Polyamines in Normal and Neoplastic Growth

Edited by
Diane H. Russell

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Polyamines in Normal and Neoplastic Growth

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Edited by

Diane H. Russell, Ph.D.
National Cancer Institute
Baltimore Cancer Research Center
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POLYAMINES IN NORMAL AND NEOPLASTIC GROWTH

List of Contributors

Juhani Ahonen
Department of Medical Chemistry, University of Turku, Turku, Finland

Uriel Bachrach
Department of Molecular Biology, Hebrew University, Hadassah Medical School, Jerusalem, Israel

William T. Beck
Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510

Miriam Ben-Joseph
Department of Molecular Biology, Hebrew University, Hadassah Medical School, Jerusalem, Israel

Anne Blackledge
Biochemistry Group, University of Sussex, School of Biological Sciences, Falmer, Brighton, Sussex, England

Craig V. Byus
Department of Biochemistry, University of New Hampshire, Durham, New Hampshire 03824

E. S. Canellakis
Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510

Seymour S. Cohen
Department of Microbiology, University of Colorado Medical Center, Denver, Colorado 80220

Gordon L. Coppoc
Department of Veterinary Physiology and Biochemistry, Purdue University, Lafayette, Indiana 47907

E. Delain
Laboratoire de Microscopie Electronique, Institut Gustave Roussy, 94, Villejuif, France

M. Drue Denton
Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229

Arnold S. Dion
Molecular Biology Section, Institute for Medical Research, Camden, New Jersey 08103

Robert H. Fillingame
Department of Biochemistry, University of Washington, School of Medicine, Seattle, Washington 98195

Robert C. Gallo
Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014

Charles W. Gehrke
Department of Agricultural Chemistry, University of Missouri, Columbia, Missouri 65201

Eduard Gfeller
Department of Anatomy, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Wade Gibson
The University of Chicago, Chicago, Illinois 60637

Helen S. Glazer
Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229

Kenneth D. Graziano
Section of Immunology and Cell Biology, Baltimore Cancer Research Center, Baltimore, Maryland 21211

Luce Gresland
Unité de Biologie Moléculaire, Groupe de Recherche No. 8 du C.N.R.S., Institut Gustave Roussy, 94, Villejuif, France

Bruce Hacker
Division of Oncology, Albany Medical College, Albany, New York 12208

Pekka Hannonen
Department of Medical Chemistry, University of Helsinki, Helsinki, Finland

Sami I. Harik
Department of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Inez A. Hawk
Section on Enzymology and Drug Metabolism, Laboratory of Pharmacology, Baltimore Cancer Research Center, National Cancer Institute, Baltimore, Maryland 21211

Olle Heby
Baltimore Cancer Research Center, National Cancer Institute, Baltimore, Maryland 21211

Edward J. Herbst
Department of Biochemistry, University of New Hampshire, Durham, New Hampshire 03824

Brigid L. M. Hogan
Biochemistry Group, School of Biological Sciences, University of Sussex, Falmer, Brighton, Sussex, England

Erkki Hölttä
Department of Medical Chemistry, University of Helsinki, Helsinki, Finland

J. Huppert
Unité de Biologie Moléculaire, Groupe de Recherche No. 8 du C.N.R.S., Institut Gustave Roussy, 94, Villejuif, France

Juhani Jänne
Department of Medical Chemistry, University of Helsinki, Helsinki, Finland

Leon T. Kremzner
Department of Neurology, College of Physicians & Surgeons of Columbia University, New York, New York 10032

Kenneth C. Kuo
Department of Agricultural Chemistry, University of Missouri, Columbia, Missouri 65201

Phoebe S. Leboy
Department of Biochemistry, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Carl C. Levy
Laboratory of Pharmacology, Baltimore Cancer Research Center, National Institutes of Health, Baltimore, Maryland 21211

Carol-Ann Manen
Department of Zoology, University of Maine, Orono, Maine 04473

Michael R. Mardiney, Jr.
Section of Immunology and Cell Biology, Baltimore Cancer Research Center, Baltimore, Maryland 21211

Laurence J. Marton
Laboratory of Pharmacology, Baltimore Cancer Research Center, Baltimore, Maryland 21211

William E. Mitch
Laboratory of Pharmacology, Baltimore Cancer Research Center, Baltimore, Maryland 21211

Dan H. Moore
Department of Cytological Biophysics, Institute for Medical Research, Camden, New Jersey 08103

David R. Morris
Department of Biochemistry, University of Washington, School of Medicine, Seattle, Washington 98195

