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

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LIST OF CONTRIBUTORS

Darrell D. Belke

Cardiovascular Disease Research Group
Faculty of Medicine
The University of Alberta
Edmonton, Alberta, Canada

Merle L. Blank

Oak Ridge Associated Universities
Medical Sciences Division
Oak Ridge, Tennessee

L. Maximilian Buja

Department of Pathology
and Laboratory Medicine
The University of Texas Medical School
Houston, Texas

Patrick C. Choy

Department of Biochemistry and
Molecular Biology
Faculty of Medicine
The University of Mannitoba
Winnipeg, Mannitoba, Canada

David L. DeWitt

Department of Biochemistry
Michigan State University
East Lansing, Michigan

William Dowhan

Department of Biochemistry and
Molecular Biology
The University of Texas Medical School
Houston, Texas

Math J.H. Geelen

Department of Veterinary Basic Sciences
Division of Biochemistry
Utrecht University
Utrecht, The Netherlands

Howard Goldfine
Department of Microbiology
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania

Yusuf A. Hannun
Department of Medicine
Duke University Medical Center
Durham, North Carolina

D. Corinne Harrison-Shostak
Department of Cell Biology and Anatomy
University of North Carolina, Chapel Hill
Chapel Hill, North Carolina

Grant M. Hatch
Department of Biochemistry and
Molecular Biology
Faculty of Medicine
The University of Mannitoba
Winnipeg, Mannitoba, Canada

Brian Herman
Department of Cell Biology and Anatomy
University of North Carolina, Chapel Hill
Chapel Hill, North Carolina

Supriya Jayadev
Department of Medicine
Duke University Medical Center
Durham, North Carolina

Ten-ching Lee
Oak Ridge Associated Universities
Medical Sciences Division
Oak Ridge, Tennessee

John J. Lemasters
Department of Cell Biology and Anatomy
University of North Carolina, Chapel Hill
Chapel Hill, North Carolina

Gary D. Lopaschuk
Cardiovascular Disease Research Group
Faculty of Medicine
The University of Alberta
Edmonton, Alberta, Canada

Ricky Y.K. Man
Department of Biochemistry and
Molecular Biology
Faculty of Medicine
The University of Mannitoba
Winnipeg, Mannitoba, Canada

Janet H. Mar

Department of Medicine
The University of Texas Health Science Center
Houston, Texas

Jeanie B. McMillin

Department of Pathology
and Laboratory Medicine
The University of Texas
Health Science Center
Houston, Texas

Joseph C. Miller

Department of Pathology
and Laboratory Medicine
The University of Texas Medical School
Houston, Texas

Anna Liisa Nieminen

Department of Cell Biology and Anatomy
University of North Carolina, Chapel Hill
Chapel Hill, North Carolina

William L. Smith

Department of Biochemistry
Michigan State University
East Lansing, Michigan

Lilian B.M. Tijburg

Unilever Research Laboratory
Vlaardingen
Vlaardingen, The Netherlands

Fred Snyder

Oak Ridge Associated Universities
Medical Sciences Division
Oak Ridge, Tennessee

Lambert M.G. van Golde

Department of Veterinary Basic Sciences
Division of Biochemistry
Utrecht University
Utrecht, The Netherlands

P. Sebastiaan Vermeulen

Department of Veterinary Basic Sciences
Division of Biochemistry
Utrecht University
Utrecht, The Netherlands

Moseley Waite

Department of Biochemistry
Wake Forest University Medical Center
Winston-Salem, North Carolina

Haichao Wang

Department of Cell Biology and Anatomy
University of North Carolina, Chapel Hill
Chapel Hill, North Carolina

Xue Feng Wang

Department of Cell Biology and Anatomy
University of North Carolina, Chapel Hill
Chapel Hill, North Carolina

PREFACE

During the last several years substantial growth in the research of lipid metabolism and lipid second messengers has occurred. In large part, these advances have resulted from the application of a wide variety of molecular biologic techniques to the examination of the kinetics of lipid metabolic enzymes and the study of structure-activity relationships on cellular physiologic function. Our knowledge of the importance of covalent modification of lipid synthetic and metabolizing enzymes in intact cells has expotentially expanded and studies on the role of specific regulatory aspects of lipid metabolism can now be routinely performed in molecular detail. Through the cloning, engineering, and insightful execution of critical experiments, the field has witnessed the refinement of old concepts and the emergence of new ideas and new paradigms. As always, it is through the careful testing of these new hypotheses and the reexamination of prior dogma that the next decade will witness an ever greater penetrance of the depth of our scientific understanding of lipid metabolism, membrane structure and function, and the mechanisms of generation of lipid second messengers in intact cells after cellular stimulation. While a change in the political, economic, and social climate has indeed refocused and restructured many practical aspects of this process of investigation, the emergence of new technologies has facilitated our dealing with these pressures to result in a collective enterprise whose progress in many respects surpasses that manifest in years past. The power of the scientific approach is ever increasingly fueled by the force of novel technologies and the innovation of human spirit. The field of membrane biochemistry and lipid metabolism continues to expand in new

