Physiol. Res. 44: 53–60, 1995

Molecular Aspects of Regulation of Cardiac Contraction

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Received November 18, 1994
Accepted December 1, 1994

Summary

Contractility of the myocardium is determined by the interaction of contractile (actin and myosin) and regulatory (troponin and tropomyosin) proteins in the presence of calcium ions and chemical energy. The formation of the actomyosin complex is affected by the modulatory (C-, F-, M-proteins, actinins) and interstitial proteins (different types of collagens, glycoproteins, glycosaminoglycans, elastins). Cardiac hypoxia is accompanied by qualitative and quantitative changes in both collagenous (change in the proportion of different extracellular matrix proteins) and non-collagenous proteins (formation of different isomyosins and isoforms of actins or regulatory proteins). This remodelling of cardiac musculature influences significantly the process of contraction and relaxation in the hypoxic myocardium.

Key words

Contractility of myocardium – Isomyosins – Contractile proteins – Regulatory proteins – Modulatory proteins – Collagen – Extracellular matrix proteins – Cardiac hypoxia

Introduction

The contractile properties of the myocardium are determined by interaction of major contractile proteins (actin and myosin), regulatory proteins (troponin [Tn] and tropomyosin [TM]) and calcium ions (Fig. 1, upper part). A necessary precondition for cardiac contraction is the ability of myosin to split ATP; the terminal phosphate is the primary source of energy for cardiac contraction. This reaction should be tightly coupled with the resynthesis of ATP from ADP by the creatine phoshotransferase reaction (creatine phoshate + ADP = ATP + creatine). A set of contractile proteins transforms the chemical energy of ATP in the myofibrillar system to the mechanical work of the myocardium. Regulatory proteins bound to the actin filament (inhibit the ATP-driven actomyosin cycle during relaxation by either blocking the attachment of myosin cross-bridges to actin or inhibiting subsequent reactions of the bound cross-bridges (Ebashi 1984, Swynghedauw 1986, Alpert *et al.* 1992, Taylor 1992, Malhotra 1994). Cardiac relaxation/contraction are generally acknowledged to result from Ca uptake and release by the sarcoplasmic reticulum. The Ca uptake occurs through the activity of a Ca- and Mg-dependent ATPase that translocates calcium from the cytosolic compartments to the inner structure of the reticulum; this process could be modified *via* phoshorylation of phosholamban (Krause *et al.* 1973, Tada *et al.* 1982).

Historical remarks

First reports about myosin were published more a than century ago (Kühne 1859, 1863 – quoted according to Lowey 1972), but the biochemical analysis of myosin started much later (Engelhardt and Ljubimova 1939, for review see Swynghedauw 1986). The use of other isolation system led to the discovery of actin (Straub 1942, 1943), tropomyosin (Bailey 1948), the troponin complex (Ebashi *et al.* 1969) and other minor proteins of the myofilaments (e.g. Masaki and Takaiti 1974, Hartzell 1984). Progress in new separation techniques and their combination (chromatography, electrophoresis, immunological procedures, histochemistry and electron microscopy) made it possible to isolate different isomyosins or isoforms of contractile, regulatory and modulatory proteins (these proteins have a different structure but they originate from the same genome (Swynghedauw 1986).