Susan Murden
Biochemistry Group, University of Sussex, School of Biological Sciences, Falmer, Brighton, Sussex, England

Donald L. Nuss
Department of Biochemistry, University of New Hampshire, Durham, New Hampshire 03824

Gavril W. Pasternak
Department of Pharmacology, Johns

Hopkins University School of Medicine, Baltimore, Maryland 21205

Pamela Piester
Department of Biochemistry, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Gérard A. Quash
Biochemistry Department, University of the West Indies, Kingston, Jamaica

Aarne Raina
Department of Biochemistry, University of Kuopio, Kuopio, Finland

Bernard Roizman
Departments of Microbiology and Biophysics, The University of Chicago, Chicago, Illinois 60637

Diane H. Russell
Laboratory of Pharmacology, Baltimore Cancer Research Center, National Cancer Institute, Baltimore, Maryland 21211

Ted T. Sakai
Department of Microbiology, University of Colorado Medical Center, Denver, Colorado 80220

Amelia Schenone
Ben May Laboratory, The University of Chicago, Chicago, Illinois 60637

Stephen C. Schimpff
Medicine Section, Baltimore Cancer Research Center, National Cancer Institute, Baltimore, Maryland 21211

Morton Schmukler
Laboratory of Pharmacology, Baltimore Cancer Research Center, Baltimore, Maryland 21211

Nikolaus Seiler
Max-Planck-Institut für Hirnforschung, Arbeitsgruppe Neurochemie, 6000 Frankfurt, Germany

Edward G. Shaskan
Section of Neurosciences, Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island 02912

Richard M. Simon
Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20014

Frank G. Smith
Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229

Solomon H. Snyder
Departments of Pharmacology and Psychiatry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Ching-Hsiang Su
Department of Microbiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

James G. Vaughn
Amino Acid Analyzer Applications Division, Beckman Instruments, Incorporated, Palo Alto, California

T. Phillip Waalkes
Department of Health, Education and Welfare, National Institutes of Health, National Cancer Institute, Bethesda, Maryland 20014

Thomas Walle
Department of Pharmacology, Medical University of South Carolina, Charleston, South Carolina 29401

George Weber
Department of Pharmacology, Indiana University School of Medicine, Indianapolis, Indiana 46202

H. G. Williams-Ashman
Ben May Laboratory, The University of Chicago, Chicago, Illinois 60637

Kwang B. Woo
Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20014

Christopher C. Wylie
Department of Anatomy, University College London, London, England

David C. Zellner
Department of Internal Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45229

Robert W. Zumwalt
Department of Agricultural Chemistry, University of Missouri, Columbia, Missouri 65201

Contents

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Polyamines in Growth—Normal and Neoplastic

Diane H. Russell

Laboratory of Pharmacology, Baltimore Cancer Research Center, National Cancer Institute, Baltimore, Maryland 21211

It is indeed the fulfillment of much hope and effort that brings us together today. We are beginning a two-day program concerning polyamines, small organic cations which are prime candidates for many regulatory roles in the control of the growth process. This is a rather lofty position for the polyamines. These compounds are the most maligned of amines, as they bear names such as putrescine, spermidine, and spermine. These symbols immediately invoke two images, one being putrefaction, the other being male genital function. The early work on polyamines left many biochemists with the impressions that these cations were the end product of a degradative pathway and that the instances of polyamine occurrence in mammalian systems were keyed to bacterial decay or to excretion into seminal fluid. The early efforts of Celia and Herbert Tabor of the National Institutes of Health in elucidating the biosynthetic pathway in bacteria and the work of Seymour Cohen and his group in linking polyamine biosynthesis to RNA metabolism have provided the backbone for the expansion of polyamine research into the mammalian system (1, 2). This expansion was further catalyzed by an article by Dykstra and Herbst (3) expressing the relationship between spermidine synthesis and RNA synthesis in regenerating rat liver. Somehow the stigma of polyamines being involved in decay began to fall away as Dykstra and Herbst showed that the uptake of putrescine and its conversion into spermidine in partially hepatectomized rats was a major event. The large accumulation of spermine in seminal fluid has never been explained and remains one of the unanswered questions.

