Biochemical Pharmacology, Vol. 26, pp. 121–124. Pergamon Press, 1977. Printed in Great Britain.

BIOSYNTHESIS AND METABOLISM OF PUTRESCINE IN THE RAT PLACENTA*

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(*Received* 5 *May* 1976; *accepted* 7 *July* 1976)

Abstract—High levels of ornithine decarboxylase are found in the developing rat placenta. Peak activities are observed around day 17 of pregnancy, after which there is a slight decline in activity up to the time of parturition. Most of the ornithine decarboxylase activity is found in the fetal placenta, whereas in the maternal placenta the activity declines steadily throughout the latter part of pregnancy. At term over 90 per cent of the placental ornithine decarboxylase is found in the fetal segment. The putrescine content of the fetal placenta is three to ten times higher than that of the maternal tissue from day 15 onward. In contrast to the pattern of distribution for ornithine decarboxylase, diamine oxidase is found mainly in the maternal placenta, and the activity remains high throughout pregnancy. *S*-adenosyl methionine decarboxylase is also present in the placenta with significantly higher levels being found in the fetal tissue. The activity of this enzyme, however, was at all times much lower than that of ornithine decarboxylase and diamine oxidase. The alignment of the metabolic pathways favoring the metabolism of putrescine via oxidative deamination rather than through conversion to spermidine suggests that putrescine may have a role to play in the developing rat placenta. The results also indicate that the high levels of diamine oxidase activity found in the placenta are probably associated more with putrescine than with histamine metabolism.

The increasing use of plasma diamine oxidase determinations as a diagnostic tool both for the detection of pregnancy [1–4] and as a monitor of fetal health [5] has accelerated the urgency for establishing the functional significance of this enzyme. Indirect support for histamine being the physiological substrate for diamine oxidase has been provided by the suggestions that histamine is involved in decidual formation [6–8], as a mediator of estrogen action [9, 10] and also by the marked changes in histamine biosynthesis occurring during pregnancy in rodents [11, 12]. There are some difficulties, however, in seeking a closer correlation between histamine and diamine oxidase during pregnancy. In the human, for example, there is very little histamine biosynthesis in the fetus, although high levels of diamine oxidase are found in the tissues and in the plasma [13]. On the other hand, high histidine decarboxylase activity is found in the rat during pregnancy but it is restricted almost completely to the fetal liver, whereas high levels of diamine oxidase are found in the uterus, placenta and amniotic fluid as well as the plasma [13]. Furthermore, the high levels of diamine oxidase in both the tissues and the plasma do not prevent substantial amounts of histamine made by the fetus from escaping in the urine unchanged [11]. Changes in diamine oxidase during pregnancy, therefore, may be related to some amine other than histamine. In this report, we present evidence that diamine oxidase in the placenta is closely allied to changes in ornithine decarboxylase and suggest that putrescine, rather than histamine, is probably the substrate for diamine oxidase in this tissue. A preliminary report has been published [14].

* Supported by U.S. Public Health Service Grants HD06387 and 07476.

MATERIALS AND METHODS

Animals. Female Sprague–Dawley rats (Charles River) weighing between 120 and 150 g were used in these experiments. The animals were housed in light- and temperature-regulated quarters and fed a diet of commercial rat chow. The stage of the cycle was determined by microscopic examination of vaginal smears, and animals in estrus were mated for 24 hr. Males were then removed and this was taken as day 1 of pregnancy.

Animals were killed by cervical dislocation and the tissues removed and cleaned. The tissues were minced with scissors and diluted 9:1 in assay buffer for ornithine decarboxylase and *S*-adenosyl methionine decarboxylase or 49:1 for diamine oxidase assays as described below. The tissue was homogenized in a Polytron (Brinkmann) and centrifuged at 10,000 *g* for 50 min. The supernatant solution was strained through nylon gauze and used for analysis.

Chemicals. DL[1-^{14}C]ornithine monohydrochloride (1.0 mCi/m-mole), DL[5-^{14}C]ornithine monohydrochloride (2.7 mCi/m-mole), *S*-adenosyl-L-[1-^{14}C]methionine (9.1 mCi/m-mole) and [^{14}C]putrescine dihydrochloride (26.4 mCi/m-mole) were obtained from New England Nuclear Co. Non-isotopic chemicals were obtained either from CalBiochem or Nutritional Biochemicals.

Ornithine decarboxylase was assayed by measuring the rate of $^{14}CO_2$ evolution from carboxyl-labeled substrate using minor modifications of methods previously described [15]. The reaction is carried out in a Warburg flask and the evolved carbon dioxide is trapped on NCS-impregnated filter paper strips. The assay medium contained tissue extract, 0.2 μmole DL[1-^{14}C]ornithine, 0.2 μmole pyridoxal phosphate, 2 μmoles ethylenediamine tetra-acetic acid (EDTA), 5 μmoles dithiothreitol and 0.1 M phosphate buffer,

pH 7.2, in a final volume of 2 ml. Incubation was carried out in air at 37° for 30 min. The reaction was stopped with 0.2 ml of 1 M citric acid injected through a rubber septum in the side arm and shaken for a further 30 min. The filter paper was then transferred to a counting vial containing 10 ml of a toluene/ethanol scintillator and counted. Production of $^{14}CO_2$ was linear with time over at least 60 min and for both fetal and maternal placenta enzyme activity was proportional to the amount of tissue over the dilution range 4:1 to 39:1. Samples were routinely assayed at two dilutions, usually 9:1 and 19:1 and the corrected values did not differ by more than 10 per cent.