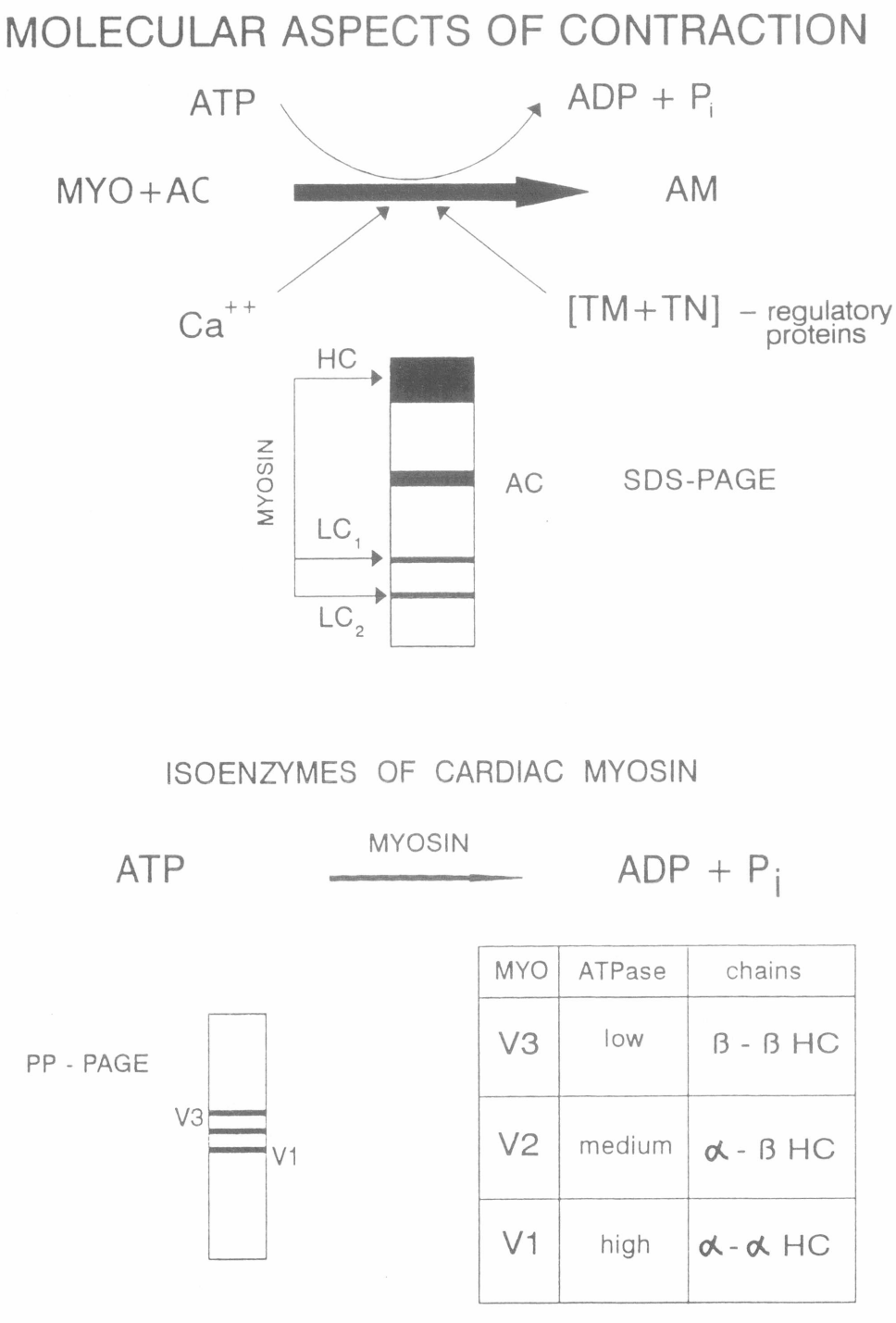

Fig. 1
Molecular aspects of contraction. Upper part: Contractility of the myocardium is determined by the interaction of myosin (MYO) and actin (Ac) in the presence of Ca ions released from sarcoplasmic reticulum and the process is activated by energy derived from metabolic pathways (ATP). The contraction is regulated by the regulatory proteins: tropomyosin (TM) and troponin (Tn). Electrophoresis of the fraction of contractile proteins (in the presence of sodium dodecylsulphate-SDS) shows heavy chains (HC), light chains (LC_1, LC_2) of myosin and actin (AC). Lower part: Native electrophoresis of cardiac myosin on polyacrylamide gel (PAGE) in the presence of pyrophosphate (PP) shows three isomyosins (V_1-V_3) with different ATPase activity; heavy chains (HC) are either homodimers (α-α, β-β) or heterodimers (α-β).

