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EDITORIAL

Biochemical Characteristics of Cardiac Collagen and Its Role in Ventricular Remodelling Following Infarction

V. PELOUCH, R. JIRMÁŘ[1]

Institute of Physiology, Academy of Sciences of the Czech Republic and [1]The Second Internal Clinic, Third Medical School, Charles University, Prague

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Until recently, the cardiac muscle was considered as a purely muscular organ. Molecular regulation of contractility was related to the interaction of contractile proteins (myosin and actin) with ATP and calcium ions under the control of regulatory (tropomyosin and troponin) and modulatory (C-, F-, M-proteins, actinins) proteins. More recent papers (Caufield *et al.* 1985, Weber *et al.* 1988, Eghbali and Weber 1990, Borg and Terracio 1990, Weber 1992) have shown that the pump efficiency of the myocardium is more complex since the interaction of myocyte and extracellular matrix proteins and their relative proportion determines the contractility and viscoelasticity of the cardiac muscle under both physiological and pathophysiological conditions. This article is, therefore, concerned with the qualitative and quantitative changes of cardiac collagenous proteins and their remodelling in the infarcted overloaded myocardium. Some clinical aspects of this topic are discussed elsewhere (Jirmář and Pelouch 1993).

Biochemical and structural characteristics of the interstitial compartment

There are two distinct cardiac protein compartments: *a) space of non-collagenous proteins*: a mixture of two different types of proteins localized either in myofibrils or cytoplasm: 1. sarcoplasmic proteins which participate in the production of chemical energy – the enzymes of oxidative and anaerobic metabolism which are localized in the cytosolic and/or mitochondrial compartments, 2. contractile proteins which participate in the consumption of chemical energy – see above; all items are localized in myocytes. Biochemical characteristics of different proteins from the space of the non-collagenous compartment have been described in different review articles (e.g. Swynghedauw 1986, Sabry and Dhoot 1989, Schwartz *et al.* 1992, Malhotra *et al.* 1992, Pelouch *et al.* 1993a), *b) space of collagenous proteins:* extensive and highly organized interstitium – network of extracellular matrix structures formed by different collagenous proteins (Weber *et al.* 1988, Weber 1992, Speiser *et al.* 1991 a,b, Imataka *et al.* 1989). Proteosynthesis of both types of proteins is either coordinated (e.g. ontogenetic growth of cardiac muscle, first stage of cardiac hypertrophy, compensated heart) or uncoordinated (e.g. decompensated hypertrophied heart, myocardial failure).

Collagenous structures

The extracellular collagenous structures in the mammalian heart have been delineated by electron microscopy (Caufield 1983, Caufield *et al.* 1985, Robinson 1990, Factor 1990, Weber *et al.* 1992). Several components (capillaries, intercellular substances, fibroblasts, interstitial fluid and proteins) participate in the function of the interstitial compartment. They play more than a passive role because they influence essential developmental processes of the cardiac muscle, as well as normal functions of the myocardium (e.g. cell-to-cell interactions, cell sorting, migration). Specific receptors of the extracellular matrix – integrins – have been discovered (Hynes 1987).

Collagen is the major organic constituent of the connective tissue matrix of the myocardium. The