directions thrust upon us by a new generation of students and investigators and it is this infusion of new thoughts and new ideas that will assure the continued growth of our field in the years to come.

Richard W. Gross
Editor

CONTENTS

RELATIONSHIP OF LIPID ALTERATIONS AND IMPAIRED CALCIUM HOMEOSTASIS DURING MYOCARDIAL ISCHEMIA

L. Maximilian Buja and Joseph C. Miller

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I. INTRODUCTION

Coronary heart disease can lead to a major reduction in myocardial blood flow which induces severe myocardial ischemia due to oxygen and substrate deprivation (Reimer and Jennings, 1986). These profound metabolic alterations lead to damage of the cardiac myocyte (Figure 1). Progressive alterations in myocardial electrolytes, including deranged calcium homeostasis, are characteristic of the pathogenesis of ischemic injury in the heart (Buja et al., 1988). There is also considerable evidence that progressive derangements in lipid metabolism are involved in the development of membrane damage and altered membrane function (Buja, 1991). In addition, changes in the cytoskeleton lead to destabilization of the membranes making them susceptible to rupture from cell swelling or other mechanisms (Buja et al., 1993). The damage may be produced either by abnormal actions of hydrolytic enzymes, such as phospholipases and/or proteases, by chemical or physical disruption, or by a combination of these factors. From the cumulative body of clinical and experimental observations, one must conclude that damage to cell membranes plays a key role in the transition from reversible to irreversible myocardial injury (Reimer and Jennings, 1986). Unlike apoptosis, the critical determinant of necrotic cell death produced by ischemia involves irreversible damage to the integrity and function of cellular membranes (Buja et al., 1993). In contrast, apoptotic cell death involves primary intracellular events, including activation of proteases and nuclear damage due to activation of an endonuclease and subsequent double stranded DNA cleavage (Buja et al., 1993; Patel et al., 1996).

Thus, a general relationship is apparent between altered intracellular calcium homeostasis and altered lipid metabolism in the cells of ischemic tissue. The purpose of this presentation is to review evidence for the nature of the alterations in calcium and lipid metabolism in ischemia and related forms of injury, and discuss the possible interrelationships between these two phenomena.

A. Myocardial Calcium Regulation

Intracellular calcium ion concentration is a key regulator of myocardial function. Electrical stimulation leads to calcium influx across the sarcolemma, calcium-induced calcium release from the sarcoplasmic reticulum, and an increase in calcium concentration at the myofibrils which triggers myocyte contraction during systole;

