Smooth muscle contraction is mediated by an ATP-dependent interaction between actin and myosin: Ca2+ regulates the actin-myosin interaction. The weighty mode of regulation is that of phosphorylation of myosin, i.e., Ca2+ binds to calmodulin so that it activates myosin light chain kinase, resulting in phosphorylation of myosin in its light chain. The phosphorylated myosin is in an active form so that it is able to interact with actin.

The mode of myosin phosphorylation has been well studied on a biochemical basis and is assumed to operate during smooth muscle contraction. However, pharmacological studies on actual contraction suggest another important mode of regulation: smooth muscle contracts under conditions where myosin light chain kinase does not work. A few Japanese pharmacologists have been aware of the complexity of the regulatory mechanisms and are devoted to finding alternatives. This book reviews these attempts.

Recent technical progress in smooth muscle research including measurement of intracellular Ca2+, skinning of cell membrane, preparation of single cells, development of calmodulin- and myosin light chain kinase-inhibitors, and adoption of molecular cloning techniques has
made these alternatives more concrete. This progress is also reviewed. It is our hope that this book will contribute to the better understanding of regulation by Ca$^{2+}$ of smooth muscle contraction and will stimulate research by scientists worldwide in this area of smooth muscle research.

We wish to express our gratitude to the authors who so willingly contributed to this volume. Our thanks are also due to the Ministry of Education, Science and Culture of Japan and the Naito Foundation who have made this publication financially possible.

November 1994
Maebashi Kazuhiro KOHAM
Kobe Kooichi SAID

Introductory Remarks

SETSURO EBASHI

National Institute for Physiological Sciences, Okazaki,
Aichi 444, Japan

Vertebrate skeletal muscle (fast, white) is the most differentiated tissue, not only among muscles but all tissues of the entire body. Until 1970 most studies on muscle contraction at the subcellular and molecular level were carried out with this muscle and smooth muscle was looked upon as a subsidiary material. There were several reasons scientists kept their distance from smooth muscle. One of them was its complexity as well reflected in Lord Adrian’s witty remark, “Keep off smooth muscle”, in sharp contrast to the simplicity of skeletal muscle.

This biased attitude of muscle scientists can be compared to that of neurophysiologists early in this century, who viewed electrophysiology as being of prime importance. Some physiologists even ignored the chemical transmission in the neuromuscular junction of skeletal muscle, holding instead to the idea of electrical transmission.

The current begun by studies on chemical transmission has now become one of the central themes of biological science under the term “signal transduction” or “transmembrane signalling”, and a number of young and ambitious scientists are enthusiastically engaged in this. In a similar way, studies on smooth muscle are also now very popular,
attracting a much larger population of researchers than is skeletal muscle research. The advantage of smooth muscle is that contractile processes are intricately related to subjects of great popularity in today’s cell and molecular biology including signal transduction. In other words, the advances in biological science have now reached a level high enough that we are able to deal with this difficult material. This, then, is the time to evaluate the fruits of skeletal muscle research from a historical point of view. As stated, the highly differentiated state of skeletal muscle has made it possible to exhibit the essential features of muscle contraction in a simple and concise manner for instance, the discovery of the sliding mechanism, one of the most fascinating concepts ever formulated in biological science, owing to its simple, beautiful arrangement of myofilaments. This was not the case with smooth muscle (even now, the question of whether or not the sliding mechanism is operating has not clearly been answered, although several pieces of circumstantial evidence have been presented). The Ca2+ concept, now widely accepted as a fundamental principle of all cells, originated from skeletal muscle research (1, 2). At the time the Ca2+ concept of early stage was proposed and nearly established (1960), i.e., Ca2+ was recognized as the final triggering factor for the contractile processes, scientists in fields other than muscle had virtually no interest in Ca2+. Smooth muscle researchers were naturally concerned with Ca2+, but for most of them it was merely one of essential factors in the bathing solution (this reminds us of the Heilbrunn attitude (1937) (3) to Ringer: he did not consider Ringer as pioneering in the work along his line, but classified Ringer’s Ca2+ as one of the “environmental conditions”).

