Advances in Lipobiology
Volume 1 · 1996

ADVANCES IN LIPOBIOLOGY

Editor: RICHARD W. GROSS
Department of Bioorganic Chemistry
and Molecular Pharmacology
Washington University School of Medicine
St. Louis, Missouri

VOLUME 1 • 1996

JAI PRESS INC.

Greenwich, Connecticut *London, England*

55 Old Post Road, No. 2
Greenwich, Connecticut 06836

JAI PRESS LTD.
38 Tavistock Street
Covent Garden
London, WC2E 7PB
England

ISBN: 1-55938-635-5

Manufactured in the United States of America

ADVANCES IN LIPOBIOLOGY

Volume 1 • 1996

LIST OF CONTRIBUTORS

Rosa J. Buxeda	Department of Food Science Rutgers University New Brunswick, New Jersey
George M. Carman	Department of Food Science Rutgers University New Brunswick, New Jersey
Rosalind A. Coleman	Department of Pediatrics Duke University Medical Center Durham, North Carolina
Rosemary Cornell	Department of Chemistry Simon Fraser University Burnaby, British Columbia, Canada
David A. Ford	Department of Internal Medicine Washington University School of Medicine St. Louis, Missouri
Rene A. Frenkel	Department of Biochemistry University of Texas Southwestern Medical School Dallas, Texas
John A. Glomset	Howard Hughes Medical Institute Research Laboratories University of Washington Seattle, Washington
Richard W. Gross	Department of Bioorganic Chemistry and Molecular Pharmacology Washington University School of Medicine St. Louis, Missouri

Tada-atsu Imaizumi
Department of Internal Medicine and Biochemistry
University of Utah School of Medicine
Salt Lake City, Utah

Suzanne Jackowski
Department of Biochemistry
St. Jude Children's Research Hospital
Memphis, Tennessee

John M. Johnston
Department of Biochemistry
University of Texas Southwestern Medical School
Dallas, Texas

James B. Lefkowith
Department of Medicine
Washington University School of Medicine
St. Louis, Missouri

Michelle Lennartz
Departments of Physiology and Cell Biology
Albany Medical College
Albany, New York

Christina C. Leslie
Department of Pediatrics
National Jewish Center
Denver, Colorado

Dennis C. Liotta
Department of Chemistry
Emory University
Atlanta, Georgia

Thomas M. McIntyre
Department of Internal Medicine and Biochemistry
University of Utah School of Medicine
Salt Lake City, Utah

Alfred H. Merrill, Jr.
Department of Biochemistry
Emory University School of Medicine
Rollins Research Center
Atlanta, Georgia

Hisashi Narhara
Department of Biochemistry
University of Texas Southwestern Medical School
Dallas, Texas

Joseph T. Nickels, Jr.
Department of Food Science
Rutgers University
New Brunswick, New Jersey

Norman S. Radin
Nephrology Division
University of Michigan Medical Center
Ann Arbor, Michigan

Sasanka Ramanadham
Washington University School of Medicine
St. Louis, Missouri

Ronald T. Riley
Toxicology and Mycotoxins Research Unit/USDA-ARS
Athens, Georgia

Charles O. Rock
Department of Biochemistry
St. Jude Children's Research Hospital
Memphis, Tennessee

Richard Schulz
Departments of Pediatrics and Pharmacology
University of Alberta
Edmonton, Alberta, Cananda

Diana M. Stafforini
Department of Internal Medicine
University of Utah School of Medicine
Salt Lake City, Utah

Pamela J. Trotter
Department of Medicine
National Jewish Center
Denver, Colorado

John Turk
Washington University School of Medicine
St. Louis, Missouri

Dennis Voelker
Department of Medicine
National Jewish Center
Denver, Colorado

Robert A. Wolf
Department of Medicine
Washington University School of Medicine
St. Louis, Missouri

Yoshiji Yamada
Department of Internal Medicine and Biochemistry
University of Utah School of Medicine
Salt Lake City, Utah