discovery of collagen isotypes has led to the recognition that there exist a whole family of collagens (each having distinctive structural and metabolic characteristic). It is currently recognized that at least 12 different collagen types are coded by a family of about 20 very complex collagen genes. They contain more than 50 different exons; about half of the exons corresponding to the triple-helical domain of the collagens has an identical length of 54 bp. Other exons that are coding for other segments of the triple-helical domain of the protein have a length of 162, 108, 99, or 45 bp. When the structures of different interstitial collagen genes were compared, it was found that the size distribution of the exons coding for the triple-helical domain of the collagen polypeptides are, with one exemption, identical: equivalent amino acid segments in the domain of these collagens are encodes by exons of equal size in each gene, despite considerable variations in the sequences of these exons (Laurent 1987, deCrombrugghe and Schmidt 1987). During processing within the nucleus, these sequences must be specifically excised and strands subsequently ligated before transcription of mRNA in the rough reticulum. The initial transcript has a short sequence of amino acids at the N-terminal end. Procollagen chains contain extension peptides at both the N- and C-terminal ends. However, neither long repeated structure of Gly-X-Y form typical for body collagen, nor the formation of triple helix are present. These peptides are removed by specific proteases that act either at the plasma membrane or after secretion of the molecule from the cell. The collagen structures are packed into vacuoles and then, they enter through invaginations of the plasma membrane, into the extracellular space where the collagenous structures are formed. There are several post-translation events. Collagen contains hydroxylated proline and lysine residues, which are produced by prolyl- and lysyl-hydroxylases. With the exception of collagen I, all collagen types contain disulphide bonds between cysteine residues. Furthermore, glucose and galactose residues are added (by specific transferases) to hydroxylysines. Covalent cross-links between chains of adjacent molecules stabilize collagen material.

Formation of cardiac extracellular matrix during ontogeny

In the early stages of development, formation of the extracellular matrix affects the differentiation of precordial mesoderms, migration of endothelial cells of the atrioventricular region, angiogenesis, and differentiation of distinct regions of the cardiac muscle (atrial and ventricular musculature). Further formation of the extracellular matrix (ECM) involves numerous interactions among collagenous proteins at at least four different morphological levels: epimysium, perimysium, endomysium and basement membrane. Perimysium consists of large bundles of a collagen network which connects the epimysium (collagenous network surrounding cardiac muscle) with the endomysium (collagenous structure which surrounds groups of myocytes and individual myocytes). There are different cardiac contacts: myocyte-myocyte, myocyte-capillary and the weave network (Weber *et al.* 1988, Borg and Terracio 1990). A similar collagenous structure is also tightly bound to the basement membrane (a specialized layer of sarcolemma that surrounds muscle cells). All these mentioned components, including the basement membrane, are composed of different types of collagenous proteins. The synthesis of individual collagen types (Miler and Gay 1987, Weber 1992, Weber *et al.* 1992), glycoproteins (Speiser *et al.* 1991a,b, Engel 1992) and proteoglycans (Mori and Honda 1982, Ruoslahti and Yamagushi 1991) localized in the cardiac ECM network has been documented. The formation of the connective tissue network is associated, after septation in the late foetal development, with differentiation of the myocardium; the three dimensional network involves interactions among fibroblasts, different proteins of ECM and specific sites localized on myocytes. During the neonatal developmental period, connective tissue is rapidly formed; different neurohumoral factors affect the postnatal growth of cardiac muscle due to higher proteosynthesis of both collagenous and non-collagenous proteins (Pelouch *et al.* 1987, 1992a, Carver *et al.* 1991). Myocyte-myocyte and myocyte-capillary connections are probably coordinated with the growth of individual myocytes and vascularization of the myocardium. These collagenous contacts are formed till the adult phase of growth; collagen fibrils go from fibroblasts to the Z band on the cell surface of the myocytes (Factor 1990, Robinson 1990). Fibroblasts produce interstitial collagens in both late foetal, neonatal and adult stages of cardiac development (Borg *et al.* 1983, Eghbali *et al.* 1988, Eghbali and Weber 1990). The weave network, poorly developed at birth, is clearly evident starting from the 4th postnatal day (Borg *et al.* 1983, Eghbali *et al.* 1988, Weber 1989, Weber *et al.* 1987, 1988, 1992). Early postnatal myocardium, however, is capable of procollagen synthesis (Borg and Terracio 1990); its mechanism is a matter of speculations (Blumenfeld 1988, Blumenfeld and Seifter 1990, Carver *et al.* 1991). The collagens are a large family of related extracellular proteins that exhibit diverse molecular and functional characteristics. They have a distinctive amino acid composition (the lengthy Gly-X-Y triplex regions spanning a sequence of 1014 amino acid residues plus a prevalence of prolyl and hydroxyprolyl residues in the X and Y position specify the triple-helical conformation and account, to some extent, for its stability – Miller and Gay 1987). The hydroxyproline assay is therefore used as a quantitative marker of the collagenous matrix.

