weight (6.87 ± 1.76 units/100g body for lactating rats versus 2.50 ± 0.65 for control rats, mean ± s.e.m. of six rats in each group). No changes in glutaminase activity were observed when the results were expressed relative to unit weight or unit protein of the small intestine. The increased capacity of the small intestine to utilize glutamine was reflected by increased glutamine uptake from the circulation as detected by arteriovenous difference measurements across the portal drained viscera (Table 1). This was accompanied by increased output of ammonia into the portal blood (arteriovenous difference measurements of −0.491 ± 0.093 μmol of NH₃/ml versus −0.104 ± 0.080 μmol of NH₃/ml, means ± s.e.m. for ten lactating and six control rats respectively).

Utilization of large amounts of glutamine by the lactating mammary gland was confirmed (Table 1). Thus, during lactation the enlarged small intestine and the mammary gland both contribute to increased demand for glutamine.

Dietary glutamine is catabolized by enterocytes and does not enter the circulation unmodified. It is logical, then, that lactation must be accompanied by either increased synthesis or decreased utilization of glutamine in other tissues. The major sites of glutamine synthesis in the body are skeletal muscle, liver, and possibly adipose tissue. No indication of compensatory changes in glutamine metabolism during lactation could be detected by arteriovenous difference measurements across liver and hindquarters (mainly skeletal muscle) (Table 1). Similarly the capacity of these tissues to synthesize glutamine, as determined by glutamine synthetase activity, was not affected. The activity of phosphate-activated glutaminase in liver and kidney, two potential sites of glutamine utilization, was also unchanged during lactation (results not shown).

These results do not allow identification of the site of glutamine production during lactation. The most likely sites, liver and skeletal muscle, cannot be ruled out from the data presented here, because simple arteriovenous difference measurements for both tissues are difficult to interpret. The large skeletal muscle mass means that changes in glutamine output may be below the level of detection (Lund & Watford, 1976; Lemieux et al., 1980). Adipose tissue has been shown to possess glutamine synthetase activity and to synthesize glutamine in vitro (Tischler & Goldberg, 1980). The ultimate sources of carbon and nitrogen for such glutamine are amino acids derived from the diet. During lactation there is a large increase in total body fat and therefore the potential of this tissue for glutamine synthesis may be important.

This work was supported in part by NIH grant AM-32894.


Received 3 June 1986

Alanine: glyoxylate and serine: pyruvate aminotransferases in primary hyperoxaluria type 1

CHRISTOPHER J. DANPURE and PATRICIA R. JENNINGS

Division of Inherited Metabolic Diseases, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, U.K.

Primary hyperoxaluria type 1 is an autosomal recessive disease caused by an abnormality in glyoxylate metabolism. This leads to increased synthesis and excretion of glycolate and oxalate. Clinically the disease is characterized by recurrent calcium oxalate kidney stones, resulting in progressive renal insufficiency and death usually before the age of 20 years (Williams & Smith, 1983). The condition of 10–30% of the patients can be improved by pharmacological doses of pyridoxine (Ludwig, 1963).

The basic defect in the disease was previously considered to be a deficiency of cytosolic 2-oxoglutarate: glyoxylate carboligase (Koch et al., 1967). However, various studies have cast doubt on this (Schlossberg et al., 1970; O’Fallon & Brosemer, 1977; Danpure et al., 1986) and recently we have shown that the livers of two patients with primary hyperoxaluria type 1 have an almost complete absence of peroxisomal alanine: glyoxylate aminotransferase (Danpure & Jennings, 1986). It has been shown previously (Noguchi et al., 1978) that, in experimental animals, there are two forms of alanine: glyoxylate aminotransferase, one of which is identical with serine: pyruvate aminotransferase.

Investigation of two livers from patients with primary hyperoxaluria type 1 (one pyridoxine-resistant and the other unknown), compared with five control livers, showed that there was a marked deficiency in the activity of serine: pyruvate aminotransferase (EC 2.6.1.51) as well as alanine: glyoxylate aminotransferase (EC 2.6.1.44) (mainly peroxisomal) (Table 1). The activities of glutamate: glyoxylate aminotransferase (EC 2.6.1.4) (cytosolic), aspartate: 2-oxoglutamate aminotransferase (EC 2.6.1.1) (mitochondrial and cytosolic) and the peroxisomal marker enzymes α-amino acid oxidase (EC 1.4.3.3) and catalase (EC 1.11.1.6) were normal or near normal.

The activity of alanine: glyoxylate aminotransferase in normal liver could be enhanced two to three times by incubating in 40 μM-pyridoxal phosphate and was not significantly inhibited by heating to 60 °C for 10 min. However, the residual activity in the liver of the patient with the pyridoxine-resistant form of the disease could not be increased by pyridoxal phosphate up to 400 μM and was significantly inhibited (by about 60%) by heating. These data and the subcellular distribution of the alanine: glyoxylate aminotransferase in the hyperoxaluric liver (mainly cytosolic; see Danpure & Jennings, 1986) strongly suggest that most of this residual activity is due to the reactivity of the cytosolic glutamate: glyoxylate aminotransferase towards alanine.

The deficiency of both alanine: glyoxylate and serine: pyruvate aminotransferase activities in the hyperoxaluric livers provides support for the previous suggestion (Noguchi et al., 1978) that they are due to the same protein.

In the liver the peroxisomes are likely to be a major site of production of glyoxylate, from glycolate (using 1,2-hydroxyacid oxidase) and glyoxylate (using α-amino acid oxidase) (Tolbert, 1981). The recent finding by Nakatani et al.
Homogentisate oxidation in isolated liver cells

Liver cells were prepared from fed rats (male Sprague-Dawley, 180–220 g body weight) as previously described (Elliot et al., 1976). Metabolic integrity of cells was assessed by measurement of ATP content (Dickson & Posgon, 1977). Radiolabelled phenylalanine and tyrosine were purified as described in Salter et al. (1984). Rates of catabolism are expressed as nmol/mg dry wt. of liver cells.

Oxidation of homogentisate derived from phenylalanine can be estimated in two ways. During hydroxylation of [4-14C]phenylalanine, tritium migrates to the 3-position forming [3-3H]tyrosine (Guroff et al., 1967) which is metabolized to either [4-14C]homogentisate or [6-14C]homogentisate; subsequent metabolism releases tritium as 3H2O. Flux through homogentisate oxidase can therefore be determined from analysis of 3H2O release (Fisher & Posgon, 1984a). Alternatively, homogentisate oxidation can be followed by parallel measurement of 14CO2 production and non-aromatic products from [U-14C]phenylalanine and [1-14C]phenylalanine. This latter approach is also the basis of the measurement of tyrosine-derived homogentisate oxidation in this case [U-14C]tyrosine and [1-14C]tyrosine are used (Salter et al., 1984).

After incubation of liver cells with 0.05 mm-phenylalanine, these independent methods give estimates of homogentisate oxidation which are not significantly different (H-method: 1.36 ± 0.16; 14C-method: 1.74 ± 0.22 nmol/