Adenylosuccinase deficiency: a patient with impaired erythrocyte activity and anomalous response to intravenous fructose

C. SALERNO[1]*, C. CRIFÒ[2] and O. GIARDINI[3]

[1]*Department of Human Biopathology,* [2]*Department of Biochemical Sciences,* [3]*Institute of Paediatrics, University of Rome La Sapienza, Italy*

Correspondence: Department of Biochemical Sciences, University of Roma La Sapienza, p.le A. Moro, 5, 00185 Roma, Italy

Summary: Clinical and biochemical data are presented on an Italian patient with adenylosuccinase deficiency of both erythrocytes and mixed peripheral blood lymphocytes. The erythrocyte enzyme showed normal substrate affinity, but decreased thermal stability. The patient displayed an anomalous response to an intravenous fructose tolerance test with a rise in plasma $[Mg^{2+}]$ and $[K^+]$ and a drop in plasma levels of inorganic phosphate, glucose, urate and succinylnucleosides upon fructose injection.

Adenylosuccinase (adenylosuccinate lyase; EC 4.3.2.2) deficiency (McKusick 103050) is an autosomal recessive disorder involving the purine nucleotide cycle and the *de novo* purine pathway (Jaeken and Van den Berghe 1984; Salerno et al 1993; Van den Berghe et al 1993; Krijt et al 1994). Most patients have severe psychomotor retardation often associated with epilepsy and autistic features. In their biological fluids two normally undetectable compounds, namely succinylaminoimidazole carboxamide riboside (SAICA riboside) and succinyladenosine (S-Ado), accumulate which are the dephosphorylated substrates of adenylosuccinase. Measurements of adenylosuccinase activity in biopsy specimens show that the degree of enzyme deficiency is variable in the patients and not present in all tissues. Normal levels of erythrocyte enzyme activity have hitherto been reported. A point mutation resulting in the Ser[413]Pro substitution has been observed in the cDNA clones from lymphoblasts of a patient (Stone et al 1992).

Several hypotheses have been put forward to explain the clinical and biochemical heterogeneity in this disease (Jaeken et al 1988). It has been proposed that SAICA riboside and S-Ado could be neurotoxic either by interfering with glucose metabolism or by mimicking the structure of adenosine, a putative neuromodulator (De Volder et al 1988). However, experiments with isolated hepatocytes (Van den Berghe et al 1993) as well as with crude membrane fractions of rat cerebral cortex (Vincent and Van den Berghe 1989) failed to support these suggestions.

In this paper, a patient is described with succinyladenosinuria and decreased adenylosuccinase activity in both erythrocytes and mixed peripheral blood lymphocytes. We also report an anomalous response of the patient to intravenous fructose tolerance test that could suggest an impairment of the purine nucleotide cycle.

CASE REPORT

The propositus (at present 10 years old) is the second daughter of healthy, unrelated Italian parents. Their first daughter is healthy. Delivery was uneventful after a normal term pregnancy. A few months after birth, motor restlessness, hypertonicity and frequent crying attacks were noticed. Eye contact was difficult, while reaction to auditory stimuli was exaggerated. At age 9 months, absences were noticed. She stood unsupported at 15 months and walked after 2 years of age. At the age of 5 years, generalized convulsions were observed for the first time. Growth including head circumference, muscle tone and reflexes were normal; the gait was awkward. Bouts of extreme physical agitation, especially involving arms and legs, were common. Intermittent external strabismus with problems of convergence were frequently observed. Episodes of enuresis and encopresis are rare. Speech is unintelligible, in short sentences of incorrect structure. Since her parents believed that animal protein and gluten cause worsening of the symptoms, the protein content in the diet is severely restricted and the plasma amino acid pattern was usually normal. However, we recently found abnormally high values of alanine (1219 μmol/L, reference range 123–629), asparagine (322 μmol/L, reference range 36–144), glycine (851 μmol/L, reference range 107–320), glutamate (307 μmol/L, reference range 15–250), proline (547 μmol/L, reference value 40–332) and serine (516 μmol/L, reference range 76–171) in the serum after an overnight fast.

