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Changes in Ornithine Decarboxylase Activity in Rat Intestines during Aging[1]

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SUMMARY

The ornithine decarboxylase (ODC) activity of rat intestines, liver, and brain was found to vary dramatically as animals develop and age. Unusually high activity was present in the small intestines of adult animals. The ODC activity of the small intestines approximated that of fetal tissues and of regenerating rat liver. Putrescine, spermine, and spermidine levels of fetal and adult animal tissues were determined. In all tissues but the stomach mucosa, high putrescine levels correlated with high ODC activity. However, the total polyamine concentrations of the stomach and colon could not be correlated with ODC levels, and no simple relationship between polyamine levels or ODC levels and cellular proliferation in the gut was found.

INTRODUCTION

The metabolism and function of the polyamines, putrescine, spermidine, and spermine, have been the subject of much recent investigation. Although their exact metabolic role remains uncertain, numerous data suggest a correlation between polyamine synthesis and accumulation and renewed or rapid cell growth (3, 22-24).

Increased ODC[3] activity has also been correlated with rapid cell growth. Low ODC levels are generally found in nongrowing tissues and high levels have been found in chick and rat fetuses, amphibian embryos, regenerating liver, and malignant cells (9, 24-26). High levels of ODC activity have been found in several rat sarcoma and hepatoma lines (8, 26), and ODC levels have been correlated with the growth rates of several rat hepatomas (28). In addition, *in vivo* levels of ODC have been shown to be increased by growth stimuli such as growth hormones in the liver (11) and by phytohemagglutinin in cultured cells (14).

We have studied the changes in ODC activity that occur in several rat tissues as the animals develop from fetus to adult. A particular emphasis was placed on the ODC activity of the intestinal epithelial cells, since they have a high proliferative rate in the adult animals. This report shows the changes in ODC activity that occur in the gastrointestinal tract during cellular development and aging and explores further our initial finding of high ODC levels in the small intestines and low colonic levels in adult rats (1). The observed changes in intestinal ODC activity are compared with enzyme level changes occurring in the brain and liver. Some properties of the intestinal enzyme were studied and, in addition, the polyamine levels in adult and fetal animal tissues and in colon tumors were determined.

MATERIALS AND METHODS

Chemicals. The following compounds were purchased from Calbiochem (La Jolla, Calif.): DTT, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer, spermidine, and spermine. Pyridoxal phosphate, ninhydrin spray, and putrescine were obtained from Sigma Chemical Co. (St. Louis, Mo.). The L-[1-^{14}C]ornithine (58 mCi/mmole), [^{14}C]spermidine (122 mCi/mmole), [^{14}C]spermine (112 mCi/mmole), [1,4-^{14}C]putrescine (60 mCi/mmole), and NCS tissue solubilizer were obtained from Amersham/Searle Corp. (Arlington Heights, Ill.). The DL-[5-^{14}C]ornithine (3.29 mCi/mmole) was supplied by New England Nuclear (Boston, Mass.), and the SA-2 cationic resin chromatography paper was from H. Reeve Angel & Co. (Clifton, N. J.). The fluorescamine was purchased from Hoffmann-LaRoche Inc. (Nutley, N. J.).

Animals. Experiments were done using CFN (Wistar) rats obtained from Charles River Breeding Laboratories (New City, N. J.). Newborn and older animals were female, but fetal animals were not sexed and colon tumor samples were obtained from male animals that had received 21 weekly 2-mg/kg s.c. injections of dimethylhydrazine (colonic ODC levels in adult male animals are essentially identical with those in female animals). Partial hepatectomies were done as described by Higgins and Anderson (7) using 200-g animals that were killed 16 to 18 hr after the operation. Adult animals were fed *ad libitum* on a commercial laboratory chow until they were killed by decapitation between the hr of 10 a.m. and 12 noon.