Table 1
Myocardium contains different types of collagenous structures (epimysial, perimysial and endomysial fibres) formed by different collagens, glycoproteins, glycosaminoglycans and elastins. The biochemical structure of different cardiac collagen types is summarized here. The major types present are collagen I and III (see text and Fig. 1).

Group	Cardiac collagen types	Biochemical characterization
1	I, III, V	chains of M_r 95 000 or greater presence of a lengthy (cca 300 nm) uninterrupted helical domain major fibrils and fibre-forming molecular species possible lateral aggregation
2	IV, VI, VII, VIII	chains of M_r 95 000 or greater but molecules are characterized by the presence of several helical domains separated by sequences incompatible with helix domains impossible lateral aggregation
3	IX, X	chains of M_r less than 95 000

Collagen types

There are at least two types of collagenous protein structures: a) *fibrillar collagens* (collagen types: I, II, III, V) that have uninterrupted triple helices and comprise the structural framework of many connective tissues, b) *nonfibrillar collagens* (collagen types: IV, VI, VIII) containing interruptions with the triple helix - promoting Gly-X-Y repeats (Table 1).

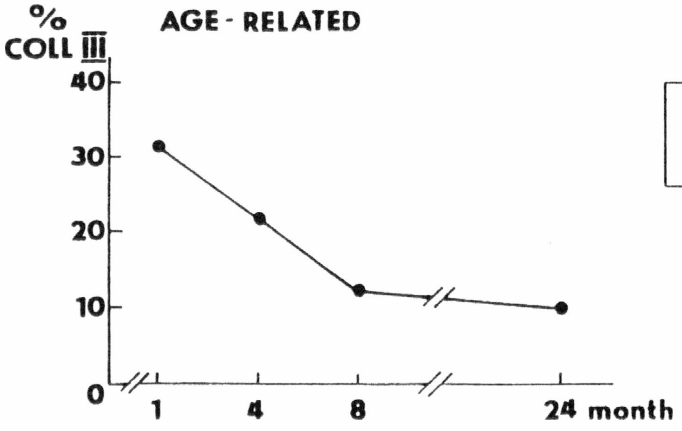
Both types of collagen have been detected in the cardiac muscle. Collagen I and collagen III represent more than 80 % of all collagenous types (McClain 1974, Medugorac 1983, Pelouch *et al.* 1980, 1985, 1987). Minority types of collagens are collagen types IV, V, VI, VIII, IX and X. The molecular weight of most collagen chains is around 95 000 (Miler and Gay 1987, Weber *et al.* 1987, 1989, Kawahara *et al.* 1990, Iruela-Arispe and Sage 1991, Speiser *et al.* 1991a,b). The collagen I forms larger stiff fibres, collagen III constitutes a fine network (for the biochemical characterization of both collagens see Fig. 1 and Table 1). The functional significance of the heterogeneity of collagen types is not clear. The relatively higher percentage of collagen III is found in younger stages of cardiac development, whereas at adult myocardium collagen III represents less than 10 %. The percentage of both cardiac collagens is, however, species-dependent. A significantly higher synthesis of collagen III is also observed at early stages of cardiac hypertrophy (Weber *et al.* 1988, 1990, Blumenfeld 1988, Blumenfeld and Seifter 1990, Eghbali and Weber 1990). The concentration of collagen has been estimated in ventricular parts of normal and hypertrophied cardiac muscle; however, the results are not consistent. At present little is known about collagen synthesis even in the normal ventricular myocardium. However, collagen growth appears to be a function of different stimuli, e.g. age, species, rapidity at which cardiac overload or hypoxia occur. On the other hand, only a few data are available for atrial musculature. The concentration of hydroxyproline is higher in rabbit atrial musculature (Caspari *et al.* 1977, Imataka *et al.* 1989). Recently, higher concentrations of collagenous protein have also been described in human atrial musculature, having a significantly higher amount of pepsin-insoluble material (Pelouch *et al.* 1988, 1993b). The response of collagenous proteins in atrial and ventricular musculature to an overload may, therefore, be different.

