Gatfield *et al.*, 1975). Patients with this syndrome exhibit mental retardation, seizures and episodic attacks of ataxia and lethargy. The basic metabolic defect has been suggested to be defective transport of ornithine by hepatic mitochondria (Shih *et al.*, 1982). Recently, Hommes *et al.* (1982) demonstrated that the cause of hyperornithinaemia is a transport defect of ornithine across the inner mitochondrial membrane.

We examined ornithine metabolism in cultured skin fibroblasts from a patient using a double-label assay. A liver sample was obtained from this patient by open biopsy and the free amino acid contents in the whole tissues and of hepatic mitcochondria were measured.

CASE REPORT

The patient, a 46-year-old man, was born in 1936 after an uncomplicated pregnancy and delivery. The parents were unrelated and had eight children. Of these, two died of unknown aetiology at the age of 3 years. The patient's neonatal period was uneventful and we have few details of his childhood history. So far as we have been able to ascertain, however, he has been slightly mentally retarded since childhood. At the age of 39 years, he suddenly began to suffer from severe headaches and unconsciousness, which continued for a month. He was admitted to our hospital in October 1978 because of hyperammonaemia and hyperornithinaemia.

Routine peripheral blood analyses and urine analyses were within normal limits as were the results of liver function tests. Blood ammonia levels were in the range of 150–370 μg/100 ml (normal 30–80). Ophthalmologic examinations showed no abnormality. Computer tomography of the brain showed cortical atrophy. Histologically, liver tissues of the patient were nearly normal and electron microscopically there were no abnormal findings in hepatic mitochondria.

MATERIALS AND METHODS

Quantitative analyses of amino acids in serum, urine and liver tissues were performed by means of an automatic amino acid analyser (Jeol Co. 6AH type). Ornithine loading tests were performed by oral adminstration of 100 mg L-ornithine HCl/kg body weight after overnight fasting. Urea cycle enzymes in an open biopsy of the liver from the patient were principally assayed by the method of Brown and Cohen. Ornithine metabolism in cultured skin fibroblasts was estimated by the double-label assay (L-[^{14}C(U)]ornithine, L-[3,4,5-^{3}H(N)]leucine) method of Shih *et al.* (1982)

RESULTS AND DISCUSSION

The patient's serum levels of ornithine were in the range of 580–700 μmol/l (normal, 50–82), whereas the levels of other amino acids remained within the normal range. The patient had ornithinuria and massive homocitrullinuria. Oral loading tests of L-ornithine indicated that the serum levels of ornithine were strikingly high during both fasting and loading and these did not decrease to the basal line after 6 h. On the other hand, the blood ammonia levels of the patient during the ornithine loading test clearly decreased from 165 μg/100 ml (before loading) to 87 (4 h after the load), which was confirmed repeatedly. The enzyme activities of the urea cycle in liver tissues of the patient were all within normal limits. The concentrations of amino acids in whole liver homogenates and hepatic mitochondria are shown in Table 1. Concentrations of ornithine in hepatic mitochondria of the patient were apparently low, whereas concentrations of ornithine in whole liver tissues were significantly elevated. The results of ornithine metabolism using a double-label assay are shown in Table 2. Incorporation of the ^{14}C-label of ornithine into protein in cultured skin fibroblasts of the patient was significantly lower than in controls.

Ornithine is one of the important substrates of the urea cycle and it converts to citrulline in the presence of ornithine transcarbamylase. The biochemical findings observed in our patient were as follows; (1) serum

Table 1 Molar ratio of free amino acids in whole liver homogenates and hepatic mitochondria

	Whole liver homogenates		*Hepatic mitochondria*	
	Patient	*Control*	*Patient*	*Control*
Orn/His	5.35	1.54	2.20	3.11
Orn/Lys	3.42	0.53	0.66	1.02
Orn/Met	11.36	4.18	1.41	6.01
Orn/Leu	4.25	0.86	0.39	1.09
Orn/Phe	9.20	2.36	0.99	2.70
Orn/Ala	0.64	0.17	0.44	1.15
Orn/Ser	2.53	1.16	0.78	2.23
Orn/Gly	1.51	0.11	0.98	0.81
Orn/Cys	15.98	0.76		
Orn/Ileu	7.62	2.36		
Orn/Tyr	9.00	1.79		
Orn/Pro	5.23	0.64		
Orn/Val	15.40	1.16		
Orn/Asp	1.37	2.09		
Orn/Thr	3.71	1.03		
Orn/Glu	0.61	1.32		
Orn/Gln	0.78	0.38		

Table 2 Incorporations of ^{14}C-label of ornithine and ^{3}H-label of leucine into protein in cultured skin fibroblasts

	$^{14}C/^{3}H$ $(cpm\,h^{-1}$ $(10^5\ cells)^{-1})$	*Ratio*
Patient	109/1438	0.08
Control 1	204/637	0.32
2	382/969	0.39

concentrations of ornithine were always elevated, (2) the patient had hyperammonaemia which decreased with oral administrations of ornithine, (3) the enzyme activities of the urea cycle in liver tissues were within normal limits, (4) incorporation of ^{14}C-label of ornithine into protein in cultured skin fibroblasts was decreased, (5) concentrations of ornithine in hepatic mitochondria were significantly low, whereas concentrations of ornithine in whole-liver homogenates were strikingly high.

From these experimental data, the possible mechanism of hyperammonaemia and hyperornithinaemia might be considered to be defective transport of ornithine in hepatic mitochondria.

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