Steven H. Zeisel
Department of Pediatrics
Duke University Medical Center
Durham, North Carolina

Guy A. Zimmerman
Department of Internal Medicine and Biochemistry
University of Utah School of Medicine
Salt Lake City, Utah

PREFACE

The last decade has witnessed explosive advances in our understanding of the role of membranes, lipid second messengers, and lipid metabolism in the molecular mechanisms regulating cell growth, differentiation, and ligand-regulated cellular activation. In large part, these insights have been facilitated by the exploitation of new methodologies including genetic engineering (molecular biology), analytical instrumentation (biophysical chemistry), and computer technology (molecular modeling and drug design). While the application of these methodologies to their parent disciplines has resulted in the easily detectable emergence of new concepts and principles, their central role in the growth of lipobiology has been less evident, especially to students embarking on new scientific careers. Accordingly, it was envisaged that a series of cohesive didactic discussions identifying rapidly-evolving scientific arenas in lipobiology would serve to illustrate the dynamic nature of the field and encourage students to participate in this rapidly-evolving discipline focused at the interface of lipid chemistry and biology. This series was created to provide a forum for leading scientists in the field of lipobiology to: (1) broadly interpret the potential significance of recent findings in the area of lipid structure and function, (2) identify what does (and what does not) constitute "proof of concept," and (3) provide a critical foundation for evaluation of experimental results and strategies in these rapidly evolving arenas. While some historical information has been presented, the purpose of this series is to provide a medium for discussion of emerging concepts by experts in the field.

It is my sincere hope that the extensive efforts by the contributors in this edition to illustrate the recent growth in the scope of investigations in lipid research will be recognized by the next generation of scientists and encourage them to fulfill the promise of our conjoint expectations.

Richard W. Gross
Editor

CONTENTS

REGULATION OF MAMMALIAN CTP:
PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE

Rosemary B. Cornell

Advances in Lipobiology
Volume 1, pages 1–38.

ISBN: 1-55938-635-5

I. ROLE OF CT IN PC SYNTHESIS

CTP:phosphocholine cytidylyltransferase (EC. 2.7.7.15, CT) is an important regulatory enzyme in phosphatidylcholine (PC)[1] metabolism. It catalyzes the transfer of a cytidyl group from CTP to phosphocholine to form CDPcholine, the head group carrier molecule. CDPcholine is subsequently attacked by diacylglycerol (DAG), releasing CMP and forming PC. This pathway for forming PC is the dominant pathway in all animal cells (see Figure 1).

A. CT Catalyzes a Rate-Limiting Reaction

The ratio of the metabolite concentrations in a pathway can indicate the slowest step. The pool sizes of the choline containing metabolites in the CDPcholine pathway have been analyzed in liver (Sundler et al., 1972), lung (Post et al., 1984), skeletal muscle cells (Sleight and Kent, 1980), HeLa cells (Vance et al., 1980; Wang

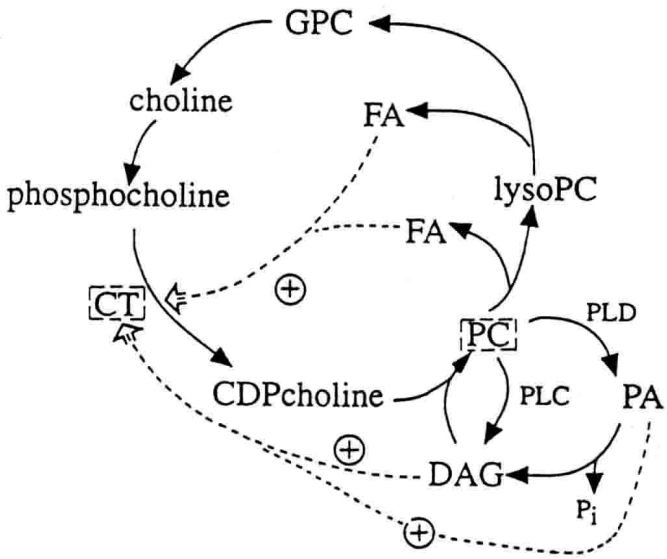

Figure 1. · CT is activated by at least three products of PC catabolism. The evidence for activation by PA is based on *in vitro* effects only.