Isomyosins of the cardiac muscle

Myosin is the major component (m. w. 480 kDa) of the A-zone (thick filament) of the cardiac muscle). The protein has a hexameric structure which contains two chains, α-helical rod-shaped regions about 150 nm long. There are two globular regions "heads" (the site of the biological activities) localized on one end of the myosin molecule. The second end is a filamentous "tail" (a coil made up of two α-helical peptides). Each myosin head is composed mainly of two heavy chains (HC) and two pairs of light chains (LC_1, LC_2) (Fig. 1. upper part) with molecular weights in the range of 200 and 20 kDa, respectively. There are different types of HM dimers (either homo- or hetero-dimers). The heterogeneity of rat ventricular myosin was established by using pyrophoshate gel electrophoresis (Hoh *et al.* 1978). The electrophoretic pattern of the rat cardiac muscle myosin consists (Fig. 1. lower part) of three types of dimers (bands on ELFO are (labeled as V1, V3 [homodimers], and V2 [heterodimer]). The proportion of these bands has a characteristic profile. Newborn rat myocardium contains only V3 isomyosin (with a low ATPase activity), whereas only the V1 isoform was detected in the period of weaning (this isoform has the highest ATPase activity). During the postnatal period of rat maturation, the ventricular myosin ATPase level decreases due to the reappearance of synthesis of V2 and V3 isomyosins (Lompre *et al.* 1981, Pelouch *et al.* 1978, 1992, Brooks *et al.* 1987). The nature of the trigger for the synthesis of individual isomyosins is still a matter of speculations (agents such as thyroxin, catecholamines and insulin cannot be ruled out). Developmental changes in the heart isoprotein composition were described in different animal species. On the other hand, human cardiac myosin contains only V3 isomyosin (Swynghedauw 1986, Bandman 1985). The main regulatory mechanism of human cardiac myosin in healthy and diseased myocardium, therefore, cannot be a change of the isomyosin profile but rather the change of the ratio between HC and LC or the qualitative characteristics of myosin subunits (overloaded human ventricular myocardium may contain light myosin chains of atrial origin and *vice versa* – Cummins 1982, Hirzel *et al.* 1985, Sweeney and Stull 1986, Pelouch *et al.* 1995).

Regulatory and modulatory proteins

Tropomyosin (TM) and troponin (Tn) comprise the key regulatory protein complex (Fig. 2, upper part) controlling cardiac contraction. The existence of different α and β TM is well established (m. w. 33 000 and 34 000, αTM and βTM having only slightly different amino acid compositions). The TM molecule is made up of two identical or different helical peptides (it is a homo- or heterodimer). There are tissue (e.g. atrio-ventricular difference), ontogenetic and species specific TM differences (e.g. the beta isoform predominates in slower-beating hearts). Two-dimensional analysis of cardiac TM (isoelectric focusing-SDS) can separate two forms of both α and β of TM; the more acidic components (α^* and β^*) are phoshorylated. The degree of phoshorylation of TM appears to be developmentally regulated, low phoshate binding having been observed in aged animals (Humphreys and Cummins 1984, Malhotra 1994).

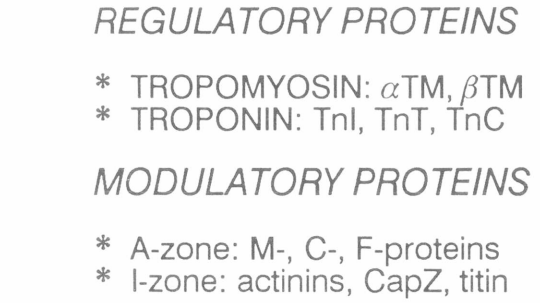

Fig. 2
Regulatory and modulatory proteins of cardiac musculature. Regulatory proteins of myocardium are formed by different isoforms of cardiac tropomyosin (TM) or troponin (Tn); they regulate the formation of actomyosin. Modulatory proteins are localized in both A-zone and I-zone of myofibrils; they participate in the contractile process and probably link metabolic pathways (production of ATP) with the hydrolytic sites on myosin molecules. For details see the text.

