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Research Article

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Development of Tyrosine Aminotransferase and p-Hydroxyphenylpyruvate Dioxygenase Activities in Fetal and Neonatal Human Liver

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A B S T R A C T In livers of fetuses of 220–340 g body wt, total cytosolic tyrosine aminotransferase activity was 1.0 nmol of product/mg of protein per min, and the corresponding values for autopsy livers of newborns of 740–1,475 g and 2,600–3,650 g were 1.5 and 5.7, respectively, as compared with the adult value of 12.7. On the other hand, p-hydroxyphenylpyruvate dioxygenase activity is at adult level already in fetuses <340 g body wt. The K_m value for tyrosine of tyrosine aminotransferase (1 mM) was considerably higher than the corresponding value for p-hydroxyphenylpyruvate of p-hydroxyphenylpyruvate dioxygenase (50 μM). These results suggest that tyrosine aminotransferase is the rate limiting enzyme in the catabolism of tyrosine in premature infants.

INTRODUCTION

Transient neonatal tyrosinemia is the most common disorder of amino acid metabolism encountered in human infants, occurring in as many as 30% of premature newborns (1–6). This condition is characterized by marked elevation of serum tyrosine concentration and urinary excretion of tyrosine, p-hydroxyphenylpyruvate, and p-hydroxyphenyllactate (4). The abnormality is spontaneously corrected most often during the first weeks but sometimes persists up to 2 or 3 mo of age (1–2). The condition has been reported to be potentially harmful to the central nervous system (5), and in one case, elevated concentrations of homovanillic and 5-hydroxyindoleacetic acids in the cerebrospinal fluid have been demonstrated (7).

The main catabolic pathway of tyrosine begins with transamination by hepatic cytosolic L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5) yielding p-hydroxyphenylpyruvate. This enzyme is under strict hormonal control and is inducible by corticosteroids, glucagon, catecholamines, dibutyryl-cAMP, and by tyrosine in rat (8) and in fetal human liver in organ culture (9). The following enzymes of the pathway, p-hydroxyphenylpyruvate dioxygenase (EC 1.13.11.27), homogentisate 1,2-dioxygenase (EC 1.13.11.5), maleylacetoacetate isomerase (EC 5.2.1.2), and fumarylacetoacetase (EC 3.7.1.2) are not known to be inducible by hormones. Tyrosine aminotransferase is the rate limiting enzyme of this pathway in hepatocytes prepared from adult rat liver (10) and in organ culture of fetal rat liver (11).

The metabolic basis of transient neonatal tyrosinemia is unknown. Early studies suggested that vitamin C could correct this abnormality (1–4). It was believed that ascorbic acid is a cofactor of p-hydroxyphenylpyruvate dioxygenase (12). As p-hydroxyphenylpyruvate is excreted into the urine, it was natural that it was assumed that transient neonatal tyrosinemia is due to incomplete maturation of the activity of p-hydroxyphenylpyruvate dioxygenase.

In the light of the findings of the present work and a literature survey we now challenge this concept and suggest that the transamination step is rate limiting in the catabolism of tyrosine in the premature infant. Therefore, it is probable that transient neonatal tyrosinemia is caused by delayed induction of hepatic tyrosine aminotransferase.
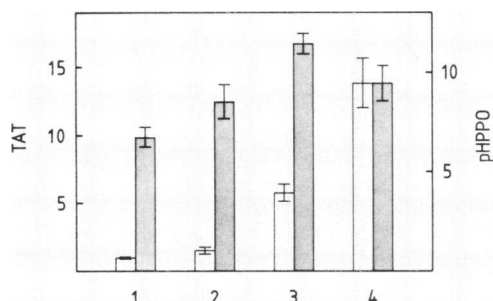
METHODS

Liver samples were obtained from legal abortions and at routine autopsies performed on the 1st and 2nd d post mortem on newborn babies and adults. A part of each sample was homogenized in 4 vol of 5 mM mercaptoethanol, 1%

FIGURE 1 Development of tyrosine aminotransferase and p-hydroxyphenylpyruvate dioxygenase activities. 1, livers of five fetuses obtained from legal abortions by section; body weights 220–340 g; 2, livers of three newborns who died of immaturity at ages of 1, 1, and 5 d (body weights 1,300, 740, and 1,475 g); 3, three newborns with body weights of 2,600, 2,650, and 3,650g who lived 1, 5, and 5 d and died of respiratory distress syndrome, pneumonia, and a congenital heart anomaly, respectively (the newborn of 2,650 g had hyperplasia of the islets of Langerhans and had the highest tyrosine aminotransferase activity); 4, seven adult livers obtained at routine autopsies. Light columns, tyrosine aminotransferase (TAT); shaded columns, p-hydroxyphenylpyruvate dioxygenase (pHPPO). Both activities are given as nanomoles of product formed per milligram of protein per minute. Vertical bars indicate SEM.