Preparation of Tissue Extract for ODC Activity. The intestinal samples from fetal and newborn animals were homogenized as intact segments in a 0.10 M Tris-HCl (pH 7.35)-1 mM DTT buffer. With older animals, the intestines were removed, placed on an ice-cold block, slit open lengthwise, and rinsed with cold 0.9% NaCl solution; then the epithelial cells were removed by scraping with a glass

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[3] The abbreviations used are: ODC, ornithine decarboxylase; DTT, dithiothreitol.

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microscope slide. The tissues were homogenized in the buffer and then centrifuged for 1 hr at 38,000 × *g*. The supernatant fraction was then used immediately for the enzyme assay.

Assay for ODC Activity. The ODC activity was determined by measuring the production of $^{14}CO_2$ from the carboxyl-labeled ornithine using a modification of the method of Russell and Snyder (25) that has been described by Ball *et al.* (2). In addition, a stoichiometric relationship between the $^{14}CO_2$ release and putrescine formation was demonstrated for the enzyme activity from regenerating liver and intestinal homogenate solutions using L-[1-^{14}C]ornithine and also DL-[5-^{14}C]ornithine as substrates. The enzyme activity is expressed as pmoles of product formed during the incubation period per mg protein in the reaction flask.

Extraction and Separation of Putrescine, Spermine, and Spermidine. Tissue samples (80 to 500 mg, dry weight) were frozen on Dry Ice and then homogenized in acid and the polyamines were extracted into butan-1-ol according to the procedure of Pegg *et al.* (19). The butanol-phase samples were evaporated to dryness on a steam bath and then dissolved in 0.5 ml water. Sample aliquots were applied to precharged SA-2 cationic exchange resin paper.

The chromatograms were run descending for 10 hr with 2 M sodium acetate (pH 4.7) as the developing solvent, a procedural modification of the method of Rinaldini (21). Polyamines in samples and controls were located on the paper by using ninhydrin spray. Samples used for fluorometric assay were not sprayed but were run alongside identical samples that were. The appropriate portions of the chromatograms were cut out, and the samples were eluted from the paper with 4 hr of shaking in 2 ml of 5 N HCl. Samples were brought to dryness twice and then stored in a vacuum desiccator. These samples were resuspended in 0.5 ml of water just prior to mixing with the fluorescamine reagent.

^{14}C-Labeled polyamines (0.1 μCi/sample) added to samples prior to homogenization in acid were extracted and recovered with better than 95% recovery. The chromatographic separation and elution from the paper were done with better than 92% recovery. Polyamine values presented in this report are only corrected for the recovery losses occurring during the chromatography and elution steps.

Fluorometric Determinations of Putrescine, Spermidine, and Spermine. Aliquots (5 to 50 μl) of the polyamine samples were added to 1.5 ml of 0.3 M borate buffer and then 0.5 ml of the fluorescamine reagent (25 mg/100 ml acetone) was rapidly added and mixed. The fluorescence intensity of each sample was determined with a Farrand spectrofluorometer as described previously (27). Putrescine determinations were done using a pH 10.0 borate buffer, and pH 10.5 buffer was used for spermidine and spermine.

Tissue sample determinations were all done in duplicate at 2 different concentrations. Sample values were compared with chromatographed and eluted standards and blanks. Standard samples gave linear results over a determined 0.4- to 65.0-nmole range.

Protein Determinations. The protein determinations were done using the method of Lowry *et al.* (16) with bovine serum albumin as standard.

RESULTS

Some Properties of the Intestinal Enzyme. The ODC activity of the small intestines of adult rats has been found to be remarkably high (1). This activity was, however, found to be dependent upon quick isolation of the epithelial cells and upon a thorough washing of the gut lumen.

The intestinal ODC activity, like that in other tissues, was found to be dependent upon the presence of pyridoxal phosphate. Dialysis of the intestinal supernatant solution against a 0.05 M Tris-HCl-1 mM DTT buffer resulted in a 97% loss of activity that was restored with added pyridoxal phosphate. In addition, as with the liver enzyme, a thiol reagent (1 mM DTT) gave an approximate 2-fold stimulation in intestinal enzyme activity and helped to stabilize the enzymatic activity.

