ATHEROSCLEROSIS, HYPERTENSION AND DIABETES

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Prof. Setsuro Ebashi, MD, PhD
Okazaki, Japan

A TRIBUTE TO PROFESSOR SETSURO EBASHI, MD, PhD

This book is dedicated to Professor Setsuro Ebashi to recognize his outstanding achievements in the area of Cardiovascular Science and Medicine. Nowadays even students know well that Ca^{2+} plays an important role in cellular activities. However, not many people know that we are greatly indebted to Professor Setsuro Ebashi who made the most important contribution to the establishment of the role of Ca^{2+} as the second messenger first in skeletal muscle.

Deeply impressed by the molecular mechanism of contraction, especially the demonstration of ATP-induced contraction of glycerinated muscle, described in "Chemistry of Muscular Contraction" by A. Szent-Gyorgyi (1949), young Dr. Ebashi determined to work in the field of muscle contraction. The question he first raised was the following. Although ATP induces contractile response of actomyosin system, the removal of ATP does not cause relaxation, which is quite different from acetylcholine-induced contraction of living muscle, where the removal of acetylcholine results in relaxation. He thought that there must be something in living muscle to cause relaxation, which was lost during the preparation of the glycerinated muscle or other actomyosin systems. He started to search for the factor in homogenized muscle and soon found a factor that caused relaxation of glycerinated muscle in the presence of ATP and reported to a meeting of a Japanese muscle physiology group in 1952. Later he realized that Marsh had already reported a similar factor in 1951. However, this was not a disappointment but an encouragement to Dr. Ebashi because it proved that his direction of research was right. Having inquired into the nature of the relaxing factor, he showed in 1955 that the essential component of the relaxing factor was in the particulate fraction, against the general belief at that time that it may be soluble ATP-regenerating enzyme(s).

As for the mechanism of relaxation, Dr. Ebashi conclusively proved in early 1960s that removal of Ca^{2+} from the medium by the relaxing factor is the cause of relaxation. The proof consisted of his several important discoveries: • the relaxing factor strongly takes up Ca^{2+} from the medium into its lumen in the presence of ATP; • chelating agents also cause relaxation and their potency is proportional to their affinity to Ca^{2+} in the ionic condition of the relaxation experiments; • a minute amount of Ca^{2+} is necessary for the contractile reaction of well-washed Ca^{2+}-free natural actomyosin system, and, therefore, the removal of Ca^{2+} from the medium causes the relaxation. (Although physiologists had recognized the contraction-inducing action of Ca^{2+}, it had not been recognized by biochemists before Dr. Ebashi, because Ca^{2+} had been thought to have no effect on the contractile reaction since all the bio-

chemical experiments had been done in the presence of a sufficient amount of Ca^{2+} contamination from reagents and/or exuded from glassware of the day.) Dr. Ebashi further demonstrated that the particulate relaxing factor has a vesicular structure under electron microscopy, indicating that it is fragmented sarcoplasmic reticulum. Since relaxation is the reverse of contraction, these findings led to a clear picture of excitation-contraction coupling: excitation somehow exerts an influence on the sarcoplasmic reticulum to cause a release of Ca^{2+} it accumulated during relaxed state, and the Ca^{2+} released in turn activates the contractile reaction.

In spite of such clear evidence, it took some time for everybody to be convinced of the vital role of Ca^{2+} in contraction, probably because biochemists at that time firmly believed that such an important biological phenomenon as contraction must be managed by some complex organic substance produced by the relaxing factor and not by such a small common inorganic ion Ca^{2+}.

One of the objections against the Ca^{2+} theory was the fact that the effect of Ca^{2+} was sometimes variable depending on the preparation of actomyosin. If it is the important physiological mechanism, the effect of Ca^{2+} should be brought about consistently any time. Dr. Ebashi inquired into this problem and discovered 'the third protein' participating contraction (the first two being myosin and actin), which conferred the Ca^{2+} sensitivity upon the actomyosin system. He further elucidated that • the protein factor is a complex of tropomyosin and a new protein which he named troponin; • tropomyosin and troponin are associated with actin filaments in living muscle; • in the absence of Ca^{2+}, troponin in collaboration with tropomyosin exerts an inhibitory influence on actin to prevent it from interacting with myosin; and • Ca^{2+} is bound to troponin and resulting conformational change of troponin removes the inhibitory influence mentioned above to start contractile reaction. Later it was found that troponin is a heterotrimer consisting of troponin T (the tropomyosin-binding subunit), troponin I (the inhibitory subunit) and troponin C (the Ca^{2+}-binding subunit).