Triton X-100 and 50 mM sodium phosphate, pH 7.4, and the remaining part in 125 mM potassium phosphate, pH 7.6. The homogenates were centrifuged at 20,000 g for 20 min, and the supernatant of the former was assayed for p-hydroxyphenylpyruvate dioxygenase (13) and that of the latter for tyrosine aminotransferase (14, 15). Protein was estimated by the method of Lowry et al. (16). K_m values were estimated by the Lineweaver-Burk method.

RESULTS

The activities of tyrosine aminotransferase and p-hydroxyphenylpyruvate dioxygenase in fetal or newborn and adult liver samples are shown in Fig. 1. The activity of tyrosine aminotransferase was very low during the fetal period. When judging the activities in groups 1–3, one must bear in mind that aspartate aminotransferase (EC 2.6.1.1) can also transaminate tyrosine with low affinity; its contribution to adult liver tyrosine aminotransferase activity is insignificant, but in fetal liver it accounts for a considerable fraction of the low "tyrosine aminotransferase" activity (9). p-Hydroxyphenylpyruvate dioxygenase activity, on the other hand, was high even in the livers of fetuses of <340 g body wt. The fetal enzyme was inhibited by o-phenantroline, inactivated by H_2O_2 and $K_3Fe(CN)_6$, and activated by reducing agents. These properties are shared by the adult liver enzyme (17). The K_m values of p-hydroxyphenylpyruvate dioxygenase activities from fetal and adult livers towards p-hydroxyphenylpyruvate were 50 and 30 μM, respectively. The values obtained for the fetal liver enzyme were, thus, slightly higher than those for adult enzyme but we do not believe this difference to be significant. The K_m values for tyrosine of tyrosine aminotransferase from both sources were close to 1 mM; the corresponding figures for p-hydroxyphenylpyruvate of p-hydroxyphenylpyruvate dioxygenase were thus considerably lower.

DISCUSSION

The present results clearly indicate that p-hydroxyphenylpyruvate dioxygenase activity is at adult level in fetuses <340 g of body wt. In contrast, tyrosine aminotransferase activity is low and still significantly below adult levels after the fetus has reached a body weight >2,600 g. Furthermore, the K_m value of this enzyme is relatively high, which suggests that the activity at physiological substrate concentrations (4, 8) is much less than the measured values. As tyrosine aminotransferase is known to be under strict hormonal regulation, whereas the other enzymes of the pathway are not, it is probable that this enzyme is rate limiting. In fact, this has been shown to be the case in rat liver (10, 11). Therefore, we suggest that the insufficient maturation of this pathway in prematures is due to a defect in the regulation of tyrosine aminotransferase.

If tyrosine aminotransferase activity is deficient, the urinary excretion of p-hydroxyphenylpyruvate, the product of this enzyme, seems paradoxical at first glance. However, a child shown to have no tyrosine aminotransferase activity in the soluble fraction of the liver excreted large amounts of this metabolite as well (18). This may be due to nonspecific aminotransferases, such as mitochondrial aspartate aminotransferase, acting in extrahepatic and extrarenal tissues that are deficient in p-hydroxyphenylpyruvate dioxygenase activity (18). There is evidence that p-hydroxyphenylpyruvate is actively secreted by the kidney (19). It can therefore be understood that this metabolite is excreted despite insufficient hepatic tyrosine aminotransferase activity.

Several studies have suggested that administration of vitamin C normalizes serum tyrosine concentration in transient neonatal tyrosinemia (1–3, 6). However, this condition resolves spontaneously and abruptly, which can easily lead to the misinterpretation that the therapy had a curative effect. The only study in which the time course of the condition was followed showed no effect of vitamin C (20) in a very large patient material. Finally, ascorbic acid is not a cofactor of p-hydroxyphenylpyruvate dioxygenase (17).

In conclusion, we propose that transient neonatal tyrosinemia is due to a defect in the regulation of hepatic tyrosine aminotransferase.

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