MATERIALS AND METHODS

Erythrocytes were isolated from venous blood by removing white cells and platelets through an α-cellulose/microcrystalline cellulose column (Sigmacell type 50; Sigma Chemical Co., St Louis, MO, USA) as described by Beutler (1983). Mononuclear preparations containing 75–80% lymphocytes and apparently free of contaminating erythrocytes were obtained by a one-step procedure (Boyum 1968) involving centrifugation of heparinized blood layered on Histopaque-1077 (Sigma).

Partial purification of adenylosuccinase from haemolysates involved preparation of a 30–60% saturated $(NH_4)_2SO_4$ fraction and chromatography of this fraction on an Econo-Pac Q Cartridge (Bio-Rad Laboratories Inc., Hercules, CA, USA) equilibrated with 10 mmol/L KCl, 1 mmol/L dithiothreitol, 2 mmol/L EDTA, 10 mmol/L Tris-HCl buffer, pH 7.4 (buffer A). The enzyme was eluted by increasing KCl concentration to 400 mmol/L.

Adenylosuccinase activity was measured by following the conversion of adenylosuccinate into AMP in buffer A at 37°C. Enzyme activity in crude extracts was determined by measuring the absorbance at 254 nm of the reaction product after its separation from the substrate by high-performance zone electrophoresis in a fused-silica capillary (size 75 μm×50 cm; running buffer 50 mmol/L sodium borate, pH 8.5; applied voltage 30 kV). Partially purified enzyme was assayed by a spectrophotometric method (Tornheim and Lowenstein 1972) based on the decrease in absorbance at 282 nm minus that at 320 nm.

Table 1   Succinylnucleoside concentration and adenylosuccinase activity

Urine	
[S-Ado]/[creatinine] (mol/mol)	0.075
[SAICA riboside]/[creatinine] (mol/mol)	0.060
Serum	
[S-Ado] (μmol/L)	3.0
[SAICA riboside] (μmol/L)	2.5
Adenylosuccinase activity	
Erythrocytes (nmol/min per mg Hb)	0.42 (control range 1.01–1.12)
Lymphocytes (nmol/min per mg protein)	0.38 (control range 1.04–1.49)

Heat denaturation curves of adenylosuccinase were obtained using erythrocyte lysates. The packed erythrocytes were diluted in 300 volumes of distilled water, incubated for 15 min at various temperatures, and then tested for adenylosuccinase activity at 37°C. The enzyme activity was compared to that of lysates maintained at 0–5°C. The T_m value refers to the temperature causing 50% loss of enzyme activity.

SAICA riboside and S-Ado were measured in plasma and urine by high-performance liquid chromatography according to Jaeken and Van den Berghe (1984). Potassium, magnesium, inorganic phosphate, glucose, uric acid, and creatinine in plasma and urine were measured by standard laboratory methods.

The intravenous fructose tolerance test (Steinmann and Gitzelmann 1981) was performed by injecting a 20% (w/v) fructose solution over 1 min at a dose of 200 mg/kg body weight. The patient had fasted overnight. Venous blood samples were drawn at zero time and after 10, 30, 60, 90 and 120 min. The urine samples were collected during the test. All patient studies were done after informed parental consent.

RESULTS

The patient excreted approximately 0.08 mol S-Ado/mol creatinine in the urine, while S-Ado concentration in plasma was about 3 μmol/L. The molar ratio of S-Ado to SAICA riboside was about 1.2 in both the biological fluids (Table 1). Serum urate concentration was 0.12–0.24 mmol/L. The succinylpurines were undetectable in urine and plasma of the parents and a healthy sister.

