Tyrosine Aminotransferase Activity in the Rabbit Placenta

EVIDENCE FOR DECREASED INDUCIBILITY BY INSULIN AND CORTISOL AS A FUNCTION OF GESTATIONAL AGE

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Tyrosine aminotransferase is present at low activity in the rabbit placenta. The activity can be enhanced by injecting the pregnant mother with insulin or cortisol. This response decreases over the last half of gestation (14–28 days gestational age).

Tyrosine aminotransferase (EC 2.6.1.5) has received much attention in recent years as one of the few known enzymes whose synthesis can be hormonally induced. Although the origin of contemporary interest in this enzyme lay in its presumed role in the tyrosinaemia of prematurity (Kretchmer, 1969), most recent investigators have used it as a tool to study the molecular mechanisms of enzyme induction. To date the physiological significance of its inducibility remains unclear.

Our interest in studying tyrosine aminotransferase in placental tissue arose for the following reasons.

(1) Little is known of the placenta's metabolic responsibilities. Liver tyrosine aminotransferase is involved in tyrosine catabolism, and shares with other transaminases responsibility for the general redistribution of amino acid nitrogen and the shunting of amino acid carbon atoms into gluconeogenesis. As the placenta appears to transport amino acids actively from the maternal to the foetal circulation, it is of interest to know whether the placenta is involved with processing amino acids for the foetus (e.g. exchanging nitrogen and carbon skeletons among amino acids and carbohydrate intermediates so as to provide an optimal balance of metabolites). Knowledge of placental tyrosine aminotransferase activity and inducibility relative to the values in liver would be suggestive of the degree to which the placenta might play such a role.

(2) The activity of tyrosine aminotransferase in foetal liver is very low throughout gestation in many species (about 5–15% of values for adult liver in rats and humans), and increases markedly around the time of birth (Litwack & Nemeth, 1965; Kretchmer, 1969; Koler *et al.*, 1969; Luppis & Rutter, 1973). In certain instances, enzymic patterns in the placenta undergo changes with gestational age that are opposite to those of foetal liver. This has led to implications that the placenta may assume certain liver-type functions for the foetus early in gestation before the foetal liver has developed functional

competence (Villee, 1953). It was therefore of interest to know the pattern of placental tyrosine aminotransferase activity and inducibility at different gestational ages.

(3) The comparatively recent findings that the placenta is capable of interacting with various hormones (Ginsburg & Jeacock, 1969; Demers *et al.*, 1973; Menon & Jaffe, 1973) suggest that at least some aspects of placental metabolism and function may be subject to endocrine influences. Hormone-mediated enzyme induction is an indication of hormone responsiveness, which had not been explored in the placenta. Further, tyrosine aminotransferase is inducible by glucocorticoids in adult liver, and the placental capability of interacting with such steroid hormones is unknown.

Materials and Methods

Pregnant New Zealand White rabbits averaging 4 kg in weight were used in all cases. Experiments were carried out on placental tissue of three gestational ages: 14 days; 20–21 days; 27–28 days (referred to as 'term'). Samples of maternal liver were taken at the same time for comparison. Three categories of rabbits were used: untreated controls; insulin-injected (1.5 units/100 g body wt.; Iletin-U-100, Eli Lilly and Co., Indianapolis, Ind., U.S.A.); cortisol-injected (4 mg/100 g body wt.; Solu-Cortef; Upjohn Pharmaceutical Co., Kalamazoo, Mich., U.S.A.). All injections were given to the mothers intraperitoneally. Insulin injections were accompanied by a 2 ml injection of 10% (w/v) glucose to prevent shock. Insulin was administered 2.5 h before death. Cortisol was injected 4 h before death. All subsequent operations were carried out at 0–4°C unless otherwise noted.

The rabbits were killed by head concussion and the placentas were immediately excised and dropped into ice-cold 0.9% NaCl. Each placenta was quickly trimmed of extraneous membranes and blotted on

paper towelling. The combined tissues for one experiment were then weighed and homogenized in a Potter-Elvehjem homogenizer in 1.5 vol. of 0.15M-KCl containing 1mM-EDTA and 1mM-dithiothreitol. The homogenate was centrifuged at 12000g for 20min, the pellet was washed and re-centrifuged, and the combined supernatants were filtered through glass wool.

Supernatant solutions were assayed for tyrosine aminotransferase activity by a modification of Diamondstone's (1966) procedure. The assay mixture contained, in a final volume of 2ml, 2.4 μ mol of pyridoxal 5'-phosphate, 20 μ mol of 2-oxoglutaric acid, 12 μ mol of L-tyrosine, 1.8 μ mol each of EDTA and dithiothreitol, 8mg of bovine serum albumin, 7 μ mol of freshly recrystallized diethyldithiocarbamic acid, 155 μ mol of Tris (pH 7.6 with HCl) and 3.5 mg of enzyme protein. Inclusion of diethyldithiocarbamate and bovine serum albumin was found to improve the reproducibility of tyrosine aminotransferase kinetics in this system. All reagents were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.