The K_m of ODC for its substrate ornithine was determined using the enzyme supernatant fractions from regenerating liver and from the jejunum and ileum. The enzymes of both sections of the small intestines had a K_m of 31 μM for ornithine. The enzyme fraction obtained from regenerating liver had a K_m of 75 μM for ornithine before dialysis, but after dialysis the value was 30 μM. Dialysis had no effect on the enzyme activity of the small intestines, and it did not change the K_m value for ornithine.

Metabolic Regulation of ODC Activity. A number of compounds were tested for their regulatory effects on the ODC activity of the crude dialyzed supernatant fractions from regenerating liver and from the small intestines. The nucleoside triphosphates, ATP, GTP, CTP, and UTP (2 mM), all had inhibitory effects on the enzymatic activity. GTP had the strongest effect, causing about a 45% inhibition. The di- and monophosphate nucleoside compounds were less effective inhibitors, with the monophosphate compounds being the least effective.

The polyamines, putrescine, spermine, and spermidine, were also found to be inhibitory. At 1 mM concentrations, putrescine and spermine were the most effective, causing a 40% reduction in activity. The polyamine and nucleotide effects were not additive. High Mg^{2+} levels (10 mM) also inhibited the reaction by 30%, but the ion also relieved most of the nucleoside triphosphate inhibition when present at a molar ratio of 2:1 to the triphosphate compound.

Liver and intestinal enzyme fractions gave nearly identical results with all the compounds tested. These preliminary results suggest that the enzymes in these tissues are probably similar. The metabolic effects are not pronounced, however, and may have only limited physiological importance.

Enzyme Activity Changes during Aging. The ODC activity of the brain, liver, and gastrointestinal tract changed dramatically as the animals developed and aged. As previously reported by others (25), total ODC activity was found to be highest in the 11-day-old fetus and then it declined as the fetus developed (Chart 1). Initial assays were done using the total fetus; later the fetal brain, liver, and intestines were isolated and assayed separately. Activity in both the liver and intestines declined rapidly during fetal development and reached a low level just before the animals were born. The activity of the brain declined more slowly. Some increase in the ODC activity of the liver and colon occurred

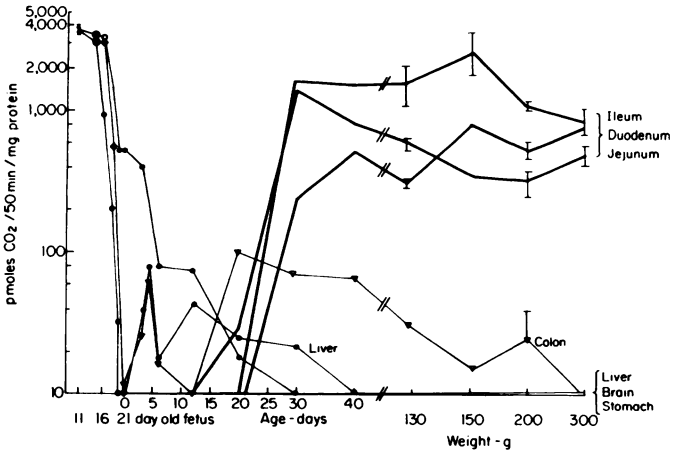

Chart 1. Changes in ODC activity in rat tissues as a function of age. The 1st point (×) represents the value for whole embryos; the next pair of points (●) represent head and body assayed separately (upper value is the head): by 15 to 16 days, the brain (○), liver (●) and fetal intestines (◆) were separated. In newborn to weanling age (21 days), the rat intestines could be divided into stomach, duodenum, jejunum, ileum, and colon (▽) sections. Only with 21-day-old and older animals were epithelial cells removed from the mucosal lining and then assayed. The results are the average of 2 to 6 separate determinations, and a few representative error ranges are shown (±S.E.).

shortly after birth, but the major change occurred in the small intestines of the weanlings. Dramatic increases in the ODC activity of the duodenum, jejunum, and ileum occurred when the animals were about 20 to 25 days old. The high levels that were achieved in the 30 to 40-day-old animals did not change much subsequently as the animals grew older. The ODC activity of the colon and liver peaked earlier and declined steadily, while ODC activity in the stomach was consistently barely measurable.