Adenylosuccinase activity in heparinized venous blood of the patient was reduced to 35–40% of that in controls. After separation of the blood components, the activity was 0.42 nmol/min per mg of Hb (reference range 1.01–1.12, $n=5$) in erythrocytes and 0.38 nmol/min per mg of protein (reference range 1.04–1.49, $n=5$) in mixed peripheral blood lymphocytes. When lysates from defective and control erythrocytes were mixed in fixed proportions, the reaction rates were as predicted from appropriately weighted averages, without evidence of inhibitory activity in the defective lysates (data not shown). The defective enzyme was as stable with time (for up to 2 days) as the controls if the whole-blood samples were stored at 0–5°C. However, the heat denaturation curve of the defective enzyme from crude erythrocyte extracts showed a shift of the T_m value from
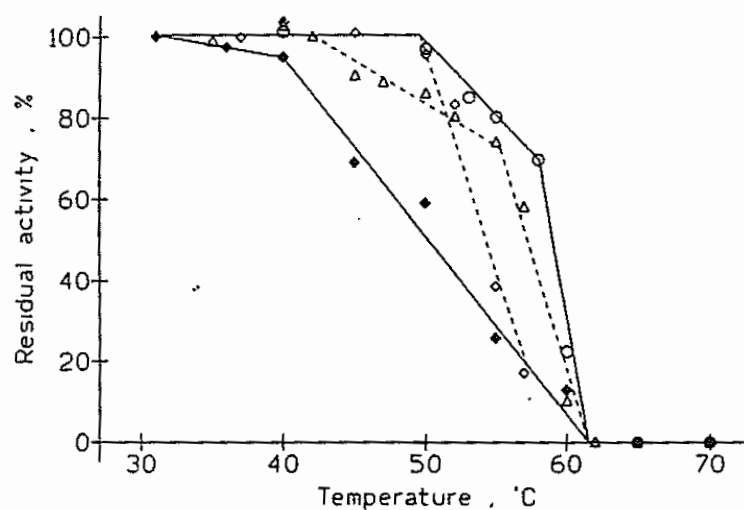
Figure 1 Heat denaturation curve of erythrocyte adenylosuccinase (◆, propositus; ◇, mother; △, father; O, control)
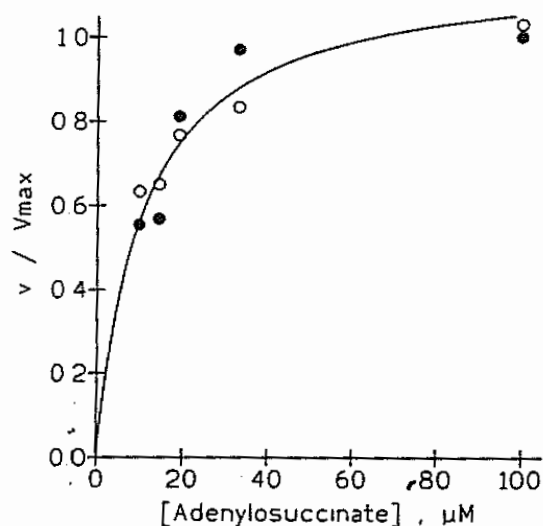

Figure 2 Initial velocity steady-state kinetics of adenylosuccinase from control (O) and defective (●) erythrocytes. Solid line shows the theoretical curve for $K_m = 8 \, \mu mol/L$

59°C to 51°C. Intermediate T_m values were obtained with erythrocytes of the parents (Figure 1).

Progressive loss of defective enzyme activity was observed during purification. The partially purified defective enzyme appeared to be unstable, since its activity decreased at 0–5°C with a half-time of about 12 h (the control enzyme retained original activity up to 3 days). The apparent K_m of adenylosuccinate for partially purified defective enzyme was not modified as compared to controls (Figure 2).