Among the discoveries mentioned above, the fact that a minute amount of Ca^{2+} causes contractile reaction of the actomyosin system was also found independently by Dr. A. Weber, and the fact that the relaxing factor can accumulate Ca^{2+} in the presence of ATP by Drs. W. Hasselbach and M. Makinose, both at about the same time. However, the discovery of troponin, the first Ca^{2+}-receptive protein, and the following elucidation of the molecular mechanism of regulation of contraction and relaxation by Ca^{2+} are Professor Ebashi's unrivaled sphere of activity. Thus, it is no exaggeration to say that Professor Ebashi is the person who opened up the present Ca^{2+} era.

Professor Ebashi was awarded numerous prizes for his great contribution including an Order of Cultural Merits and the Japan Academy Award with an Imperial gift. He is now Professor Emeritus of the University of Tokyo and of National Institute for Physiological Sciences. He is also a member of the Japan Academy, a foreign member of the Royal Society, London, a member of the National Academy of Sciences, USA, and a member of Leopoldina German Academy. He was decorated with the First Order of Merit, the highest rank of decoration in Japan in 1995.

He lives in Okazaki with his wife, Dr. Fumiko Ebashi, who supported him faithfully at home as well as in the laboratory as a coworker and a secretary. Very unfortunately he has been ill for about two years. However, he is mentally still sharp, and everybody who knows him prays earnestly for his recovery and longevity.

Makoto Endo
Moroyama, Japan

PREFACE

This text, as the title states, is a compilation of papers devoted to the study of atherosclerosis, hypertension and diabetes. These three distinct disease entities, although not entirely unrelated, are three of the most important disease conditions in the world today. As such, this volume of research papers is of obvious medical importance. The justification of the energy, time and financial resources directed towards the study of each of these three diseases requires some discussion.

The majority of papers amongst the three diseases that are discussed in this volume are dedicated to the study of atherosclerosis. This is not by accident. Cardiovascular disease is the number one killer today in the world. In the United States almost 61 million Americans have one or more forms of cardiovascular disease. These diseases claimed nearly 1 million lives in 1998 alone. Although approximately 80% of those who die of cardiovascular disease are 65 years of age or older, a significant number of people are killed by cardiovascular disease below the age of 65. Atherosclerotic heart disease in the coronary vasculature caused approximately ½ million deaths in the United States in 1998. At least 12,400,000 people are alive today in the United States with a history of myocardial infarctions or chest pain or both. Clearly, atherosclerotic disease in the heart is a major medical problem. This disease affects both men and women. Although men are more likely to experience a heart attack and are at greater risk for cardiovascular disease, more then ½ of the people alive today with a history of heart attacks or angina are females. As well, women who do have myocardial infarctions are twice as likely to die from the event within a few weeks. Atherosclerotic vascular disease is not limited to just the heart. An atherosclerotic ischemic event is the primary cause of stroke today. Although it is not well appreciated, stroke is the number 3 killer in America today and the leading cause of debilitating neurological damage. Atherosclerotic vascular disease therefore, has a cost in terms of human life, quality of life and financial burden today that no other disease can match. The seriousness of this medical problem demands research attention.