et al., 1993a), and several other cell lines (Cornell and Goldfine, 1983; Sleight and Kent, 1983a; Tessner et al., 1991). The ratio of phosphocholine to CDPcholine ranges from 10 (Post et al., 1984) to 150 (Wang et al., 1993a). This indicates a bottle-neck at the CT catalyzed reaction. Secondly, ^{3}H-choline pulse-chase studies have directly indicated that the conversion of phosphocholine to CDP-choline is the slowest step in the pathway (Vance et al., 1980; Pritchard and Vance, 1981; Post et al., 1982; Cornell and Goldfine, 1983). In addition there are several examples of changes in the rate of PC synthesis which correlate with changes in the relative ratio of phosphocholine:CDPcholine or changes in the turnover rate of phosphocholine. For example, fatty acid stimulation of PC synthesis in HeLa cells caused a decrease in phosphocholine and an increase in CDP-choline such that the ratio decreased from 150 to 12 (Wang et al., 1993a). Phospholipase C (PLC) treatment of chick myoblasts stimulated PC synthesis and caused a 60% decrease in phosphocholine and a 2.5-fold increase in CDPcholine (Sleight and Kent, 1980). PC synthesis was elevated in lung from prematurely born rats due to activation of CT. The phosphocholine pool size decreased at least fourfold (Possmayer et al., 1981; Weinhold et al., 1982). Inhibitors of cholesterol synthesis inhibited PC synthesis in L_6 myoblasts and led to an increase in the ratio of phosphocholine:CDPcholine, and an increase in the turnover of phosphocholine (Cornell and Goldfine, 1983). Poliovirus infection (Vance et al., 1980) or phorbol ester (Paddon and Vance, 1980) stimulated PC synthesis twofold in HeLa cells and increased the phosphocholine turnover rate twofold.

B. Evidence for Other Rate-Limiting Steps

The reaction catalyzed by CT may not be the rate-limiting step under every condition. The concentration of DAG, the substrate for the terminal reaction, can be the rate-limiting factor. PC synthesis was inhibited when hepatocytes were treated with cAMP analogues, however there was no effect on CT activity. Rather, the DAG content of cellular membranes decreased probably due to inhibition of fatty acid production (Jamil et al., 1992). Replenishment of the DAG restored the PC synthesis rate in a direct concentration-dependent manner. Secondly, PC synthesis was stimulated only threefold when CT was over-expressed in COS cells, although the amount of the active form of CT increased nearly 20-fold. The CDPcholine concentration increased 12-fold, indicative of a bottleneck at the terminal step. Increasing the supply of DAG stimulated PC synthesis fourfold (Walkey et al., 1994).

The choline kinase catalyzed step can also be rate limiting. This conclusion is based on a change in the specific activity of choline kinase that correlates with the change in the PC synthesis rate (e.g., Warden and Friedkin, 1985), or a change in the ratio of choline:phosphocholine in a direction opposite to that of choline flux. Regulation of PC synthesis by choline kinase or the supply of choline has been recently reviewed (Tijburg et al., 1989; Kent et al., 1991).