Troponin (Tn) consists of three subunits (TnT – troponin-binding tropomyosin, TnC – calcium-binding troponin and TnI – troponin-inhibiting formation of the actomyosin complex). These three troponin subunits interact in a cooperative manner with each other and also with TM and actin. Developmental changes of regulatory proteins are subject to neural, hormonal and metabolic control; abnormalities in myofibrillar ATPase activity (e.g. in hypertrophic, diabetic or hypertensive animals) can largely be corrected by recombinating the preparations with the TM-TM complex. It can, therefore, be concluded that regulatory proteins play a major role in the altered function of the diseased myocardium (Humphreys and Cummins 1984, Sabry and Dhoot 1989, Pelouch *et al.* 1992, Malhotra 1994).

Additional modulatory proteins (Fig. 2, lower part) have been found in myofibrils; their physiological role is still a matter of speculation. Some of them are localized in Z-lines (α-, β-, γ-actinins), others are in the Z-and/or A-zone (M-, C- and F-proteins) and I-zone (actinins, titin) of myofibrils. Modulatory proteins participate in the contractile process and they link both glycolytic (F protein *via* phoshofructokinase) and oxidative production of ATP with the hydrolytic sites on myosin molecules (M-protein *via* the phoshocreatine shuttle) – for details see Lowey 1972, Masaki and Takaiti 1974, Miyahara and Noda 1980, Miyahara *et al.* 1980, Squire 1983, Katz 1992, Morano *et al.* 1994). Modulatory proteins also form part of the myocyte cytoskeleton (e.g. titin, gelsolin, actin capping - CapZ). Some proteins may serve a dual role, contributing to signal transduction as well as to the organization and mechanical stability of cells. Although the mechanism of the cytoskeletal assembly in normal and healthy myocardium is still poorly understood, new biochemical approches may extend our understanding of the pathophysiology of cardiac contraction (Ganote and Armstrong 1993, Schaart *et al.* 1993, Morano *et al.* 1994).

Role of extracellular matrix proteins

The myocardium may be differentiated into compartments occupied by myocytes, and the interstitial space between these cells. The interstitial space (Fig. 3) is populated by several classes of cells (cardiac fibroblasts, endothelial cells, macrophages) and different extracellular matrix proteins (collagens, elastin, non-collagenous glycoproteins, proteoglycans, different growth factors and proteases – see Paulsson and Saladin 1989, Blumenfeld and Seifert 1990, Borg and Terracio 1990, Yamada 1991, Kjellén and Lindahl 1991, Weber 1992, Engel 1992, Borg and Burgess 1992/1993, Weber *et al.* 1993a,b, Pelouch and Jirmář 1993, Pelouch *et al.* 1994a). Collagens are the major organic constituent of the connective tissue; they represent a wide variety components associated with cardiac function (Fig. 3). It is currently recognized that different collagens are encoded by a family of about 20 very complex collagen genes. Collagen I and III represent more than 80 % of all collagenous types, minor collagens being IV, V, VI and VIII (Kawahara *et al.* 1990, Iruella-Arispe and Sage 1991, Speiser *et al.* 1991a,b, Bashley *et al.* 1992, Pelouch and Jirmář 1993, Pelouch *et al.* 1994a). A range of morphological, physiological and biochemical changes of extracellular components occur during ontogeny of the myocardium; a higher amount of collagen III and a lower amount of cross-links in collagens are characteristic for the young myocardium (Pelouch *et al.* 1993a,b, 1994b). The data available in the literature support the view that extracellular matrix is a dynamic entity and alterations in this structure result in the development of heart dysfunction and affect cardiac contractility. The main modulators of extracellular matrix remodelling are substances derived from the renin-angiotensin system. The connective tissue network contribute to the mechanical tension surrounding the myocytes in both neonatal development and in the response to physiological and pathophysiological stimulation (Simpson and Decker 1992/1993). Qualitative and quantitative changes of extracellular proteins will, therefore, affect the heart muscle function due to both perpendicular and lateral attachment of the connective tissue network with myocytes. A pathway enabling information to be transduced from external environment *via* specific regions of the sarcolemma to the cytoskeleton and the nucleus cannot be ruled out (Krug *et al.* 1987, Borg and Burgess 1992/1993, Pelouch *et al.* 1994a,b,).