The papers in this volume are directed towards increasing our understanding of novel ways of preventing or treating atherosclerotic disease. We also examine some of the basic mechanisms involved in the atherosclerotic process. For example, nutritional interventions are discussed that may prevent, retard or treat the atherosclerotic process. These include, vitamin therapy (like vitamin D or vitamin B in the treatment of hyperhomocysteinemia), the replacement of hydrogenated fats in the diet because of the influence on cholesterol levels, the use of antioxidants like co-

enzyme Q10 and other nutritional interventions. Several papers discuss the use of cholesterol lowering agents and their effects both in the control of cholesterol metabolism and atherosclerosis and in the surprising beneficial side-effects that these drugs have in platelet activation. Naturally, lipids themselves are a focus for research intervention. Two papers in our volume address a particular type of lipid, oxidized LDL, as a focus for interventional therapy. The identification of new oxidized LDL receptors and the mechanisms whereby oxy radicals influence cholesterol metabolism may be some of the most important sites for research study in this area that have been identified in the last two decades.

Other sites for research intervention have been identified in this text. The interaction and the use of bone marrow in the study of atherosclerosis is a novel and exciting intervention that has gained enormous popularity in the last few years. Finally, the study of endothelial cell dysfunction and angiotensin and its relationship to atherosclerosis remain exciting avenues to understanding not only how atherosclerosis may block vessels but also how these areas may influence vessel contractile function and the distribution of blood flow through a vascular system.

One of the most novel and intriguing areas of research in the 21st century with regard to cardiovascular disease has been the identified association of infection with atherosclerosis. Although, at first, this seemed to be quite an erratic departure from the dogma of atherogenesis, it is now well recognized that vascular inflammation and the changes in lipid metabolism associated with atherosclerosis may be important stimuli for the development of this disease. Involvement of PPAR-α in the vascular inflammation and a detailed treatise of the use of animals in the study of chlamydia pneumonia as an atherogenic agent are both exciting, new additions in the study of atherosclerotic vascular disease.

Hypertension is often referred to as the silent killer. It is estimated that one in four adults in the United States has hypertension. However, because hypertension has virtually no symptoms, one in three people who have high blood pressure don't even know it. This "silent disease" is deadly. Hypertension killed almost 45,000 Americans in 1998 and contributed to the deaths of 210,000 more. As many as 50 million Americans 6 years of age and older have hypertension. There are racial predispositions for high blood pressure. For example, non-Hispanic blacks and Mexican Americans are more likely to experience high blood pressure than non-Hispanic whites. High blood pressure affects one in three African Americans. Further research into the mechanisms of hypertension is clearly justified. Amazingly, in 90 to 95 percent of cases of people with high blood pressure, the cause is unknown. High blood pressure is the single most important risk factor for strokes. Obviously, the more we understand about how to reduce high blood pressure, the better we can reduce the incidence of stroke, neurological damage, and heart disease.

The papers in this volume dedicated to the study of hypertension focus on factors that may be responsible for high blood pressure. These include examining the genetic predisposition to hypertension as well as how drugs may inhibit the genes involved

in vascular hyperplasia and remodeling (two phenomena associated with hypertension). Other papers examine insulin resistance and its involvement in hypertension, and the neurological aspects associated with high blood pressure. These include the involvement of the sympathetic nervous system and hypothalamic peptides in the development of hypertension. An important paper in this volume discusses the cellular function of the endothelium and its relationship to blood pressure. The nutritional basis of hypertension is also examined and discussed. It has long been recognized that kidney dysfunction is involved in the hypertensive condition. One of the papers in this volume examines the effects of leptin on the cardiovascular system and renal function. Although the kidney has long been associated with hypertension as the primary etiological organ, the intriguing involvement of the brain and insulin resistance in the hypertensive condition is identified and discussed in two separate manuscripts. Finally, one paper has advanced novel routes of drug delivery for the treatment of hypertension.

Diabetes is another major disease in the world today. Nearly 60 million Americans have insulin resistance and 25% of these cases will go on to develop diabetes. Diabetes kills 60,000 Americans each year and it is estimated that its complications can contribute to another 190,000 deaths each year. Before the discovery of insulin, most diabetic patients died after lapsing into a coma. Today, with the conventional use of insulin therapy, diabetic patients are living longer but still die sooner than their non-diabetic counterparts. People with diabetes are 2 to 4 times more likely to have stroke or heart disease. If the heart disease does occur, it is more severe in the diabetic and more likely to develop into congestive heart failure than in the non-diabetic population. Diabetes causes lipid abnormalities that are conducive to heart disease. Decreases in high density lipoproteins and increases in low density lipoprotein and triglycerides are common in the diabetic population. Most diabetic patients (approximately 80 to 90 percent) are over weight. The complications of diabetes are not limited to just cardiovascular disease. Diabetes can cause or lead to induction of blindness, kidney disease, nerve disease and limb amputations. Research to discover new methods for the treatment and prevention of diabetes are clearly justified.