II. ROLE OF CT IN CONTROL OF LIPID SECOND MESSENGER CONCENTRATIONS

A. CT Controls the PC Metabolic Cycle

PC is the source of DAG production via phospholipase D and phosphatidic acid phosphatase in response to bombesin, epinephrine, vasopressin, cholecystokinin, and other agonists (Billah and Anthes, 1990). In these pathways, phosphatidylinositol bisphosphate- (PIP_2) specific PLC generates the first wave of DAG production, followed by more sustained production of DAG via the hydrolysis of PC. PC is also the immediate precursor to DAG via PC-specific PLC in response to interleukins 1 or 3, tumor necrosis factor α, interferon-α, and colony stimulating factor-1 (Liscovitch, 1992). In these latter signal transduction pathways no PIP_2 hydrolysis occurs; rather PC seems to be the sole generator of DAG. PC is also a source of arachidonic acid via PLA_2. Protein kinase C (PKC) appears to be both required for and activated by the sustained production of second messengers from PC. For the long-term effects of PKC activity, a prolonged production of DAG would be needed. If enhanced PC hydrolysis were to continue for several hours without any stimulation of PC synthesis this would likely lead to a fatal reduction in membrane PC content. However, in every system investigated the stimulation of PC catabolism results in an acceleration of synthesis (e.g., Sleight and Kent, 1980; Guy and Murray, 1982; Lacal, 1990). This and other data (Terce et al., 1991; Tijburg et al.,

1991; Walkey et al., 1994) provide strong evidence for a tightly controlled PC metabolic cycle in which synthesis and degradation are closely coupled (Pelech and Vance, 1989; Tronchère et al., 1994). Stimulation of PC synthesis usually involves activation of CT. Three of the products of degradation of PC are known activators of CT: fatty acids, DAG, and PA (Figure 1). In this way CT plays a regulatory role in the maintenance of PC homeostasis in cells activated by a wide variety of agonists.

B. CT Attenuates the Agonist-Induced DAG Signal

CT may also be involved in attenuation of the DAG signal. DAG can be metabolized by lipase to produce fatty acids, by kinase to produce PA, by acyltransferase to produce triacylglycerol, or by phosphotransferases to produce PE or PC. The importance of the phosphotransferase pathway in the metabolism of DAG has been illuminated recently. DAG accumulates in liver of rats in which PC synthesis has been inhibited by deprivation of choline (Blusztajn and Zeisel, 1989). Similarly, in pancreatic acini, inhibition of PC synthesis by cholecystokinin resulted in increased DAG levels, suggesting that the major clearance route for DAG in these cells is via incorporation into PC (Matozaki et al., 1991). Using labeled long-chain DAG species introduced into 3T3 fibroblasts by liposome fusion, Florin-Christensen et al. (1992) found that the predominant metabolic destiny of [1–18:0, 2–20:4]-DAG was PC. The DAGs appeared to be incorporated intact into PC, that is, without first being degraded to the free fatty acid. Increases in [DAG] stimulate DAG kinase or lipase by substrate level control, whereas increases in [DAG] may stimulate the synthesis of PC at the substrate level and/or by allosteric activation of CT. DAG has been shown to activate CT both *in vitro* (Cornell and Vance, 1987a,b; Cornell, 1991b) and *in vivo* (Rosenberg et al., 1987; Kolesnick and Hemer, 1990; Slack et al., 1991). Thus increases in [DAG] would accelerate the formation of CDPcholine, the rate-limiting substrate in the pathway, and thus the rate of the CPT-catalyzed reaction, which converts DAG to PC.

III. CT IS A REGULATED ENZYME

The activity of CT is regulated by many factors. The addition to cultured cells of phorbol esters (Pelech et al., 1984a), phospholipases (Sleight and Kent, 1980), fatty acids (Pelech et al., 1984b), diacylglycerol (Utal et al., 1991), calcium ionophore (Sanghera and Vance, 1989a), and CSF-1 (Tessner et al., 1991) all lead to stimulation of CT activity. Glucocorticoid or estrogen treatment also activates CT in developing lung (Possmayer et al., 1981; Chu and Rooney, 1985; Rooney et al., 1990; Xu et al., 1990). Treatment of cells with okadaic acid (Hatch et al., 1992), cholesterol synthesis inhibitors (Cornell and Goldfine, 1983), cholecystokinin (Matozaki et al., 1991), alkyl phosphocholine (Geilen et al., 1992), and transfection with H-*ras* (Teegarden et al., 1990) inhibit CT activity. Are these agents acting via