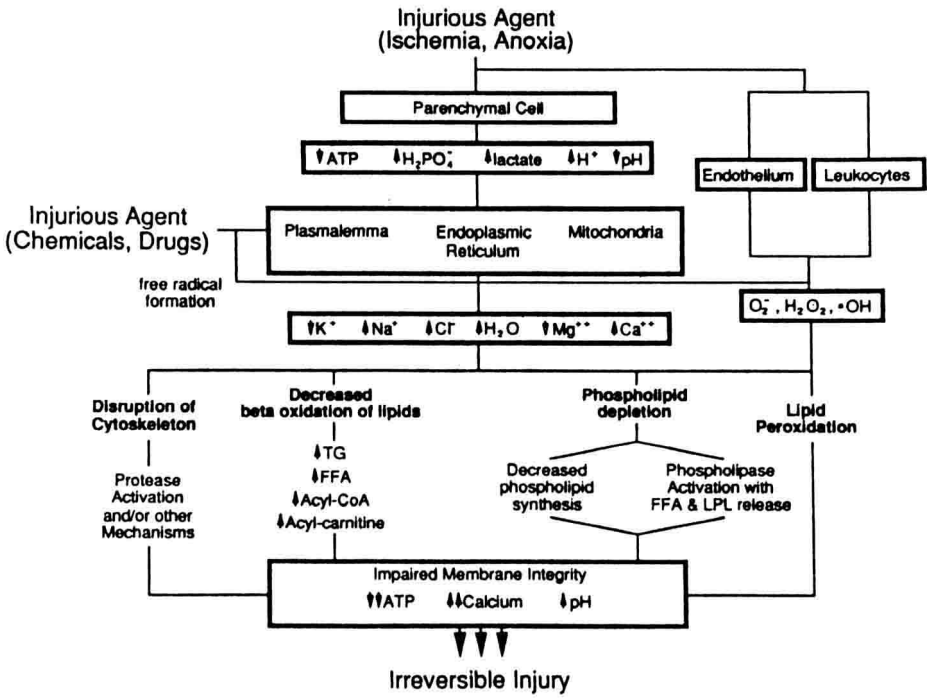

Figure 1. Scheme of the pathogenesis of irreversible cell injury. ATP, adenosine triphosphate; CoA, coenzyme A; FFA, free fatty acids; LPL, lysophospholipids; TG, triglycerides; H_2PO_4, phosphoric acid; H_2O_2, hydrogen peroxide; OH•, hydroxyl radical; H_20, water; Ca^{++}, calcium ion; Mg^{++}, magnesium ion; Cl^-, chlorine ion; Na^+, sodium ion; and K^+, potassium ion. From Buja et al. (1993).

reuptake of calcium ions (Ca^{2+}) into the sarcoplasmic reticulum and relaxation occurs during diastole (Braunwald, 1982; Barry and Bridge, 1993). Intracellular, free calcium ion concentrations ($[Ca^{2+}]_i$) normally range from approximately 50-100 nM during diastole to peaks of 500-1000 nM during systole with each contraction cycle (Figure 2). Precise documentation of $[Ca^{2+}]_i$ in normal tissue and cells, in myocardial ischemia, and related experimental models has come from technical advances in methods for measurement of calcium (Barry et al., 1987; Morris et al., 1989; Lee et al., 1988; Steenbergen et al., 1990; Koretsume and Marban, 1990; Doeller and Wittenberg, 1990; Thandroyen et al., 1992). These approaches include electron probe x-ray microanalysis to determine total cellular calcium levels and microspectrofluoroscopy using fluorescent probes, such as fura-2 and indo-1, for determination of intracellular, free calcium ion concentration, $[Ca^{2+}]_i$. These technical advances have allowed correlation of intracellular $[Ca^{2+}]_i$ levels with the severity of cell injury as discussed in the following section.

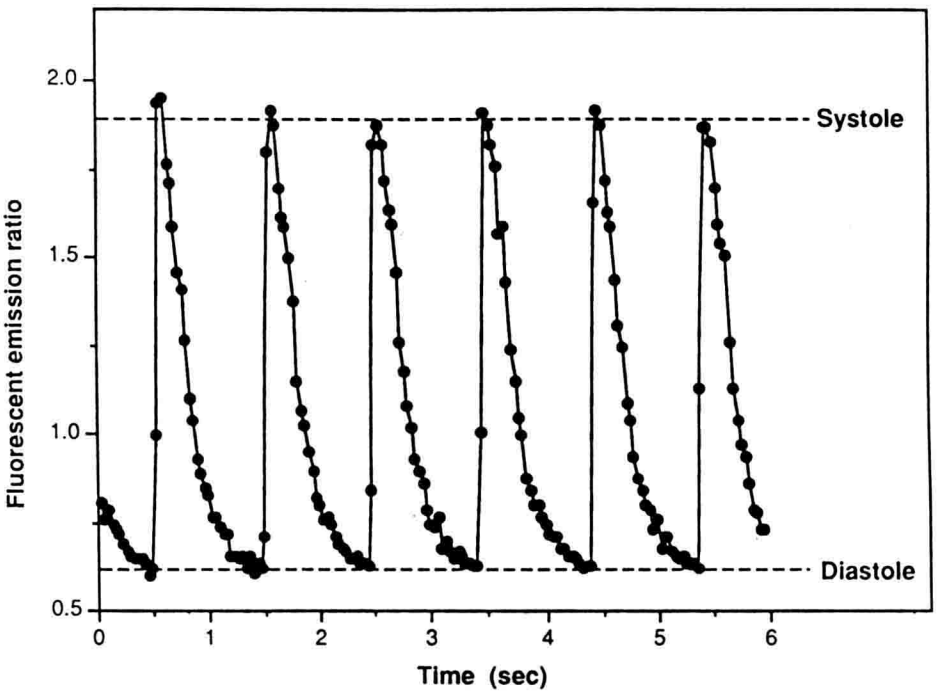

Figure 2. Normal intracellular, free calcium transients measured with fura-2 microspectrofluorometry. The fluorescence emission exceeding 470 nm produced from an alternating of 340 and 380 nm excitation was measured in cultured neonatal myocytes loaded with fura-2/AM. The 340/380 emission ratio increases proportionally with $[Ca^{2+}]_i$ associated with each contraction (systole) in the mycoytes then decreases during relaxation (diastole).