In the above case, the role of Ca2+ on contractile elements could have eventually been recognized without the prior results of skeletal muscle research, but the actin-linked nature of Ca2+ regulation would never have been revealed if the studies had been confined to smooth muscle. Muscle biochemists of the older generation had an almost religious belief in myosin—the myosin molecule could do almost anything, whereas actin was more likely only a mere mechanic: support. I must confess that I also could not shake off this belief even when troponin was separated from native tropomyosin and found to have an extraordinarily strong Ca binding capability (1965) (4). Compelled by the thought that myosin must have played its crucial role...
with the aid of troponin, I wasted nearly one year in looking for non-existent evidence for this irrational idea. If we had not conceived

INTRODUCTORY REMARKS ix

the experiment to use the hybrids of skeletal and cardiac troponins (1967) (5), I would not have reached the conclusion that troponin was the Ca2+-receptive protein itself and that myosin had no direct role in the Ca2+-regulated processes.

As shown in this book, phosphorylated myosin is the site for Ca2+ reception in the actomyosin system of Physarum polycephalum, although the phosphorylation-déphosphorylation cycle may not be involved in the physiological process. In smooth muscle, there is no need to comment on the dominant position of myosin in studies on the regulatory mechanism carried out so far. In the somatic muscle of molluscan which has attained a high degree of differentiation comparable to vertebrate skeletal muscle, the site of Ca2+ is in the myosin molecule (1970) (6). If this work had been done prior to the discovery of troponin (1965), many muscle biochemists would have been reluctant to accept the actin-linked troponin-tropomyosin system.

Thus the actin-linked system owes its recognition entirely to the research on skeletal muscle, and the development of the system in the animal kingdom appears to have been the matter of evolution. Around 1980 (7) it was believed that the actin-linked system was a phylogenetically developed device possessed only by higher animals having an exoskeleton or endoskeleton.

However, the situation has recently changed considerably. Troponin has also been found in lower animals, viz., nematodes (1981) (5) and even in molluscs (1986) (9). Even more remarkable is that there is accumulating evidence for the existence of various kinds of actin-linked regulatory systems in smooth muscle. Thus the actin-linked regulation is rather ubiquitous in its nature, although its expression is not uniform but varies phylogenetically from species to species. (Even in Physarum polycephalum, there is an indication of the presence of an actin-linked system (1991) (10), though it may not have a physiological role). These circumstances have spotlighted the urgent need for a book on this subject.

In summary, the actin-linked system is a vital feature of smooth muscle and is a more fundamental device of muscles than had ever before been realized.

REFERENCES

X S. EBAS


Contents

Preface v

Introductory Remarks S. Ebashi vii

1 Regulation of Smooth Muscle: Phosphorylation-dependent and
-independent Mechanisms H.Karaki 3

I. Ca2+ Sensitivity of Myosin Phosphorylation 3
II. Ca2+ Sensitization Which Is Not Dependent on Myosin Phosphorylation 6
III. Role of Protein Kinase C 9
IV. Mechanism of Smooth Muscle Contraction 10
Summary 11

2 Characterization of Myosin Light Chain Kinase as an Actin-binding
Protein That Regulates the ATP-dependent Interaction between Actin and Myosin of Smooth Muscle

T. Okagaki and K. Kohama 17
I. MLCK as an Actin-modulatory Protein 18
II. Regulation of Actin-myosin Interaction by Actin-binding Activity of MLCK 19
III. Inhibitory Effect of MLCK on Actin-activated ATPase Activity 20
IV. Regulatory Activity of MLCK As Examined by the Motility Assay with Myosin-coated Glass Surface 20
V. Are Phosphorylation/Dephosphorylation of Myosin Involved in the Actin-linked Inhibition Observed with the Surface Assay? 23
VI. Stimulatory Effect of MLCK in the Presence of Ca-CaM on Actin-myosin Interaction 24
VII. Physiological Involvement of the Inhibitory Effect of MLCK in Regulating the Actin-myosin Interaction of Smooth Muscle 25
VIII. MLCK Shares Actin-binding Site(s) with CaD 28
IX. Effects of CaD on the Actin-myosin Interaction 29
X. Stimulatory and Inhibitory Effects of CaP 30
XI. Regulatory Role of MLCK As Compared with That of CaD or CaP 30
Appendix I: Utility of Myosin-coated Surface Assay 32
Appendix II: Binding of Actin-filaments to the Myosin-coated Surface and Their Motility on the Surface 32
Summary 34
3 Ca2+-induced Actin-severing and Contraction of Smooth Muscle M.K. Uchida 39
I. Ca2+-independent Contraction of Smooth Muscle .... 39
II. Drugs Affecting Actin Filaments on Ca2+-independent Contraction 45
III. Morphological Evidence of Actin-severing by Ca2+ with Confocal Laser Microscopy 49
IV. Other Cases of Ca2+-inhibition of Contraction in Smooth Muscle 52
V. Contractions Observed at Various Concentrations of Extracellular Ca2+ 53
VI. Ca2+-induced Inhibition of Ca2+-induced Contraction 55
VII. Inhibitory Response in the Early Phase of Contraction of Skeletal Muscle and Secretion from Secretory Cells 59
VIII. Our Working Hypothesis: Actin-linked Regulation of Initiation of Contraction 62
4 Regulation of Smooth Muscle Contraction by Calponin Phosphorylation and Dephosphorylation