Changes of collagenous proteins in the ischaemic myocardium

There are numerous haemodynamic and neurohumoral stimuli which affect the plasticity and adaptability of both ischaemic and non-ischaemic parts of the infarcted myocardium. An increased demand for myocardial work initiates a series of compensatory physiological events (lower ejection fraction induces acute and long-term compensatory responses in the cardiac muscle – see Fig. 2). The progressive increase in the end-diastolic lengths is coupled with a large

HEART COLLAGEN

COLLAGEN	CHAINS		PRECIPITATION		
			NaCl/M	0.5M HA	1M NaCl
I	2 α_1 (I) α_2 (I)	HETEROTRIMER	0.7	+	
			2.5		+
III	3 α_1 (III)	HOMOTRIMER	0.7	+	
			1.8		+



HYPERTROPHY

DIFF. TYPES	COLL III ↑

Fig. 1
Heart collagen is composed of different collagen types; collagen I and collagen III predominated. Collagen III has three chains of the same type (homotrimer), whereas collagen I is composed of two different chains (heterotrimer). A significant decrease of the content of collagen III is detected during the early postnatal development of myocardium compared with adult myocardium. Collagen I forms thick filaments, collagen III forms a finer structure. The synthesis of collagen III is significantly elevated in the hypertrophied myocardium (first stage - developing cardiomegaly). In the failing myocardium, collagen concentration is increased but the pattern of collagen types returns back to the control level (predominantly of collagen I). Different cardiac collagens could be removed from the muscle by 0. 5 M acetic acid (HA) – pepsin (1: 10–100 enzyme/protein ratio) and then precipitated in a stepwise manner (by different concentrations of NaCl).

relative shortening of that region as a compensatory response in the myocardium with a long-lasting volume overload myocardium where eccentric hypertrophy is present. The extent and timing of these processes is specific according to the animal species (Bishop *et al.* 1990, Judgutt *et al.* 1992, Pfeffer *et al.* 1985, Pfeffer and Braunwald 1991). The reduction of the ejection fraction in patients with the myocardium damaged by infarction is compensated by acute mechanisms: the distention of viable myocardium together with augmentation of inotropic and chronotropic responses of the myocardium could restore the functional parameters of ventricles. The long-term maintenance of adequate pump activity is insufficient; enlarging the cavity size by long-term dilatation could restore the stroke volume for a short time only. On the other hand, this dilatations would increase systolic and diastolic wall stress and thus initiate the proteosynthesis in different structures and further affect ventricular enlargement (Fig. 2).

VENTRICULAR DILATATION AS AN ADAPTIVE PROCESS

Decreased ejection fraction

↓

Acute and long-term compensatory responses

a) augmentation of chronotropic and inotropic activity,
b) acute distension of viable myocardium, and
c) augmenting cavity size by long-term dilatation (this dilatation would augment diastolic and systolic stress → stimulation of ventricular enlargement)

Fig. 2
Schematic representation of adaptive processes in a non-ischaemic part of the infarcted myocardium (for details see text).

Compensatory responses of the viable myocardium are sufficient to offset the loss of myocytes; development of hypertrophy in the remaining regions of both ventricles is supposed to be an important adaptive process for the maintenance of mechanical function of the myocardium with healed infarction (Mill *et al.* 1990, Pfeffer and Braunwald 1991). Furthermore, cardiac overload induces increased myocyte size, a higher content of myofibrils (Morgan and Baker 1991), synthesis of different isoforms of contractile proteins (Swynghedauw 1986) or formation of cardiac myosin with lower ATPase activity (Pelouch *et al.* 1976). All these alterations could be affected either by protein synthesis, degradation or most probably by the combination of both processes; the described increase of the concentration of cardiac RNA is, however, a marker of higher synthesis of different protein fractions (Simpson *et al.* 1989). Ventricular overload and different types of hypoxia are also the main stimuli for collagen synthesis (Bartošová *et al.* 1969, Pelouch *et al.* 1984, 1985, 1987); the changes in collagen integrity, muscle bundle alignment, or qualitative and quantitative parameters of collagenous structures could also be detected.