Putrescine, Spermidine, and Spermine Concentrations. The polyamine levels in the epithelial cells of the gastrointestinal system of adult animals were determined and compared with those in the liver and brain. The polyamine levels of fetal liver and brain and that of malignant colon cells were also determined.

Table 1 shows that, in adult animals, the rapidly proliferating gut tissues had high polyamine levels relative to those in the liver and brain. The 3 segments of the small intestines had spermidine/spermine ratios of 1 or higher, while the stomach and colon cells had spermidine/spermine ratios of 0.3 and 0.7, respectively. The fetal brain and liver also had high polyamine levels compared with those present in the adult animal. In addition, the levels of the polyamines in 2 colon tumors were nearly identical, and they were higher than the levels in normal colon tissue.

As can be seen in Table 2, however, the polyamine levels, especially putrescine levels, cannot be systematically correlated with cellular proliferation rates or the ODC levels present in the tissues. In adult animals, the ODC level in the small intestines is high, and the putrescine level is about 10 times that of the colon, liver, and brain. However, the stomach mucosa also has high polyamine levels despite its low ODC activity.

In addition, the ODC activity of a sample of five carcinogen-induced colon tumors was assayed and was found to average 1564 ± 200 pmoles/mg protein for 50 min *versus* 4 ± 4 for normal colon tissue from animals of the same age. The polyamine levels for the colon tumors were above normal levels but even the putrescine levels were only 6-fold higher than those of normal colon, while ODC levels were nearly 400-fold higher.

DISCUSSION

The level of ODC activity found in the small intestines of adult animals is quite high relative to that present in several other tissues. The enzyme appears to be similar to that found in regenerating liver with respect to its K_m value for ornithine and the regulatory effects of the metabolites that were tested. The regulatory effects of the metabolites on the intestinal and liver enzymes are in general agreement with

Table 1

Putrescine, spermine, and spermidine levels in some rat tissues

Polyamine determinations were done as described in "Materials and Methods;" the results are presented in μmoles/g trichloroacetic acid-precipitable protein. They are the average of 4 to 6 separate determinations ± S.E. The tissue for each separate sample came from 1 to 4 rats, depending upon the tissue used. For fetal material, litter groups were pooled. The 2 colon tumors were from male animals that had been given 21 weekly injections of dimethylhydrazine (2 mg/kg body weight).

	μmoles/g dry wt		
	Spermine	Spermidine	Putrescine
15–16 day fetus			
Liver	1.27 ± 0.24	4.22 ± 0.42	0.44 ± 0.02
Brain	5.11 ± 0.54	7.09 ± 0.30	2.40 ± 0.11
200-g female rats			
Stomach	19.50 ± 2.10	5.45 ± 0.38	0.58 ± 0.10
Duodenum	3.58 ± 0.66	4.41 ± 0.41	0.57 ± 0.07
Jejunum	1.54 ± 0.23	2.65 ± 0.26	0.50 ± 0.06
Ileum	3.96 ± 0.67	3.94 ± 0.80	0.52 ± 0.07
Colon	2.25 ± 0.27	1.84 ± 0.24	0.05 ± 0.01
Liver	0.82 ± 0.10	0.63 ± 0.05	0.03 ± 0.01
Brain	0.41 ± 0.12	0.72 ± 0.20	0.03 ± 0.03
Colon tumor	3.40	3.18	0.34

Table 2

ODC activity and putrescine levels in some rat tissues

Polyamine determinations are as given in Table 1. The ODC activity is given as pmoles/mg protein for 50 min. The enzymatic activity for fetal tissues is the average for 3 litter groups of animals. Six to 8 separate determinations were made with 200-g animals. Values are the average ± S.E.