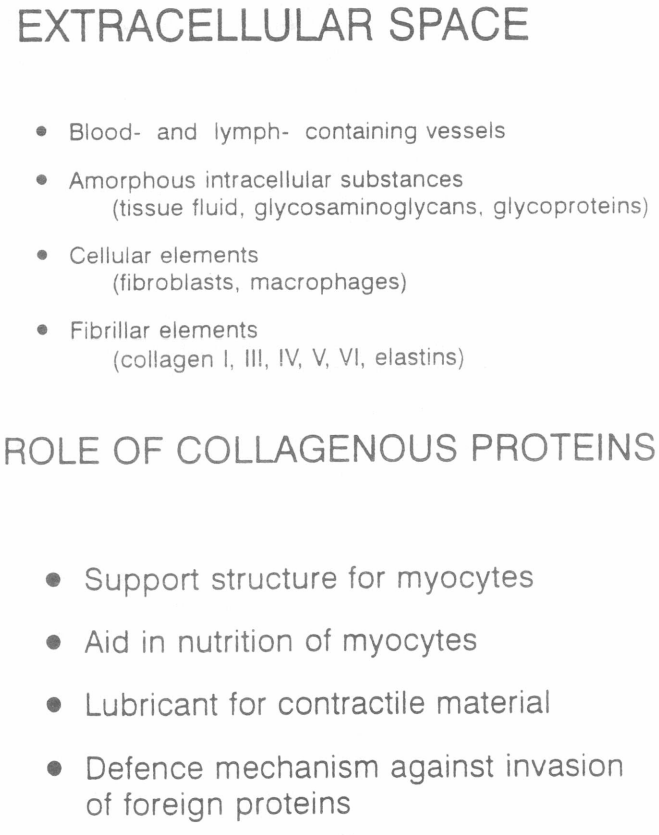

Fig. 3
Extracellular space and the physiological role of cardiac collagenous proteins. The interstitial compartment contains vessels, amorphous substances, cellular and fibrillar elements and a mixture of extracellular proteins: collagens, glycoproteins, elastins and glycosaminoglycans.