There are two types of remodelling in cardiac hypertrophy. Normal myocardial architecture which is characterized by muscle bundles held in alignment by fibrillar collagens, whereas the overloaded myocardium has muscular bundles which had either slipped toward the centre of the chamber (positive slippage) or slipped away from the centre of the chamber (negative slippage). The former motion increases wall thickness and results in concentric hypertrophy, the latter motion causes wall thinning and chamber dilatations and results in eccentric hypertrophy. Both slippages occur only when the collagen structure had been disrupted. This chamber restructuring and side to side slippage of cells occur even in the early period of damaged myocardium and persist till the is healed injured ventricle (Ollivetti *et al.* 1988, Capasso *et al.* 1990, 1992, Whittaker *et al.* 1991). These alterations (Hutchinson and Bulkley 1978) in ventricular topography and composition, with infarct expansion (referred to as ventricular remodelling – see Fig. 3) can profoundly impair the function of the infarcted left ventricle and increase early morbidity and mortality. Large transmural infarcts are characterized by complex alterations in the architecture of the ventricular wall, rearrangments of myocytes within both surviving and infarcted zones of the cardiac muscle, disproportionate regional thinning (a consequence of the slippage between muscle bundles – a reduction in the number of myocytes across the infarcted area), acute dilatations and thinning of the area of infarction (explained by additional myocardial necroses of the heart). The cellular necrosis produced by myocardial infarction results in a regional impairment of systolic function in the case of small infarcts (Zhao 1987, Whittaker *et al.* 1989, 1991).

Most of the previous biochemical studies of the infarcted myocardium have been focused on the changes of contractile proteins and the morphological structure of myocytes (for review see Swynghedauw 1986, Malhotra *et al.* 1992). Progress in both analytical and morphological approaches made it possible also to detect changes in ECM proteins of the affected myocardium. Collagenous structures have an important biological role even in the healthy myocardium: they form a supporting structure for myocytes, participate in the nutrition of myocytes and form a lubricant for contractile proteins localized in both A and I zones of myofibrils (Weber 1989). During the acute stage of myocardial infarction, collagen participates in maintaining the structural integrity of cardiac muscle. The cardiac collagen network (endomysium, perimysium, epimysium) is mechanically and structurally well able to resist infarct expansion, however, expansion does occur (preferentially by the system of collagen struts: myocyte-myocyte connections that are thought to be important in tethering the cell).

It is clear that either organization of collagenous structure or the quality and quantity of collagenous stroma can be affected in the diseased myocardium. It has been shown that the biochemical changes of extracellular matrix have a characteristic time course. Acute inflammation predominates in the first week, chronic inflammation in the second week and the higher synthesis of collagenous proteins and their deposition is elevated in the next weeks (Judgutt *et al.* 1992, Pelouch *et al.* 1992b). Scar formation

occurrs as the effect of the long-term phase of fibroblast proliferation (Fig. 3). Before and during these processes the resorption of necrotic tissue, slippage between muscle bundles and reduction in the number of myocytes across the infarcted region have been observed. Quantitative changes of ECM proteins could be detected by the measurement of hydroxyproline (as a biochemical marker of collagen). Qualitative changes of these collagenous fractions could be detected after the separation on SDS electrophoresis (5–7 % of acrylamide in either the presence or absence of mercaptoethanol – see Miller and Gay 1987). The elevated concentration of hydroxyproline in the infarcted myocardium is predominantly due to a higher concentration of insoluble collagenous proteins, while the percentage of hydroxyproline in pepsin-soluble collagen is reduced due to a greater number of cross-links in the collagen fraction (Pelouch *et al.* 1992b).