As recommended by Kenney (1959) for rat liver tyrosine aminotransferase, pyridoxal phosphate (0.24 μ mol in 0.1 ml) was added to a 5 ml sample of placental homogenate. This sample was preincubated for 5 min at 37°C, as were the assay tubes, which contained all the additions except diethyldithiocarbamate. This latter reagent, used to inhibit *p*-hydroxyphenylpyruvate oxidase, was added to each assay tube just before addition of the enzyme solution (usually 0.2 ml). The reaction mixtures were incubated at 37°C for 15 min and reactions were terminated by the addition of 0.2 ml of 10M-NaOH. After 30–45 min, the reaction tubes were read against a complete reagent blank (0.2 ml of 10M-NaOH added before the enzyme) at 331 nm.

Protein concentrations were determined by the biuret method (Gornall *et al.*, 1949).

Results

Tyrosine aminotransferase activity in the rabbit placenta varies with gestational age and is only 3–12% of that present in the maternal liver (see Fig. 2 legend). Compared with the maternal liver enzyme, the placental enzyme is more difficult to deal with experimentally because of its low activity and considerable biological variability. Efforts to effect a substantial increase in placental tyrosine aminotransferase activity by varying preparative or assay procedures have so far been unsuccessful.

The rabbit placenta was also found to contain considerable *p*-hydroxyphenylpyruvate oxidase activity. This enzyme, detected by the method of Lin *et al.* (1958), can lead to erroneously low measurements of tyrosine aminotransferase activity or erratic kinetic behaviour unless inhibited. Accordingly, diethyl-

dithiocarbamate was used to inhibit the *p*-hydroxyphenylpyruvate oxidase. *o*-Phenanthroline was also tested as an oxidase inhibitor in this system, but proved to be less satisfactory.

As shown in Fig. 1, placental tyrosine aminotransferase activity is higher after the administration of a steroid (cortisol) and a polypeptide (insulin) hormone via intraperitoneal injection to the mother. This effect decreased during the progress of gestation (Fig. 2).

Discussion

Several decades ago it was observed that babies born prematurely are prone to tyrosinaemia unless their diets are supplemented with vitamin C (Levine *et al.*, 1939). Infants carried to full term, on the other hand, have no such difficulty. At least in part, the source of this defect appears to be at the level of two enzymes concerned with tyrosine metabolism, namely tyrosine aminotransferase and *p*-hydroxyphenylpyruvate oxidase. Tyrosine aminotransferase is present at only very low activity during early gestational ages; *p*-hydroxyphenylpyruvate oxidase is an ascorbic acid-requiring enzyme.

It is now well documented in several species that tyrosine aminotransferase activity in foetal liver, scarcely detectable throughout most of gestation, increases markedly at, or shortly before, birth to attain adult activity within 24 h (Litwack & Nemeth, 1965; Kretchmer, 1969; Koler *et al.*, 1969; Luppis & Rutter, 1973). This increase results from an accelerated rate of synthesis of enzyme molecules which can be triggered in various systems by both steroid

Fig. 1. Kinetics of tyrosine aminotransferase activity in control and hormone-stimulated 14-day rabbit placenta and maternal liver

Data points represent the average values of determinations performed on tissues from 10 to 17 animals, $\pm$ S.E.M. The control liver plot is shown for comparison. ▲, Liver, control; ○, placenta, cortisol-treated; ■, placenta, insulin-treated; ●, placenta, control.

Fig. 2. Comparison of tyrosine aminotransferase activities in insulin-treated (▨), cortisol-treated (■) and untreated placentas (□) of different gestational ages, and maternal liver

Bars represent averages $\pm$ S.E.M. Circled numbers associated with each bar show the numbers of animals contributing tissues. The actual kinetic data for 14-day placenta and liver control are shown in Fig. 1. Control values in each case (nmol of *p*-hydroxyphenylpyruvate/min per mg of protein, $\pm$ S.E.M.) were 0.38 ± 0.06 (14-day placenta), 1.55 ± 0.24 (21-day placenta), 0.80 ± 0.10 (28-day placenta) and 13.39 ± 1.34 (maternal liver).

(glucocorticoid) and polypeptide (e.g. glucagon, insulin) hormones (Kenney, 1970).

Although it is known that tyrosine aminotransferase is the first step in the reaction sequence by which tyrosine is oxidized to homogentisate, the physiological significance of tyrosine aminotransferase inducibility by these various hormones is by no means clear. Tyrosine aminotransferase inducibility does not appear to be simply related to the tyrosine load on the animal and also cannot be simply related to its energetic requirements. If the concentration of the enzyme was extremely low relative to that of tyrosine, however, it could limit and thus regulate the rate of tyrosine oxidation to homogentisate. This may apply in the foetoplacental situation, since tyrosine aminotransferase activity in foetal liver and the placenta is much lower than in adult liver, and yet amino acid concentrations are generally greater in foetal than in maternal blood (Christensen & Streicher, 1948).

Presumably the most significant utilization of amino acids by the foetus is in the synthesis of new proteins. Any conversion of amino acids, mediated through transamination, into other products could be handled by the mother. This was indicated by the various reports of low tyrosine aminotransferase activity in foetal liver, and by its dramatic increase at birth when reliance on maternal systems is no longer possible. The possibility remained, however, that the placenta could act for the foetus in this enzymic capacity during the gestational period, when the foetal liver had not yet become functionally competent. Our present finding of the very low tyrosine aminotransferase activity in the rabbit placenta makes this possibility doubtful and further supports the contention that tyrosine transamination is less of a requirement for the foetus than for the adult.

The low tyrosine aminotransferase activity in the placenta may reflect intrinsic requirements of the placental tissue itself, rather than representing a function performed on behalf of the foetus.

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