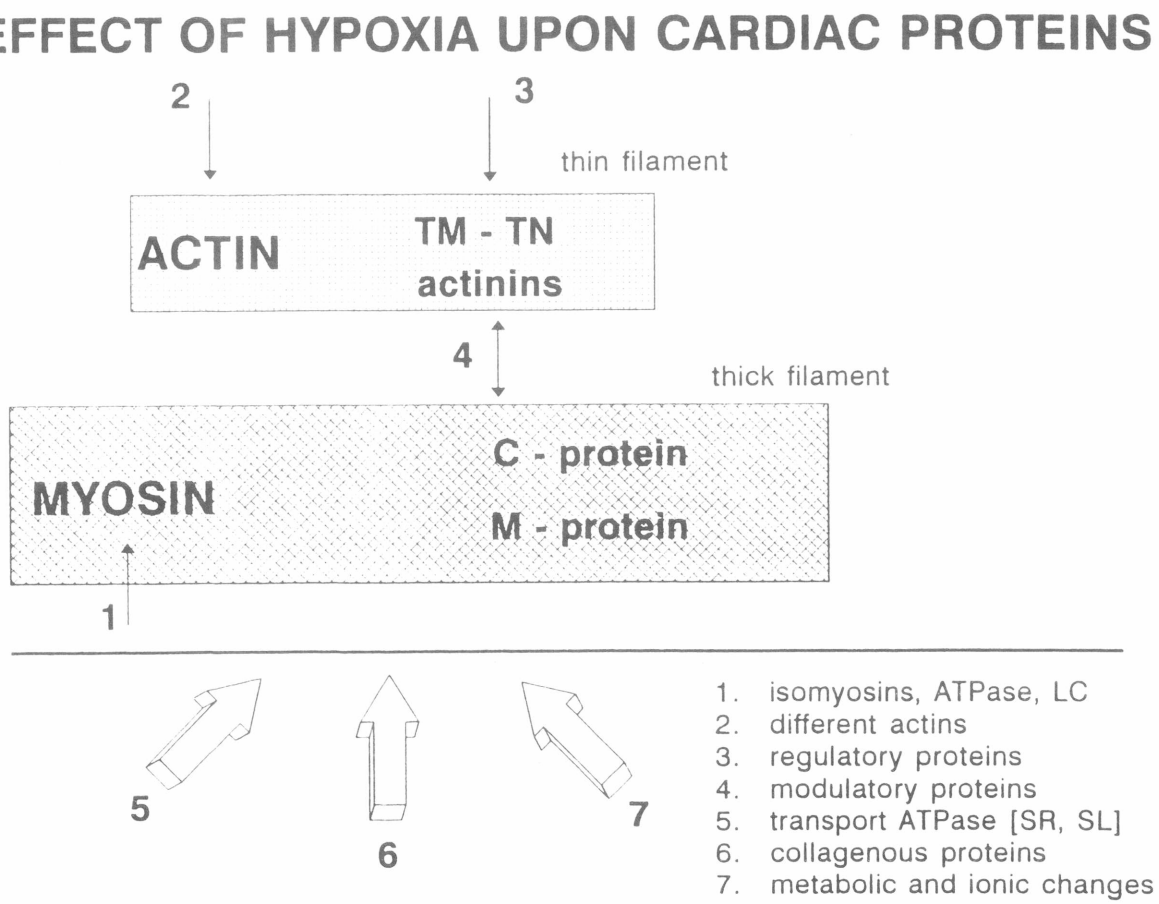

Fig. 4
Effect of hypoxia upon cardiac proteins. Contractile proteins are localized in both the thin filaments (actin, tropomyosin [TM], troponin [Tn] – regulatory proteins, actinins – modulatory proteins) and thick filaments (myosin and C-, M-protein – modulatory proteins). The effect of hypoxia on cardiac myosin can possibly involve: a) change of both isomyosin composition and myosin ATPase activity and light chains (LC) – *1.*, and b) protein synthesis of different isoforms of thin filament proteins – *2.,3.,4.* The secondary effect of hypoxia may involve the breakdown of energy sources (lower production of ATP or creatine phoshate) which affects the transport ATPase systems on the sarcolemma (SL) and the sarcoplasmic reticulum (SR) – *5., 7.* The qualitative and quantitative remodelling of the interstitial space (the collagen I/collagen III ratio and the proportion of minor collagens play a major role) is one of the major factors regulating cardiac contractility in both normal and diseased myocardium – *6.*

Effect of hypoxia on contractile and collagenous cardiac proteins

Hypoxic states of the cardiopulmonary system result from a disproportion between the amount of oxygen supplied to the cell and the amount actually required by the cell; hypoxia significantly decreases myocardial contraction (Oštádal and Kolář 1992). Fig. 4 summarizes the possible sites of action of hypoxia on cardiac contractile and collagenous proteins. The effect of hypoxia on myosin can possibly involve a change in the molecular structure of myosin (qualitative and quantitative changes in both heavy and light chains, a shift of isomyosins from V1 to V3) and/or damage of the active centre of the myosin molecule (the site of myosin Ca-ATPase activity). Altered interactions in the thin part of myofibres (I-zones) can also be expected (e.g. a change in the proportion of different actins, regulatory or modulatory protein isoforms – Vandekerckhove *et al.* 1986, Malhotra 1994). Furthermore, hypoxia may affect either cardiac metabolism (e.g. a lowered concentration of ATP and creatine phosphate as a result of inhibition of oxidative metabolic cycles, pH changes due to higher activity of glycolytic enzymes) and transport ATPase systems (localized on both the sarcolemma and the sarcoplasmic reticulum), or the accumulation of extracellular matrix proteins (e.g. a higher concentration of collagens, a change in the proportion of major and minor collagens,

morphological remodelling of the cardiac interstitial space). The complex of both hypoxia-induced molecular changes of myofibrillar and collagenous proteins (for details see Pelouch *et al.* 1984, Pelouch 1993) and cellular basis of wall remodelling in long-term pressure or volume overload (Ollivetti *et al.* 1989) significantly influences the process of contraction and relaxation in the diseased myocardium.

Acknowledgement
This work was partly supported by grants No 2039-3 and 0879-3 of the Ministry of Health of the Czech Republic.

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