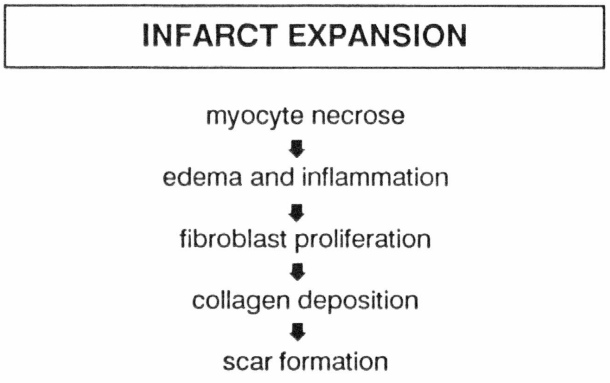


Fig. 3
Infarct expansion represents a cascade of changes localized in both myocytes and the extracellular matrix of diseased cardiac muscle. All these structural changes are combined with proteosynthesis of collagenous and non- collagenous isoforms (contractile and sarcoplasmic proteins) characteristic of an ischaemic myocardium (for details see text)

The elevation of hydroxyproline in infarct zone reaches its peak value between 3–6 weeks; a significantly higher value is detected already one week after ligation of the left coronary artery (Judgutt *et al.* 1992, Pelouch *et al.* 1992b). On the other hand, the nature of the underlying changes in human cardiac collagen derived from the ischaemic part of the myocardium remains unclear, but alterations in both degradative and synthetic processes play an important role. The thickness and collagen content in the scars after infarction might be important determinants of myocardial performance and prognosis for survival after acute ischaemic damage of the myocardium. The number of normally birefringent collagen fibres is significantly reduced as early as the first day after myocardial infarction, the loss of interstitial space and intermyocyte collagen struts is detected by the 4th day (Judgutt and Amy 1986, Whittaker *et al.* 1989, 1991, Judgutt *et al.* 1992). On the other hand, the decrease of collagen struts is consistent with the concept that expansion proceeds *via* slippage of myocytes previously tethered by the struts. There is correlation between infarct expansion and damage of the collagen structure. The heterogeneity of ventricular remodelling during the healing of myocardial infarction is due to the loss of viable myocardium; connective tissue cells enter the myocyte compartment and connect disrupted myocyte fibres (Judgutt *et al.* 1992, Zimmer *et al.* 1990, Pfeffer and Braunwald 1991, Judgutt and Amy 1986).

Changes of collagenous structures during the regression of cardiac hypertrophy

Regression of the interstitial space together with significantly lower compliance and changes of myocytes, described above, result in dilatations of the affected parts of the cardiac muscle. If the collagenous network is the major factor responsible for the maintenance of structural integrity of the cardiac muscle, then factors that are able to protect collagens against the qualitative as well as quantitative changes could diminish the process of infarct expansion. The synthesis and degradation of collagen are still a matter of speculation; the major sites of degradation are still uncertain (Laurent 1987). Apart from proteolytic events, degradation of collagens occurs at both intracellular (endoplasmatic reticulum, Golgi apparatus, lysosomes) and extracellular sites (collagenase which makes them susceptible to other neutral proteases in the extracellular space). Increased production of defective collagen stimulates lysosome-mediated breakdown; both non-lysosomal and lysosomal pathways are dependent on the relationship between the rates of synthesis and degradation of collagenous proteins (Sen and Bumpus 1979, Laurent 1987). The collagen equilibrium may be up- or down-regulated, depending on the direction of the change in the content of collagenous proteins. Significantly higher collagen deposition has been repeatedly detected during compensatory growth of the cardiac muscle, but its degradation has seldom been studied even in the control myocardium. It has been shown that degradation of newly synthetized and "mature" collagen are independent processes with a significantly different time constant (Laurent 1987). The degradation of collagenous proteins in healing of cardiac infarction could be affected by both the presence and absence of leucocytes. The effect of oxygen free radicals, which are usually generated under different pathological situations (hypoxia, ischaemia, reoxygenation) could also be taken into consideration. It has, however, been