My own interest in polyamine research occurred during my collaboration with Dr. Solomon Snyder. We were intrigued by the role of histidine decarboxylase in the rapid growth process as postulated by Kahlson (4). In discussion, we postulated that if histidine decarboxylase, an enzyme which forms histamine, a diamine, is important in rapid growth, this should be greatly enhanced in all rapid-growth systems. However, studies had indicated that this was not true. Could it be that histamine serves a function in certain rapidly growing tissues which could be served in other tissues by polyamines? Therefore, we looked at the first enzyme in the polyamine biosynthetic pathway in regenerating rat liver. In a pilot experiment, we assayed ornithine decarboxylase activity in the liver of sham-hepatectomized rats and in the liver of rats that had undergone a partial hepatectomy 24 hr prior to sacrifice. Results were rewarding. It appeared that ornithine decarboxylase activity was very low in the liver of normal rats: the counts were in the range of 200 to 400 cpm. However, the counts for the first 24-hr regenerating liver sample were around 20,000.

After finding this dramatic increase in ornithine decarboxylase activity in regenerating rat liver (5), which is rather unusual since mammalian enzymes usually fluctuate a few-fold and rarely 25-fold such as found for ornithine decarboxylase, I was astounded to find in the literature how widely polyamines had been implicated in cell regulation. The quotation that comes to mind appears in Seymour Cohen's book *The Introduction to the Polyamines* (2) at the beginning of Chapter 1: "All this has been said before—but since nobody listened, it must be said again" (André Gide). It seemed reasonable to suppose that early increases in ornithine decarboxylase activity which lead to such dramatic increases in the putrescine and spermidine pools in growing tissues had to be of great importance. First, polyamines had been implicated in growth processes. Herbst and his collaborators had found that certain bacterial mutants exhibit absolute requirements for polyamines (6). Further, polyamines were implicated by Seymour Cohen and others in the regulation of RNA synthesis (7, 8).

To summarize, then, we found that increased ornithine decarboxylase activity was one of the earliest, marked events that occurs after partial hepatectomy in the rat. Its increased activity appears to parallel the early increase in RNA synthesis, and precedes by many hours the maximal DNA synthesis that occurs in regenerating rat liver (9). We also found a close relationship between ornithine decarboxylase activity and the initiation of rapid growth in chick embryos and tumors (5).

The ability of ornithine decarboxylase activity to fluctuate rapidly in response to the introduction or withdrawal of stimuli suggests that putrescine synthesis is under strict modulation. The rapid turnover rate of hepatic ornithine decarboxylase is the most striking example of this modulation.

We found that ornithine decarboxylase activity declined rapidly in unoperated rats or in hepatectomized rats after cycloheximide administration. The decline had an estimated half-life of 11 min (10). To my knowledge, this is the most rapidly turning over mammalian enzyme known. Further, estimating the half-life of ornithine decarboxylase after growth hormone stimulation and decline, without any inhibitors, led to a similar estimation of a half-life of less than 20 min (11). The only evidence lacking, of course, was evidence of the turnover rate of the purified enzyme. Since ornithine decarboxylase has not been purified to homogeneity, it was impossible to do studies on the purified enzyme. This rapid turnover rate is of great importance because the synthesis of most mammalian enzymes is a linear function of time, whereas enzyme degradation is an exponential function of time. Therefore, rates of change of enzyme levels from one steady state to another are determined solely by the degradation rate of the enzyme. The very high degradative rate of ornithine decarboxylase suggests that its activity changes rapidly in response to stimuli for new synthesis. Taken together, these data suggest that polyamine synthesis is a finely modulated process. Moreover, they suggest that this kind of sensitive regulation of synthesis would be necessary only to control the level of compounds important in the cell stimulatory system. This is of further importance when you consider that in most mammalian tissues there are not known enzymes that degrade or metabolize the polyamines. Therefore, an overproduction of the polyamines could lead to an elevated growth rate for a particular tissue or organ. This could be catastrophic in an adult mammal, since most of the tissues and organs are in dynamic equilibrium, and are not growing *per se*. In the mammalian organism, exceptions to this generalization are proliferating surfaces such as the gut, secreting organs such as the pancreas, and abnormal growths such as cancers (discussed in detail later).

I. HORMONAL REGULATION OF POLYAMINE BIOSYNTHESIS

If polyamine biosynthesis is necessary for growth processes to occur, it should be expected that this biosynthesis would be affected by hormones that regulate growth. Indeed, this is true. Castration in the rat results in a rapid decrease in both ornithine decarboxylase activity and S-adenosyl-L-methionine decarboxylase activity in the rat ventral prostate. When testosterone is administered to the castrated rat, there is a rapid increase in the activities of both ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase (12). In young rats, ornithine decarboxylase activity exhibits an early dramatic induction after growth hormone administration, followed later by a substantial increase in the level of S-adenosyl-L-methionine decarboxylase activity (11, 13, 14). *De novo* synthesis appears to be involved in the enzyme inductions since the administration of RNA and protein inhibitors suggest that both protein synthesis and DNA-dependent