	ODC activity	Putrescine (μmoles/mg dry wt)
15–16 day fetus		
Liver	851 ± 100	0.44 ± 0.02
Brain	3200 ± 200	2.40 ± 0.11
200-g female rats		
Stomach	3 ± 3	0.58 ± 0.10
Duodenum	541 ± 81	0.57 ± 0.07
Jejunum	334 ± 50	0.50 ± 0.06
Ileum	1063 ± 70	0.52 ± 0.07
Colon	23 ± 15	0.05 ± 0.01
Liver	7 ± 7	0.03 ± 0.01
Brain	4 ± 4	0.03 ± 0.02

the findings of others for liver and prostate enzymes (4, 13, 18).

The increase in ODC activity of the small intestines that occurred during the weanling period is interesting because dramatic developmental changes occur in this tissue at this time. There is an increase in cellular proliferation in the crypts of the intestines, a 4-fold increase in the rate of cell migration from the crypt up to the villi, and an increased differentiation of villus cells (5, 6). In addition, several other enzymes have also been shown to increase in the intestines at this time, although these enzyme changes are generally far less dramatic (5, 29) than the observed changes in ODC activity. The fact that a similar increase in ODC activity does not occur in the large intestines is interesting in that the cellular turnover rate of the colonic mucosa does not change at weaning.

Jänne and Hölttä (10) have also reported increased ODC activity in the small intestines of weanling rats, but they found this increase to be a very temporary one; they did not differentiate between the 3 different sections of the small intestines, nor did they determine colonic levels. The low activity they found in adult animals may have been caused by some delay in isolating the tissue or by inadequate washing of the gut lumen before homogenization of the tissue.

The results of the polyamine determinations are in general agreement with those of others (12, 15, 19, 28), although the assay procedures were not identical. The fetal tissues assayed had high spermidine/spermine ratios and high putrescine levels compared with those of adult animals. The small intestines of adult animals had high ODC activity and high putrescine levels, about 10-fold those of brain, liver, and colon. The stomach mucosa was unique; despite its low ODC activity, it had the highest total concentrations of polyamines and the putrescine levels were just as high as those in the small intestines.

These results show that ODC levels do not always correlate with rates of cellular proliferation. Both the colonic and stomach mucosal cells have high proliferation rates that are comparable to that of the small intestines, but they have low ODC levels. In addition, the polyamine levels do not consistently correlate with ODC levels.

It is not clear how polyamine levels are maintained in the rapidly migrating and turning over colonic and stomach cells. These cells have little ODC activity, yet they are able to maintain polyamine levels similar to or higher than those present in the small intestines. ODC activity may indeed be rate limiting in the polyamine-biosynthetic pathway (20), but it appears that other enzymes in the pathway may also have an important role in regulating polyamine levels. It is likely that ODC levels are related to a variety of cellular phenomena and that the high ODC levels present in the small intestines are not due to the high proliferative rate of the mucosal cells but rather are related to the unique metabolic function of the cells. In addition, it may be that changes in the relative ratios of the polyamines in the cells and the rates of utilization of the various polyamines may be of more importance than the specific concentrations present.

It is intriguing that the colon and stomach, which have low ODC activity relative to the small intestines, also have a high incidence of tumor formation. Recently, O'Brien *et al.* (17) have reported a rapid temporary carcinogen-induced increase in ODC activity in mouse epidermis. Studies in progress in our laboratory suggest that early carcinogen-induced changes in ODC activity of the liver and large intestines can be observed that may be indicative of later malignant transformation.

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