The papers in this text on diabetes discuss the risk factors and mechanisms responsible for diabetic vascular and cardiac dysfunction. For example, the involvement of cholesterol and cardiovascular disease in diabetes is discussed. Another paper extends this to a treatise detailing the alterations in the lipid profiles found in diabetic patients and the changes in fibrinolysis. Diabetic vascular disease is discussed as is the role of the endothelium in cardiovascular disease in diabetics. In view of the association of diabetes with excessive body weight, one of the papers examines the mechanisms of adipogenesis and sheds light on the factors involved in this important process. Several papers in this text discuss diabetic heart disease. They describe the mechanisms underlying Type I and Type II diabetic cardiomyopathy, the current research on Type II diabetic heart disease, energy metabolism in the diabetic heart, and a variety of molecular mechanisms responsible for diabetic heart

disease. Another interesting paper discusses the use of vanadate as an alternative therapy to insulin in the treatment of diabetes mellitus. New ideas are presented for the mechanism involved in insulin resistance. Finally, one of the significant papers in this manuscript examines how neurotransmitters (like 5HT) may function as targets for the prevention of cardiovascular disease and diabetes.

This volume brings together nearly 40 papers to discuss the 3 diseases covered by this text; atherosclerosis, hypertension and diabetes. These manuscripts were invited from scientists who presented state of the art lectures at the XVII World Congress of the International Society for Heart Research that was held in Winnipeg, Canada in July, 2001. This Congress, which attracted approximately 2,000 participants, not only served as a venue for scientific interaction and networking but, as evidenced by this volume itself, also resulted in the generation of new science and new thought processes as they pertain to these 3 significant pathological conditions. We certainly hope that you enjoy reading these manuscripts. We believe that they lead to new insights into the management and treatment of atherosclerosis, diabetes and hypertension.

Grant N. Pierce, Winnipeg, Canada
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ATHEROSCLEROSIS, HYPERTENSION AND DIABETES

I. ATHEROSCLEROSIS AND CARDIOVASCULAR DISEASE

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PPAR-ALPHA IN LIPID AND LIPOPROTEIN METABOLISM, VASCULAR INFLAMMATION AND ATHEROSCLEROSIS

JEAN-CHARLES FRUCHART, BART STAELS, and PATRICK DURIEZ

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Summary. Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily, PPAR-alpha is highly expressed in liver, skeletal muscle, kidney, heart and the vascular wall. PPARs are activated by fatty-acid derivatives and pharmacological agents such as fibrates for PPAR-alpha. PPAR-alpha controls intra- and extracellular lipid metabolisms. Fibric acids decrease triglyceride concentrations by increasing the expression of lipoprotein lipase and decreasing apo C-III concentration. Furthermore, they increase HDL-cholesterol by increasing the expression of apo A-I and apo A-II. In addition, PPARs also modulate the inflammatory response. PPAR activators have been shown to exert anti-inflammatory activities in various cell types by inhibiting the expression of proinflammatory genes such as cytokines, metalloproteases and acute-phase proteins. PPARs negatively regulate the transcription of inflammatory response genes by antagonizing the AP-1, nuclear factor-κB (NF-κB), signal transducer and activator of transcription (STAT) and nuclear factor of activated T-cells (NFAT) signalling pathways and by stimulating the catabolism of proinflammatory eicosanoids. PPAR-alpha activators (gemfibrozil) decrease the risk of coronary heart disease in patients with normal LDL-cholesterol and low HDL-cholesterol (VA-HIT) and they slow the progression of premature coronary atherosclerosis (BECAIT) (bezafibrate), particularly in patients with type 2 diabetes (DAIS) (fenofibrate).

Key words: PPAR-alpha, Lipoproteins, Inflammation, Atherosclerosis

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