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Putrescine Metabolizing Enzyme Activities in Some Rat Tissues during Postnatal Development

JUHANI JÄNNE and ERKKI HÖLTTÄ

Department of Medical Chemistry, University of Helsinki, SF-00170 Helsinki 17, Finland

Four enzyme activities, ornithine decarboxylase (EC 4.1.1.17), *S*-adenosylmethionine decarboxylase, spermidine synthase and diamine oxidase (EC 1.4.3.6), involved in the metabolism of putrescine and spermidine have been followed in some rat tissues during the postnatal development and subsequent ageing of the animal. In thymus and small intestine, known to contain high diamine oxidase activity, ornithine decarboxylase and diamine oxidase activities showed roughly an opposite mode of behavior during the postnatal development of the rat. The activity of diamine oxidase increased markedly at the time ornithine decarboxylase activity decreased. Both S-adenosylmethionine decarboxylase and spermidine synthase activities decreased with age even though the changes were not very marked.

The biosynthesis and accumulation of putrescine and spermidine undergo dramatic changes after application of various growth stimuli, *e.g.* in rat liver after partial hepatectomy,[1–5] and in several target tissues after appropriate hormone administration.[6–10] In general, young and growing tissues are rich in spermidine and putrescine [3,11,12] whereas during ageing of the animal the tissue spermidine concentration markedly decreases.[11] The main role attributed to putrescine in mammalian tissues is to serve as the natural precursor in the synthesis of spermidine. Little is known of the metabolic fate of putrescine aside from its function in spermidine biosynthesis. Radioactive putrescine, when injected into the intact animal, was rapidly oxidized to expired carbon dioxide,[3] however, the mechanisms involved in this oxidation are not known. Recent observations indicate that the enzyme histaminase (also known as diamine oxidase, EC 1.4.3.6) may play a role in the metabolism of putrescine in some mammalian tissues. During pregnancy in the rat there is a marked rise in ornithine decarboxylase activity (EC 4.1.1.17), the enzyme responsible for the synthesis of putrescine in animal tissues, in the fetal placenta with a concomitant increase in diamine oxidase activity in the maternal placenta.[13] This may suggest that the natural substrate for the high levels of diamine oxidase activity found during pregnancy was putrescine rather than hista-

mine.[13] Furthermore, it has also been suggested that putrescine serves as the major substrate for the high diamine oxidase activity found in rat thymus.[14,15]

In the present communication we have followed the changes of enzyme activities directly or indirectly involved in the metabolism of putrescine in specific rat tissues during postnatal development and ageing of the animal. In agreement with earlier observations [14] we found high diamine oxidase activity in rat thymus and in addition found unusually high activity in the small intestine. An opposite pattern of enzyme activity changes was apparent for ornithine decarboxylase and diamine oxidase during the postnatal development. *S*-Adenosylmethionine decarboxylase and spermidine synthase, the enzymes involved in the synthesis of spermidine from putrescine and adenosylmethionine, showed much smaller changes.

MATERIALS AND METHODS

Labelled and unlabelled adenosylmethionine were synthesized and purified according to Pegg and Williams-Ashman.[16] Decarboxylated adenosylmethionine was prepared with the aid of *Escherichia coli* adenosylmethionine decarboxylase and purified on ion exchange columns and by preparative paper electrophoresis as described earlier.[17,18] DL-Ornithine-^{14}C (specific radioactivity 37 mCi/mmol) was purchased from the Radiochemical Centre, Amersham. Putrescine-1,4-^{14}C (specific radioactivity 17.5 mCi/mmol) was purchased from the New England Nuclear Corporation and purified before use on a Dowex 50-H^+ column.[19]

Female rats of the Wistar strain were used in all experiments. The rats were killed by decapitation and the tissues were immediately removed and homogenized with ice-cold 0.25 M sucrose – 0.3 mM EDTA – 1 mM 2-mercaptoethanol. Before homogenization the small intestine was opened and thoroughly rinsed with cold water. The homogenates were centrifuged for 60 min at 100 000 g_{max} and the supernatant fractions were used for enzyme assays.