RNA synthesis are necessary for these elevations to occur. Enhancements in the biosyntheses of putrescine, spermidine, and spermine can be shown also in the castrated rat uterus after estradiol administration (15–17). Further, it has been reported that cortisone has an effect on hepatic ornithine decarboxylase activity (18). The mammary gland, which is under strict hormonal control and which can be cycled through growth, lactation, and involution, exhibits intensive polyamine biosynthesis and accumulation during pregnancy and lactation, with the concentration of spermidine reaching levels above 5 mM during midlactation (19). If the number of suckling young is decreased by removing them from the mother, the amount of spermidine drops precipitously and is concomitant with dramatic drops in the amount of RNA present. To my knowledge, there are no growth processes that occur without prior stimulation of polyamine biosynthesis.

II. EMBRYONIC SYSTEMS

One of the compelling reasons to study polyamine biosynthesis and accumulation in embryonic systems is to assess polyamine metabolism in a maximally responding system. The other compelling reason, however, is to understand the growth process *per se*, which is best exemplified here. Further, the embryonic system, considered a normal growth system, most nearly parallels tumor systems. That is, "Resemblance of hepatoma to fetal tissues indicates some resemblance of all tumors to all fetal tissues, a general tendency that can be called the fetalism of tumors" (20). It appears that tumors and fetal systems resemble each other because both fetal tissues and tumors tend to be undifferentiated and therefore exhibit very similar enzyme patterns. Knox (20) states that "undifferentiated tumors are very similar to each other, as similar as some fetal tissues are to one another." Organ-specific components and great diversity disappear in undifferentiated tumors, and are only present in the highly differentiated tumors which are rather rare. These concepts which stress the similarities of tumors and fetal systems are gaining more acceptance from the scientific community. A recent report indicated that several human tumors contain the fetal form of thymidine kinase in contrast to other human tissues which have only a postnatal thymidine kinase (21). Therefore, the finding that polyamine biosynthesis and accumulation is an early marked event in all types of embryos [chick (22, 23), toad (24, 25), rat (26), and sea urchin (27, 28)] has implications for the understanding and the control of the cancer process. The same rapid synthesis and accumulation of polyamines that is exhibited by embryos is also exhibited by tumors in early growth stages (29). An effective inhibitor of putrescine synthesis or spermidine synthesis would appear to be an ideal cancer chemotherapeutic agent. If the levels of polyamines of a particular system could be lowered, it should decrease the viability of the tumor substantially.

We have screened a large number of analogues of ornithine *in vitro* for their ability to inhibit ornithine decarboxylase activity from 24-hr regenerating rat liver. Most of these analogues contained ring structures attached to the delta amino group. Only two were even moderately good inhibitors *in vitro*, α-methyl ornithine (Table 1) and *n*-methyl ornithine. At 10^{-3} M, *n*-methyl ornithine resulted in a 40% inhibition of ornithine decarboxylase. Neither of these analogues changed the ornithine decarboxylase activity during the course of L1210 leukemia in mice nor resulted in an increased survival rate for those leukemic mice receiving the drug(s).

TABLE 1. *Effect of an ornithine analogue on ornithine decarboxylase activity of 24-hr regenerating rat liver*

Inhibitor	Concentration of inhibitor (M)	% Inhibition
α-Methyl ornithine	0	0
	10^{-7}	5.3
	10^{-6}	13.3
	10^{-4}	40.5
	10^{-3}	64.6

III. PHYSIOLOGICAL SIGNIFICANCE OF POLYAMINES

It has been stated before that the accumulation of polyamines is concomitant with RNA synthesis and, of course, with protein synthesis. The correlation of polyamine synthesis with RNA synthesis is parallel in so many systems that it is hard to believe at this point that there is not a cause and effect relationship. Probably because of the tight relationship between RNA synthesis and DNA synthesis in certain systems, there appears at times to be a relationship between polyamine concentrations and DNA synthesis. However, there are systems in which you can uncouple RNA synthesis and DNA synthesis, such as in the heart undergoing hypertrophy after constriction of either the aortic or the pulmonary artery; in this case there is extensive polyamine accumulation which again correlates with RNA synthesis, but there is no concomitant DNA synthesis (30, 31).

The first clue that has come from our work as to one possible physiological role for the polyamines comes from work on developing *Xenopus laevis*. There is an anucleolate mutant of *X. laevis* which is unable to make riboso-