$[Ca^{2+}]_i$ represents only a very small fraction of total cellular calcium in the normal cell (Buja et al., 1988). Electron probe measurements determine that total cellular calcium concentrations range from approximately 5-10 mmol/kg tissue dry weight (Jones et al., 1989). Assuming the dry/wet tissue weight ratio of approximately 20% and the specific density of 1 g/ml, we can estimate that total calcium concentrations of 1-2 ug/ml, or 1-2 mM in normal myocytes. Therefore, the cytoplasmic, free calcium pool represents only 0.05-0.10% of the total calcium. This 20-fold range represents the minimum to maximum $[Ca^{2+}]_i$ in myocytes during the contraction cycle, with regulation of the contractile cycle being the primary physiological function of shifts in $[Ca^{2+}]_i$. Accordingly, the myocyte has tremendous capacity to quickly move calcium ions between the various intracellular and extracellular compartments.

The very rapid calcium fluxes in contracting myocytes are mediated by ion pumps and channels located in the cellular and organellar membranes (Braunwald, 1982; Barry and Bridge, 1993). Loss of calcium homeostasis can produce myocardial dysfunction. Function of these membrane proteins can be influenced by the properties of the membrane phospholipids which encompass them (Spector and Yorek, 1985; Philipson and Ward, 1985; Katz and Messineo, 1981; Corr et al., 1984).

B. Myocardial Phospholipid Metabolism

Cellular membranes are fluid mosaic structures composed primarily of phospholipids arranged into a molecular bilayer containing proteins which mediate membrane functions (Singer and Nicolson, 1972). Maintenance of membrane integrity is absolutely critical for cell viability; loss of either structural or functional integrity leads to irreversible cell injury (Katz and Messineo, 1981; Corr et al., 1984). Membrane homeostasis represents a balance between phospholipid anabolism and catabolism (Figure 3). Synthesis of phospholipids can occur by *de novo* synthesis or by reacylation pathways where the acyl moieties may be drawn from extracellular or intracellular sources (Katz and Messineo, 1981; Corr et al., 1984; Buja, 1991). Phospholipid catabolism in myocardium is mediated by phospholipases, lysophospholipases, and lipases yielding a diverse group of products (Buja, 1991; Gross, 1992; Wolf and Gross, 1992; Hazen and Gross, 1992a and 1992b).

The myocardium contains phospholipases in different subcellular fractions which can be distinguished functionally by differences in their substrate specificities, pH optima, and dependence on calcium for activation (Gross, 1992; Hazen and Gross, 1992b). Phospholipases of the A_1 and A_2 classes hydrolyze the fatty acyl moieties from the sn-1 and sn-2 positions of the phospholipid molecule, respectively, producing a free fatty acid and a lysophospholipid. Cells normally maintain very low levels of the monoacyl lipid species, presumably to minimize their potent detergent effects. These lipophilic products are susceptible to further degradation or may be used for resynthesis of phospholipids. Specifically, the lysophospholipids can be further hydrolyzed by lysophospholipases, and the resultant fatty acids can undergo beta-oxidation in the mitochondria to meet energy needs of the myocytes. Alternatively, regeneration of the intact phospholipid molecule can occur by the enzymatic reacylation of a high-energy fatty acyl-CoA to the lysophospholipid by a lysophospholipid acyl transferase. A deacylation-reacylation cycle is formed by the combined action of phospholipase A and lysophospholipid acyl transferase. In addition to diacyl phospholipids, myocardial membranes contain large amounts of plasmalogens (Gross, 1992). These lipids have an ether-linked fatty acid in the sn-1 position. Choline and ethanolamine plasmalogens with an arachidonyl moiety in the sn-2 position are particularly abundant species. The myocardium contains phospholipases with a selective affinity for plasmalogens (Wolf and Gross, 1985; Hazen and Gross, 1992b).