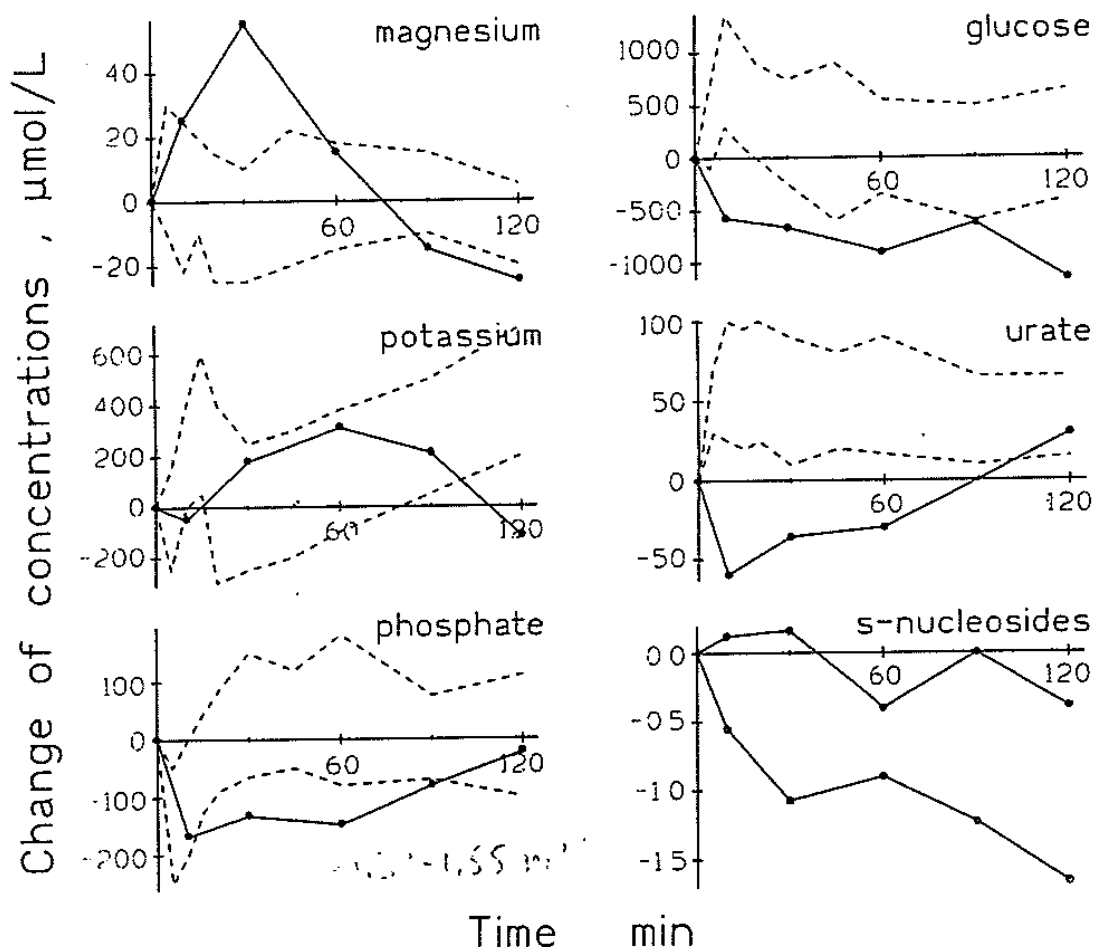Figure 3 shows the response of plasma analytes to the intravenous fructose tolerance

Figure 3 Plasma response to the intravenous fructose tolerance test. Broken lines comprise means ± 1 SD of the controls reported by Steinmann and Gitzelmann (1981). S-nucleoside curves refer to S-Ado (O) and SAICA-riboside (●)

test. The propositus had a rise of $[Mg^{2+}]$ and $[K^+]$ in the plasma during the first 60–90 min, followed by a drop of these ions below the initial values. Inorganic phosphate and urate concentrations in plasma fell at first and then increased again (in the case of urate to greater than the initial value). A slow decrease in the plasma concentrations of glucose, S-Ado and SAICA riboside was observed during the whole test. The urate/creatinine ratio in urine was roughly constant up to 5 h after the fructose injection, while a 5–15% decrease in urinary succinylnucleoside excretion was observed.

DISCUSSION

The clinical and most of the biochemical findings presented in this paper are similar to the data already reported for other cases of inherited succinyladenosinuria (Van den Berghe et al 1993). Nevertheless, the enzyme defect in our patient seems also to involve the erythrocytes, perhaps because of a markedly decreased stability in the protein. The unbalanced diet with severe protein restriction could also have contributed to revealing the erythrocyte enzyme defect. Indeed, changes in adenylosuccinase activity resulting from starvation

have been reported in rats and chickens (Brand and Lowenstein 1978); no data are available up to now for humans.