T. Tanaka, M. Naka, T. Mino, and U. Yuasa 71
I. Effects of Actin, Tropomyosin on Phosphorylation of CN by PKC 73
II. Effects of Phosphorylation on CN Binding to F-actin and Tropomyosin 73
III. Location of Phosphorylation Sites and the F-actin, Tropomyosin, and CaM-binding Regions in CN 74
IV. Sites of Phosphorylation of CN by PKC 74
V. Dephosphorylation of CN by Type 1 Protein Phosphatase (PP-1) 75
VI. Phosphorylation of CN in Intact Porcine Coronary Artery Strips 77
Summary 78

5 Modulation of Calcium Sensitivity of Smooth Muscle Contraction J. Nishimura 83
I. Brief Account of the Methods 84
1. Permeabilization by -toxin 84
2. MLC phosphorylation determinations 85
II. Modulation of Ca2+ Sensitivity 86
III. Involvement of G Proteins and PKC in the Increased Myofilament-Ca2+ Sensitivity 86
IV. MLC Phosphorylation 89
V. Modulation of Ca2+ Sensitivity by ADP and P1 91
VI. Discussion 92
1. Modulation of Ca2+ sensitivity 93
2. Involvement of G proteins and PKC in the increased myofilament Ca2+ sensitivity 94
3. Modulation of Ca2+ sensitivity by ADP and P195
4. MLC phosphorylation 96
6 Ca2+ Sensitization of Smooth Muscle in Relation to Small GTP-binding Protein K. Saida 103

I. Skinned Smooth Muscle 104
1. Saponin-treated skinned smooth muscle 104
2. -Toxin-treated skinned smooth muscle 105
3. Others 105
II. Ca2+ Sensitivity 105
1. Myofilament Ca2+ sensitivity in skinned smooth muscle 105

xv

7 Ca2+ Release Mechanisms in Single Smooth Muscle Cells

M. lino 115
I. Brief Account of the Method 116
II. Responses of Single Smooth Muscle Cells to Agonist ... 117
III. Compartments of the Ca2+ Stores 119
IV. Relation between Agonist-sensitive Store and IP3-sensitive Store 120
V. All-or-none Ca2+ Release 122
VI. Comparison between the Single Cells and Bundles .... 122
VII. Perspectives 126
Summary 126

8 Expressional Regulation of Caldesmon Isoforms in Association with Phenotypic Modulation of Smooth Muscle Cells

K. Hayashi and K. Sobue 131
I. Characterization of CaD Isoforms and Their Functions in the Actomyosin System 131
II. Primary Structures and Domain Maps of h- and /-CaDs- 132
III. Expressional Change of h- and /-CaDs in Association with Phenotypic Modulation of Smooth Muscle Cells 134
IV. Genomic Structure of CaD Gene and the Expressional Regulation 137
V. Conclusion 138
Summary 139

9 Vascular Smooth Muscle Calponin K. Takahashi 145

I. Calponin Structure 145
II. Homology with the Proto-oncogene Product and a Yeast Gene CDC24 148
III. Expression of Calponin in Normal and Diseased Arteries 149

CONTENTS XV
IV. Intracellular Expression of Calponin in Contractile Vascular Smooth Muscle Cells 151
Summary 152

Subject Index 157