ENZYMATIC ANALYSIS OF CITRULLINEMIA (12 CASES) IN JAPAN


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INTRODUCTION

Citrullinemia, first described by McMurray et al.¹, is considered a rare hereditary disorder of the urea cycle caused by a deficient activity of argininosuccinate synthetase (ASS). Shih² reviewed 12 cases in 1975. In Japan, however, more than 40 cases of citrullinemia have been reported. Most of them were characterized by higher age of onset and moderately high level of serum citrulline³ in contrast to neonatal onset and extremely high concentration of serum citrulline of classical-or neonatal-type citrullinemia described by McMurray et al. and others. These findings suggest that there may be some heterogeneities in citrullinemia. So we analyzed the properties of ASS in the liver and other organs of 12 cases of citrullinemia in Japan.

MATERIALS AND METHODS

Patients

The patients included in this report are twelve adults (18-48 years old) whose surgical or autopsy specimens of the liver and other organs were sent to our laboratory. All the patients suffered from disturbance of consciousness such as disorientation, restlessness and sleeplessness³. They were diagnosed as citrullinemia on the basis of laboratory findings of high serum citrulline (more than 100 nmol/ml as compared with control values of 20-40 nmol/ml) with normal levels of the other amino acids and hyperammonemia with almost normal liver function tests, as briefly summarized in Table 1. No one had a family history of hereditary disorder, although the mother and the son of
patient No. 3 showed a slightly higher level of serum citrulline (52 nmol/ml). Histological examination of the liver specimens of all the patients revealed moderate fatty infiltration or mild portal fibrosis. Detailed clinical descriptions of patients No. 14, 25, 36, 67, and 78 have been reported and those of the other patients are in preparation for report.

**Determination of Enzyme Activity**

The liver, kidney and brain were homogenized in 9 vols. of 0.15 M KCl containing 0.05 M Tris-HCl, pH 7.5, with a Teflon homogenizer. The supernatant was prepared by centrifugation at 100,000 xg for 30 min. and used for the determination of ASS, argininosuccinase and arginase. For the determination of the activities of carbamylphosphate synthetase and ornithine transcarbamylase, the liver was homogenized in 9 vols. of 0.02 M Tris-HCl, pH 7.2, and 20% glycerol containing 2 mM dithiothreitol and 0.2% cetyltrimethylammonium bromide. Lysis of cultured fibroblasts was achieved by freeze-thawing two times in 50 mM Tris-HCl, pH 8.5. The enzyme assays were performed on the crude homogenate immediately after lysis of the cells. ASS activity in the brain and the cultured skin fibroblasts was determined by the radiochemical method of Schimke at pH 8.5 and 37°C, and that in the liver and the kidney was determined by the modified method at pH 7.5 and 25°C. The activities of the other urea cycle enzymes were determined essentially according to the method of Schimke.

**RESULTS**

**Activities of Urea Cycle Enzymes in the Liver of the Patients**

The ASS activities in the liver of the twelve citrullinemic patients were decreased to 2 to 50% of the control, while the other urea cycle enzymes were almost normal (Table 1). The liver of patient No. 7 had a considerably low activity of carbamylphosphate synthetase. This may be attributable to the storage of the liver sample for long time, since the high level of serum citrulline found in his clinical data seems to conflict with the deficiency of carbamylphosphate synthetase. In some cases, the activity of ornithine transcarbamylase was higher than the control.

These results indicate that the defect of ASS is characteristic to these citrullinemic patients.

**Kinetic Analysis of ASS in the liver**

The kinetic properties of hepatic ASS from all the patients except No. 3 and 6 were very similar to those of the control enzyme, and the Km values of the control enzyme, 0.049 mM, 0.033 mM and 0.24 mM for citrulline, aspartate and ATP, respectively, were in