As far as the intravenous fructose tolerance test is concerned, the decrease in serum phosphate concentration does not markedly differ from what is observed in some of the controls and reflects, at least in part, the conversion of fructose to fructose-1-phosphate and the rephosphorylation of ADP (Steinmann and Gilzelmann 1981; Van den Berghe et al 1977). By contrast, the increase in plasma [Mg^{2+}] is significantly above the control levels and could be taken as an indirect evidence of ATP loss (Mäempää 1972). ATP depletion could be also suggested by the increase in plasma [K^+] and by the slow decrease in blood glucose concentration. While the rise in plasma potassium is probably due to a transient impairment of the ATP-dependent pumps, the fructose-induced hypoglycaemia, exceptionally observed in healthy subjects (Schartz et al 1964), is generally attributed to defective gluconeogenesis that, in turn, seems to depend, at least in perfused rat liver (Woods et al 1970), on reduced ATP concentration. The transient decrease in plasma urate level upon fructose injection is somewhat unexpected, taking into account the well known hyper-uricaemic effect of fructose in patients with hereditary fructose intolerance as well as in some of the normal subjects (Steinmann and Gitzelmann 1981). A possible explanation is that the plasma urate concentration reflects the effects of both an impairment of the *de novo* purine synthesis (due to the lack of ATP) and an increased degradation of adenine nucleotide. The slow decrease in plasma succinylnucleoside level could be taken as further evidence for the reduced *de novo* purine synthesis. On the whole, the chemical signs induced by fructose seem to support the hypothesis of an impairment of the purine nucleotide cycle in patients with succinyladenosinuria. This hypothesis has already been discussed in explaining the muscular wasting and growth failure of another patient with the same enzyme defect (Van den Berghe and Jaeken 1986; Van den Berghe et al 1993).

REFERENCES

Beutler E (1983) *Red Cell Metabolism*. Orlando: Grune & Stratton, 8–18.

Boyum A (1968) Separation of leukocytes from blood and bone marrow. *Scand J Clin Lab Invest* 21: 77–83

Brand LM, Lowenstein JM (1978) Effect of diet on adenylosuccinase activity in various organs of rat and chicken. *J Biol Chem* 253: 6872–6878.

De Volder AG, Jaeken J, Van den Berghe G et al (1988) Regional brain glucose utilization in adenylosuccinase-deficient patients measured by positron emission tomography. *Pediatr Res* 24: 238–242.

Jaeken J, Van den Berghe G (1984) An infantile autistic syndrome characterized by the presence of succinylpurines in body fluids. *Lancet* 2: 1058–1061.

Jaeken J, Wadman SK, Duran M et al (1988) Adenylosuccinase deficiency: an inborn error of purine nucleotide synthesis. *Eur J Pediatr* 148: 126–131.

Krijt J, Sebasta I, Svehlakova JA, Zeman J (1994) Adenylosuccinate lyase deficiency in a Czech girl and two siblings. In Sahota A, Taylor M, Weber G, eds. *Abstracts of the Eight International Symposium on Purine and Pyrimidine Metabolism in Man*. Bloomington: Indiana University Press, 46.

Mäempää PH (1972) Fructose-induced alterations in liver polysome profiles and Mg levels. *FEBS Lett* 24: 37–40.

Salerno C, Lomonte A, Giardini O, Crifò C (1993) Adenylosuccinate lyase deficiency: a case report. *Pharmacy World & Science* 15: F11.

Schartz R, Gamsu H, Mulligan PB, et al (1964) Transient intolerance to exogenous fructose in the newborn. *J Clin Invest* 43: 433–439.

Steinmann B, Gitzelmann R (1981) The diagnosis of hereditary fructose intolerance. *Helv Paediatr Acta* 36: 297–316.

Stone RL, Aimi J, Barshop BA, et al (1992) A mutation in adenylosuccinate lyase associated with mental retardation and autistic features. *Nature Genetics* 1: 59–63.

Tornheim K, Lowenstein JM (1972) The purine nucleotide cycle. The production of ammonia from aspartate by extracts of rat skeletal muscle. *J Biol Chem* 247: 162–169.

Van den Berghe G, Jaeken J (1986) Adenylosuccinase deficiency. *Adv Exp Med Biol* 195A: 27–33.

Van den Berghe G, Bronfman M, Vanneste R, Hers HG (1977) The mechanism of adenosine triphosphate depletion in the liver after a load of fructose. *Biochem J* 162: 601–609.

Van den Berghe G, Van den Bergh F, Vincent MF, Jaeken J (1993) The biochemical aspects of ASase deficiency. In Gresser U, ed. *Molecular Genetics, Biochemistry and Clinical Aspects of Inherited Disorders of Purine and Pyrimidine Metabolism*. Berlin: Springer Verlag, 140–143.

Vincent MF, Van den Berghe G (1989) Influence of succinylpurines on the binding of adenosine to a particulate fraction of rat cerebral cortex. *Adv Exp Med Biol* 253B: 441–445.

Woods HF, Eggleston LV, Krebs HA (1970) The cause of hepatic accumulation of fructose 1-phosphate on fructose loading. *Biochem J* 119: 501–510.