BIOPHYSICS AND BIOCHEMISTRY

Ornithine Decarboxylase and Malignant Growth

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Polyamines, including the diamine putrescine, have piqued the interest of many scientists. Not only have original and review papers dealt with this problem of late, but regional and international meetings have been held and periodicals issued devoted to it [2, 5, 13, 14, 21]. Two circumstances explain this upsurge of interest. First, polyamines seem to be directly involved in the regulation of cell metabolism, in the processes of nucleic acid and protein synthesis among others. Specifically, the presence of a certain polyamine concentration is obligatory during various stages of animal cell mitosis and differentiation [8,12, 15,16,19]. Second, some authors report that a specific level of polyamines both in the course of normal growth and differentiation of eucaryotic cells and in the course of malignant degeneration and malignant growth is maintained and regulated by a specific ratio of polyamine synthesis and degradation enzymes [1,5,9,17,18,21]. Convincing evidence of a drastic increase in the activities of enzymes catalysing polyamine biosynthesis and of a sharp decrease to almost undetectable levels of the activities of polyamine degradation catalyzing enzymes, of diamine oxydase in particular, has been obtained by many researchers [10, 19] and in our laboratory as well [3, 6, 7]. These results are indicative only of the presence of deviations in polyamine metabolism in the course of malignant degeneration, playing a still unknown though possibly significant role in tumor development.

The hypothesis, however, that the key enzymes of polyamine synthesis and degradation and the levels of the polyamines themselves may be useful biological markers of malignant growth and diagnostic tools is not open to question. In view of this, we undertook the present research aimed at elucidating the relationships between tumor cell malignancy and the activity of ornithine decarboxylase (ODC), the key enzyme in polyamine synthesis.

MATERIALS AND METHODS

ODC activity was measured in the liver of intact adult mice and rats, in regenerating liver tissue 16-18 h after partial hepatectomy, in newborn rat liver, chicken embryonal (6-day) tissue, in rat and murine transplanted hepatomas with different growth rates, and in two Morris hepatoma cell lines, strains 8994 and 7777. In addition, the enzymic activity was measured in rat liver tissue in the course of nitrosopiperidine (NP)-induced chemical carcinogenesis, in transplanted rat G-27 hepatoma, and in the liver of tumor-bearing rats fed a vitamin B_6-deficient diet. The time for the preparation of homogenates from all the examined tissues had to be minimized because of the very short half-life of ODC, and the specimens were prepared on ice within several minutes after the

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TABLE 1. Ornithine Decarboxylase Activity in Various Liver and Tumor Tissue Preparations ($M \pm m$, $n=6$)

Tested tissue	Putrescine increment in nmol/h/mg protein
Adult rat liver	0.95±0.15
Newborn rat liver	0.93±0.26
Regenerating rat liver	8.19±1.21
Chicken embryonal (6 days) tissue	16.57±1.57
Morris hepatoma cells, strain 8994	2.81±0.50
The same, strain 7777	3.36±0.47
G−27 rat hepatoma	5.29±1.05
G−27 hepatoma in B_6 deficiency	1.74±0.14
Liver of tumor−bearing rats with B_6 deficiency	0.72±0.10

TABLE 2. Ornithin Decarboxylase Levels in Murine Transplanted Hepatomas with Various Growth Rates ($M \pm m$, $n=6$)

Tested tissue	Putrescine increment in nmol/h per mg protein
Adult mouse liver	0.64±0.11
Hepatoma 22a	0.88±0.20
Hepatoma 60	0.89±0.12
Hepatoma 61	1.80±0.51
Hepatoma 48	1.39±0.32
Hepatoma 46	2.46±0.82

tissues were removed from the body or from the nutrient medium.

ODC activity in the supernatant of the examined homogenized tissue was measured by two methods (spectrophotometrically and fluorometrically) developed in our laboratory [3, 4]. L-ornithine was used as substrate. Enzymatic activity was expressed by the putrescine increment in nmol/h/mg protein; protein was measured by Lowry's method [11].

RESULTS

Table 1 shows a marked rise of the ODC level in all hyperproliferative tissues, including rat regenerating liver tissue, chicken embryonic liver, and Morris hepatoma cells grown in tissue culture, this enzyme level surpassing severalfold that in the liver of adult and even newborn rats.

The highest ODC levels were found in chicken embryonal tissue (18.6±1.57 nmol/h/mg protein) and in regenerating liver tissue (8.2±1.5 U). Another important fact shown in Table 1 is almost the same ODC activity in intact rat liver and in the liver of tumor-bearing rats fed a B_6-deficient diet (0.95±0.15 vs. 0.72±0.10 U, respectively). At the same time, the enzyme activity in tumor (hepatoma G-27) tissue varied considerably if the animals were kept under different conditions: ODC activity in hepatomas in rats fed a B_6-deficient diet was approximately three times lower (1.74±0.14 U) than in hepatomas of rats fed a normal control diet. These data once again confirm the significance of pyridoxal phosphate as an ODC coenzyme.

The data presented in Table 2 indicate appreciable differences in ODC activity in mouse hepatomas in relation to the rate of tumor cell growth. In some slowly growing hepatomas the ODC levels (0.88±0.20 U) were similar to control values (0.64±0.11 U), whereas in rapidly growing tumors (strains 61, 48, and 46) this activity surpassed the control levels two to four times.

ODC activity measurements over the course of NP carcinogenesis (Table 3) showed the maximal values to be attained after nine months of malignant degeneration (0.73±0.12 U), this being followed by a slow drop of the ODC level, which was almost twice lower than its maximum by the end of the experiment (17 months); still, even after 17 months, the ODC content was almost three times as high as its level in intact animal liver. These results, together with our previous findings obtained in a study of nitrosoethyl-induced carcinogenesis [2,3], suggest that a de novo ODC synthesis rather than its activation occurs in the course of malignant degeneration of liver cells.

TABLE 3. Ornithine Decarboxylase Activity in Rat Liver in the Course of Nitrosopiperidine Carcinogenesis (in nmol putrescine in one hour per mg protein, $M \pm m$, $n=8$)

Tested tissue	Period after NP injection, months	ODC levels measured by	
		spectrophotometry	fluorometry
Intact rat liver	9	0.13±0.01	–
Intact rat liver	17	0.17±0.10	0.49±0.13
Rat liver after NP injection	9	0.73±0.12*	–
Rat liver after NP injection	17	0.36±0.05	1.30±0.39*

Note. Asterisk shows values with $p<0.05$.

The results of this research, together with published data [8, 17, 20], indicate a close relationship between ODC activity and polyamine synthesis with respect to cell growth and mitosis. The constant significant increase of ODC suggests a possible specific role of this enzyme in active cell proliferation, and specifically in the process of oncogenesis. ODC participates in cell differentiation and growth regulation, being maximally active in the case of rapid cell growth. Experiments with chemical carcinogenesis have yielded direct experimental evidence of ODC induced synthesis rather than of activation of the initial enzymatic activity. A noticeable reduction of ODC induced synthesis was demonstrated both in the liver and in hepatoma of rats fed a diet deficient in vitamin B_6, this indicating the significance of pyridoxal phosphate as an ODC coenzyme. These data confirm the previously suggested hypothesis that ODC induced synthesis may be regarded not only as an early, but, possibly, as an obligatory biochemical marker of any hyperproliferative process, carcinogenesis included.

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