S-adenosyl-L-methionine decarboxylase was assayed using a procedure identical to that described for ornithine decarboxylase, except that the incubation mixture contained 0.2 μmole *S*-adenosyl-L[1-^{14}C]methionine in place of the labeled ornithine and the addition of 2.5 μmoles putrescine.

For the assay of histidine decarboxylase, the labeled substrate was [carboxyl-^{14}C]L-histidine (0.2 μmole) and incubation was carried out for 2 hr. Other conditions were the same as for ornithine decarboxylase. Enzyme activity for all three decarboxylases is expressed as nmoles CO_2 g^{-1} hr^{-1}.

Diamine oxidase was assayed as described previously [16]. The reaction is carried out in screw cap culture tubes at 37° for 2 hr. Each tube contains 1 ml of enzyme extract, 0.57 μmole [^{14}C]putrescine and 0.1 M phosphate buffer, pH 7.2, to a final volume of 2.0 ml. At the end of the incubation period a mixture of aminoguanidine sulfate (227 μg) and sodium bicarbonate (200 mg) is added to stop the reaction and adjust the pH. The end product, Δ^1-pyrroline, is extracted into 2 × 10 ml aliquots of a toluene–PPO (i.e. 2,5-diphenyloxazolyl) counting solution. The aqueous phase is removed by freezing and the two toluene extracts are pooled and counted in a scintillation counter.

Table 1. Distribution of ornithine and histidine decarboxylases between the fetus and the placenta on day 17 of pregnancy*

Tissue	Enzyme activity (nmoles g^{-1} hr^{-1} ± S. E. M.) Ornithine decarboxylase activity	Histidine decarboxylase activity
Fetus	16 ± 3.5	133.4 ± 31.3
Placenta	536 ± 131	12.1 ± 1.3

* Placentas from each litter were pooled and each value is the mean obtained from five different litters.

Table 2. Development of ornithine decarboxylase activity in rat placenta*

Day of pregnancy	Ornithine decarboxylase activity (nmoles g^{-1} hr^{-1} ± S. E. M.)
13	125 ± 23
15	517 ± 75
17	560 ± 90
19	390 ± 54

* Placentas from each litter were pooled. Each value is the mean obtained from five different litters.

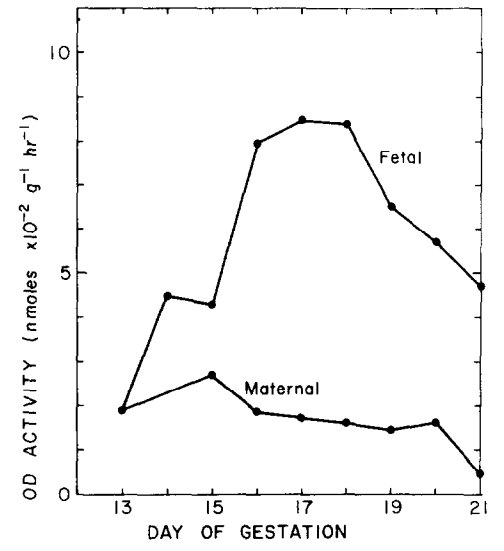

Fig. 1. Ornithine decarboxylase activity in the fetal and maternal placenta during pregnancy. Each result is the mean of five animals.

Putrescine was assayed using a modification of the enzyme method originally outlined by Harik and Snyder [17]. Briefly, the procedure takes advantage of the findings reported by Janne *et al.* [18] that the *S*-adenosyl-L-methionine decarboxylase from baker's yeast requires putrescine for its activity to be expressed. Enzyme activity, expressed as $^{14}CO_2$ evolved from *S*-adenosyl-L[1-^{14}C]methionine, is plotted against known concentrations of putrescine and sample content determined by comparison with the standard curve. The standard curve of $^{14}CO_2$ production against putrescine concentration is linear up to 1 nmole and the sensitivity is about 20 pmoles. The incubation volume is 120 μl and the reaction is carried out in the side arm of a Warburg flask in a shaking water bath at 37° for 30 min. The $^{14}CO_2$ evolved is trapped on NCS-impregnated filter strips placed in the center well. The procedure for stopping the reaction and counting is the same as described earlier for the other decarboxylase assays.