shown that superoxide anion or hydroxyl radicals in the presence of oxygen could liberate small hydroxyproline-containing fragments from collagen. On the other hand, hydroxyl radicals trigger polymerization of collagen in the absence of oxygen (Momnbloisse and Borel 1992). These differences could contribute to the changes of collagenous proteins in both infarcted and non-infarcted parts of the diseased myocardium.

Modulators of collagenous protein synthesis

The cellular necrosis produced by myocardial infarction results in a regional impairment of systolic function. In the case of small infarcts, a compensatory reaction in the region of the viable myocardium is able to compensate the loss of myocytes. On the other hand, more extensive infarction is combined with remodelling of cardiac tissue: a) distortion of the chamber contour, b) infarct region thins and elongates, c) loss of myocytes. Furthermore, the elevation of ventricular systolic pressure together with coronary perfusion pressure activates the proteosythesis of contractile and collagenous proteins in both ischaemic and non-ischaemic parts of the cardiac muscle. All these changes have important prognostic implications for survival.

Different substances have been used to determine the manner and the extent of collagen remodelling of the diseased myocardium; these include angiotensin-converting enzyme (ACE) inhibitors. Components of the renin-angiotensin system, including renin, angiotensinogen, angiotensin-converting enzyme, angiotensin I and II and conversion of angiotensin I to angiotensin II have been detected in the myocardial muscle (Yamada *et al.* 1991, Hirsch *et al.* 1991). A beneficial effect of different ACE inhibitors on this conversion in myocardial ischaemia has been described in perfused rat cardiac muscle (Linz *et al.* 1988). At present, major effort is focused on the clinical impact of ACE inhibitors. It can be summarized that therapy with ACE inhibitors prolongs survival, the greatest benefit occurring in a medium infarct with less dilated left ventricles, where markedly lowered left ventricular filling pressure was observed (Pfeffer and Braunwald 1991). Much less is known about the effect of ACE inhibitors on cardiac collagenous and non-collagenous proteins. There is evidence that the renin-angiotensin system is responsible for fibroblast proliferation (Jalil *et al.* 1989, Krimpen *et al.* 1991, Keeley *et al.* 1991, 1992, Weber 1988, 1992b). Beside circulating angiotensin, myocardial collagen accumulation is also affected by the retention of water or sodium ions and contribution of hypokalaemia; all these items are potential candidates for abnormal collagen remodelling (Brilla *et al.* 1990, Cappaso *et al.* 1990, Olivetti *et al.* 1990, Anversa *et al.* 1986, Weber *et al.* 1988). Furthermore, it has been shown that the administration of ACE inhibitors, either partial or complete, reverses the changes of myosin isoenzymes, volume density of collagen, elevation of blood pressure and hormone disbalances observed in untreated experimental animals (cardiac necrosis induced by ligation of the left coronary artery – Michel *et al.* 1988). Recognition of these modulatory factors which either remodel both myocyte and non-myocyte cells or induce the promotion of different isotypes of contractile and collagenous proteins in the damaged part of the myocardium, may permit the development of pharmacological strategies as how to prevent structural changes or to restore the function and structure to normal range when abnormal tissue heterogeneity had already occurred.

It can thus be concluded that the study of quantitative as well as qualitative changes of synthetic and degradative processes of cardiac collagenous proteins, together with morphological, functional and clinical observations, could shed a new light on the extracellular protein space that is one of the major factors regulating cardiac contractility in both normal and diseased myocardium.

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Reprint Requests

Dr. V. Pelouch, Ph.D., Institute of Physiology, Academy of Sciences of the Czech Republic, 142 20 Prague 4, Vídeňská 1083, Czech Republic.