Diamine oxidase was assayed in the presence of 0.4 mM putrescine-1,4-^{14}C by the method of Okuyama and Kobayashi [20] as modified by Tryding and Willert.[21] The incubation time was 60 min at 37°.

The activity of ornithine decarboxylase was assayed in the presence of 2 mM L-ornithine-^{14}C [22,23] and that of adenosylmethionine decarboxylase in the presence of 0.2 mM adenosylmethionine and 2.5 mM putrescine.[18,24] Spermidine synthase activity was assayed in the presence of 0.5 mM putrescine-1,4-^{14}C and 0.1 mM decarboxylated adenosylmethionine.[18,25]

RESULTS AND DISCUSSION

The different enzyme activities involved in the biosynthesis of putrescine and spermidine together with the activity of diamine oxidase in rat thymus during the postnatal development and subsequent ageing of the animal are shown in Fig. 1. Ornithine decarboxylase activity was found to be remarkably high in young rats; in fact, the maximum activity was found at the age of one month, with a rapid decrease thereafter. The activity of diamine oxidase followed an opposite pattern, *i.e.* a marked increase at the time ornithine decarboxylase activity decreased. Using crude thymic extracts as the source of enzyme the diamine oxidase was roughly characterized. The production of toluene-extractable radioactive material was linear for at least 60 min. The enzyme showed a broad pH optimum between 7.0 and 7.6 in potassium phosphate buffer. An apparent K_m value for putrescine was approximately

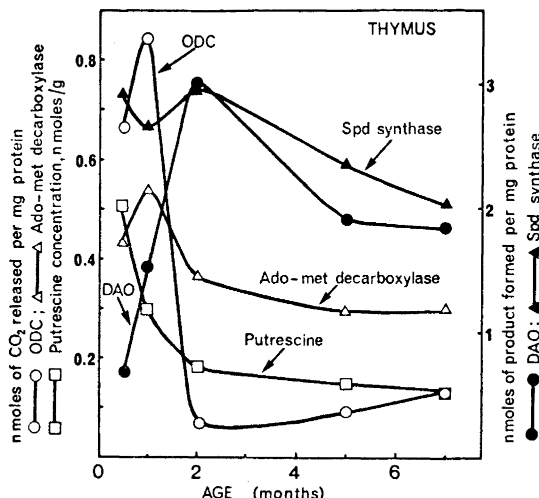

Fig. 1. Diamine oxidase (DAO), ornithine decarboxylase (ODC), *S*-adenosylmethionine (Ado-met) decarboxylase, spermidine (Spd) synthase activities and putrescine concentration in rat thymus during postnatal development. The enzyme activities were assayed under standard incubation conditions as described in Materials and methods. Each value represents a pooled sample obtained from at least six rats.

0.1 mM. Spermidine and spermine were apparently not oxidized by the enzyme since the inclusion of 1 mM spermidine or spermine (unlabelled) to the incubation mixture did not decrease the formation of radioactive product from putrescine-1,4-^{14}C.

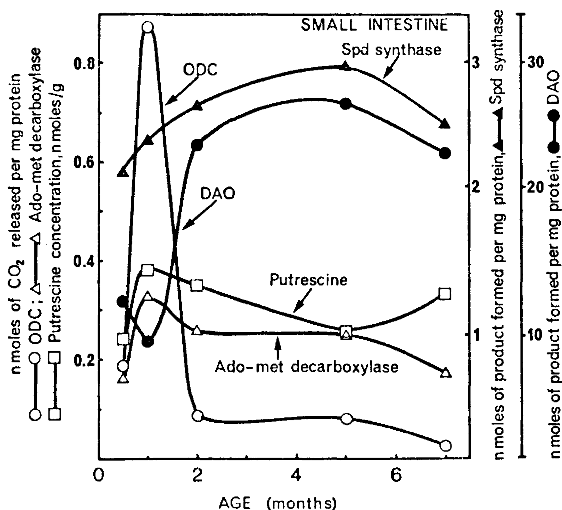

Fig. 2. Putrescine metabolizing enzyme activities and the concentration of putrescine in rat small intestine during postnatal development. For details, see legend of Fig. 1.