RESULTS

When ornithine decarboxylase activities of the rat embryo and the placenta were determined at day 17

Table 3. Putrescine levels in the fetal and maternal placenta at different days of pregnancy*

Day of pregnancy	Putrescine content (nmoles g^{-1} ± S. E. M.) Maternal placenta	Fetal placenta
15	17.1 ± 2.7	350.5 ± 30.2
17	28.5 ± 4.5	292.6 ± 29.0
19	14.7 ± 1.9	172.5 ± 11.7

* Each result is the mean obtained from five different animals.

Table 4. Diamine-metabolizing enzymes in the placenta on day 18 of pregnancy*

Tissue	Enzyme activity (nmoles g^{-1} hr^{-1} ± S. E. M.) Ornithine decarboxylase	Diamine oxidase	*S*-adenosyl methionine decarboxylase
Whole placenta	450 ± 27	60,000 ± 5,500	19.2 ± 4.1
Fetal placenta	840 ± 82	4,200 ± 390	46.3 ± 7.0
Maternal placenta	120 ± 19	70,000 ± 8,540	9.2 ± 3.1

* Placentas from each litter were pooled. Each value is the mean of four animals.

of pregnancy, most of the enzyme activity was found in the placenta (Table 1). During the latter third of pregnancy, the activity of the whole embryo remained low, a finding in agreement with earlier observations [19]. In contrast most of the histidine decarboxylase activity was found in the fetus. Ornithine decarboxylase activity in the placenta rose steadily up to about day 17 and then declined before parturition (Table 2). The values at day 19 were consistently and significantly ($P < 0.01$) lower than those observed at day 17 of pregnancy. The activity of ornithine decarboxylase in the placenta represents the highest levels of activity of this enzyme that we have found in any tissue. It is some ten times higher than that found in the ovary after stimulation with human chorionic gonadotrophin [20].

When the fetal and maternal placenta were studied separately, most of the ornithine decarboxylase activity was found in the fetal placenta (Fig. 1). The activity in this tissue increased up to about day 18 of gestation. The growth of the fetal placenta is also rapid during this period. From day 15 the ornithine decarboxylase activity in the maternal placenta declined steadily throughout pregnancy and at term constituted less than 10 per cent of the ornithine decarboxylase found in the whole placenta. From day 15 the distribution of ornithine decarboxylase is increasingly in favor of the fetal placenta.

The marked difference in the putrescine-synthesizing capability between fetal and maternal placenta is also reflected in the difference in putrescine content between the two tissues (Table 3). At each of the 3 days examined, i.e. 15, 17 and 19 days of pregnancy, the putrescine content of the fetal placenta ranged from three to eight times that of the maternal placenta.

Rat placenta also contains large amounts of diamine oxidase [21]. The changes in activity during pregnancy are similar to those described for ornithine decarboxylase, i.e. a peak around 18 days followed by a slight decline before parturition. As shown in Table 4, over 80 per cent of the activity is found in the maternal placenta. *S*-adenosyl methionine decarboxylase was also studied during this period. More activity was found in the fetal placenta than in the maternal placenta, but the levels of activity were low compared to ornithine decarboxylase activity.

DISCUSSION

In mammals, putrescine is formed after decarboxylation of ornithine by the enzyme ornithine decarboxylase and this is a key enzyme in the biosynthesis of the polyamines spermidine and spermine. Putrescine is incorporated directly into spermidine, and the propylamine moiety is derived from the decarboxylation of *S*-adenosyl methionine. Many studies have sought to establish a relationship between polyamines and nucleic acids but their precise role is uncertain [22].

Ornithine decarboxylase is widely distributed in tissues, and high levels of activity are found in the regenerating rat liver [23], in certain tumors [15] and in several tissues after hormonal administration [24, 25]. The high levels of ornithine decarboxylase in the rat placenta and its localization to the fetal segment of that tissue provide, therefore, another example of the association of a rapidly growing tissue and high rates of polyamine biosynthesis. In most mammalian systems, putrescine serves mainly as a precursor for spermidine. However, the activity of *S*-adenosyl methionine decarboxylase, and hence the synthesis of spermidine, was very low compared to the activity of ornithine decarboxylase. Since high levels of diamine oxidase are found in the maternal placenta, and putrescine but not spermidine is a substrate for this enzyme, the alignment of metabolic pathways indicates a preference for the metabolism of putrescine via oxidative deamination rather than conversion to spermidine. There is a possibility, therefore, that putrescine may be functionally operative in addition to or in preference to serving as a precursor for spermidine. If this is correct, then spermidine, spermine and putrescine should be regarded as a group of compounds each of which may, under conditions as yet ill-defined, have its own specific function in the regulation of cellular activity.

A similar situation to that observed here for the rat placenta has been reported by Atkins and Beaven [26] in the thymus gland. In that tissue high levels of ornithine decarboxylase are found in the lymphocytes, and diamine oxidase is found in the thymic stroma. These studies, together with those reported in this paper, indicate that diamine oxidase in some instances is probably associated more with putrescine metabolism than it is with histamine metabolism, since in both the thymus [27] and the rat placenta histidine decarboxylase activity is low.

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