Adenosylmethionine decarboxylase, most probably the rate limiting enzyme in the synthesis of spermidine in animal tissues,[26,27] behaved much like ornithine decarboxylase even though the changes in enzyme activity during development were not marked. Spermidine synthase activity seemed to decrease with increasing age. The concentration of putrescine in the thymus continuously decreased with age.

The corresponding enzyme activities in the small intestine are shown in Fig. 2. The pattern of the enzyme activities in this tissue differed only slightly from that found in the thymus; an opposite pattern of ornithine decarboxylase and diamine oxidase activities could be seen again.

It is of interest to note that two tissues, namely thymus and small intestine tissue, which had high ornithine decarboxylase activity also contained high diamine oxidase activity. At the time when ornithine decarboxylase activity dropped most markedly, *i.e.* between the first and the second month of the life, the activity of diamine oxidase increased sharply (Figs. 1 and 2). The role of diamine oxidase activity in the metabolism of putrescine is not known as yet. The changes in ornithine decarboxylase and diamine oxidase activities as well as putrescine concentration in thymus during the postnatal development are interesting (Fig. 1) as the thymus of the young rat appears to contain exceptionally high concentration of putrescine and also high ornithine decarboxylase activity. Between the first and second months of the life the ornithine decarboxylase activity decreased to a fraction of the activity found in young animals and, in addition, the concentration of putrescine was decreased to less than half the concentration found in very young rats. During the same period the activity of diamine oxidase more than tripled. It is possible that the putrescine content of thymus is regulated by the activities of both ornithine decarboxylase and diamine oxidase. The patterns of ornithine decarboxylase and diamine oxidase activities were similar in small intestine

Table 1. Putrescine and spermidine synthesizing enzyme activities and diamine oxidase activity in liver and spleen during the postnatal development of the rat. The enzyme activities are expressed as pmoles of product formed per mg protein per 30 min (ornithine decarboxylase, Ado-met decarboxylase and spermidine synthase) or 60 min (diamine oxidase). Ado-met = *S*-adenosylmethionine. For details, see legend of Fig. 1.

	Age of the animal (months)	Diamine oxidase	Ornithine decarboxylase	Ado-met decarboxylase	Spermidine synthase
Liver	0.5	< 1	111	128	1612
	1	9	87	362	1509
	2	< 1	36	284	1237
	5	5	10	309	1117
	7	< 1	16	234	1229
Spleen	0.5	21	274	365	2232
	1	51	49	325	1932
	2	14	38	192	1344
	5	10	10	174	1518
	7	20	26	139	1315

and thymus; however, it seems more likely that the high diamine oxidase activity in small intestine functions to metabolize exogenous rather than endogenous putrescine.

Liver and spleen contained very low diamine oxidase activities at any time during development (in kidney and brain the activity was not measurable) as compared with the high activity found in thymus and small intestine (Table 1). Also in these tissues (liver and spleen) ornithine decarboxylase and adenosylmethionine decarboxylase activities decreased after the first month of life. The activity of spermidine synthase also tended to decrease with age, however, the changes were not very marked.

In all tissues studied spermidine synthase activity assayed under optimal conditions was much greater than adenosylmethionine decarboxylase activity further suggesting that adenosylmethionine decarboxylase activity together with the concentration of putrescine is the rate limiting step in the enzymatic synthesis of spermidine in mammalian tissues.[27]

The stimulation of putrescine and spermidine synthesis seems to be, almost without exception, associated with a rapid growth of animal tissues. The significance of these phenomena for the general metabolism of an animal cell is not known. However, a better understanding of the metabolism of these compounds may also provide further insight to the physiological function of the polyamines.

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