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The ageing spontaneously hypertensive rat as a model of the transition from stable compensated hypertrophy to heart failure

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KEY WORDS: Compensated cardiac hypertrophy, heart failure, cardiac remodelling, extracellular matrix, ventricular stiffness, spontaneously hypertensive rat.

Spontaneously hypertensive rats (SHR) of advanced age exhibit depressed myocardial contractile function and ventricular fibrosis, as stable compensated hypertrophy progresses to heart failure. Transition to heart failure in SHR aged 18–24 months was characterized by impaired left ventricular (LV) function, ventricular dilatation, and reduced ejection fraction without an increase in LV mass. Studies of papillary muscles from SHR with failing hearts (SHR-F), SHR without failure (SHR-NF), and age-matched Wistar Kyoto (WKY) rats allowed examination of changes in the mechanical properties of myocardium during the transition to heart failure. Papillary muscles of SHR-F exhibited increased fibrosis, impaired contraction, and decreased myocyte fractional area. These findings in papillary muscles were correlated with a higher concentration of hydroxyproline and increased histological evidence of fibrosis in the LV free wall. While a depression in active tension accompanied these structural alterations in papillary muscles, it was not evident when active tension was normalized to myocyte fractional area. Together, these data suggest that individual myocyte function may be preserved but that myocyte loss and replacement by extracellular matrix contribute substantially to the decrement in active tension. An absent or negative inotropic response to isoproterenol is observed in SHR-F and SHR-NF papillary muscles and may result in part from age-related alterations in β-adrenergic receptor dynamics and a shift from α- to β-myosin heavy chain (MHC) protein. During the transition to failure, ventricles of SHR exhibit a marked increase in collagen and fibronectin mRNA levels, suggesting that an increase in the expression of specific extracellular matrix genes may contribute to fibrosis, tissue stiffness, and impaired function. Transforming growth factor-β_1 (TGF-β_1) mRNA levels also increase in SHR-F, consistent with the concept that TGF-β_1 plays a key regulatory role in remodelling of the extracellular matrix gene during the transition to failure. The renin-angiotensin-aldosterone system is also implicated in the transition to failure: SHR treated with the angiotensin converting enzyme inhibitor captopril starting at 12 months of age did not develop heart failure during the 18–24 month observation period. Captopril treatment that was initiated after rats were identified with evidence of failure led to a reappearance of α-MHC mRNA but did not improve papillary muscle function. Research opportunities include investigation of apoptosis as a mechanism of cell loss, delineation of the regulatory roles of TGF-β_1 and the renin-angiotensin-aldosterone system in matrix accumulation, and studies of proteinase cascades that regulate matrix remodelling.

Introduction

Heart failure is a debilitating, progressive, and fatal disease[1]. Understanding the mechanistic basis of heart failure has remained a largely intractable problem facing medicine and science. Heart failure has many causes and, perhaps, numerous primary inciting factors (Table 1)[1,2]. Hypertension and cardiac hypertrophy are commonly associated with heart failure[2] and are two features that characterize spontaneously hypertensive rats (SHR). The virtues and limitations of SHR as a model of clinical hypertension have been thoroughly debated[3,4]. The potential of ageing SHR as a model of heart failure has also been recognized[5–7], and SHR of advanced age have been used to characterize the transition from stable compensated hypertrophy to heart failure[8–13]. The purpose of this review is to summarize the knowledge gained from this body of work and to examine its implications for future research.

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Table 1 Hypothetical mechanisms of myocardial failure

Insufficient Ca^{2+} available to the myofilaments following excitation
Intrinsic alterations in contractile proteins
A reduction in myofilament Ca^{2+} binding or contractile response to Ca^{2+}
Impaired autonomic modulation during stress
Insufficient energy supply
Extracellular matrix changes
Supraoptimal cell Ca^{2+} loading
Myocyte dropout

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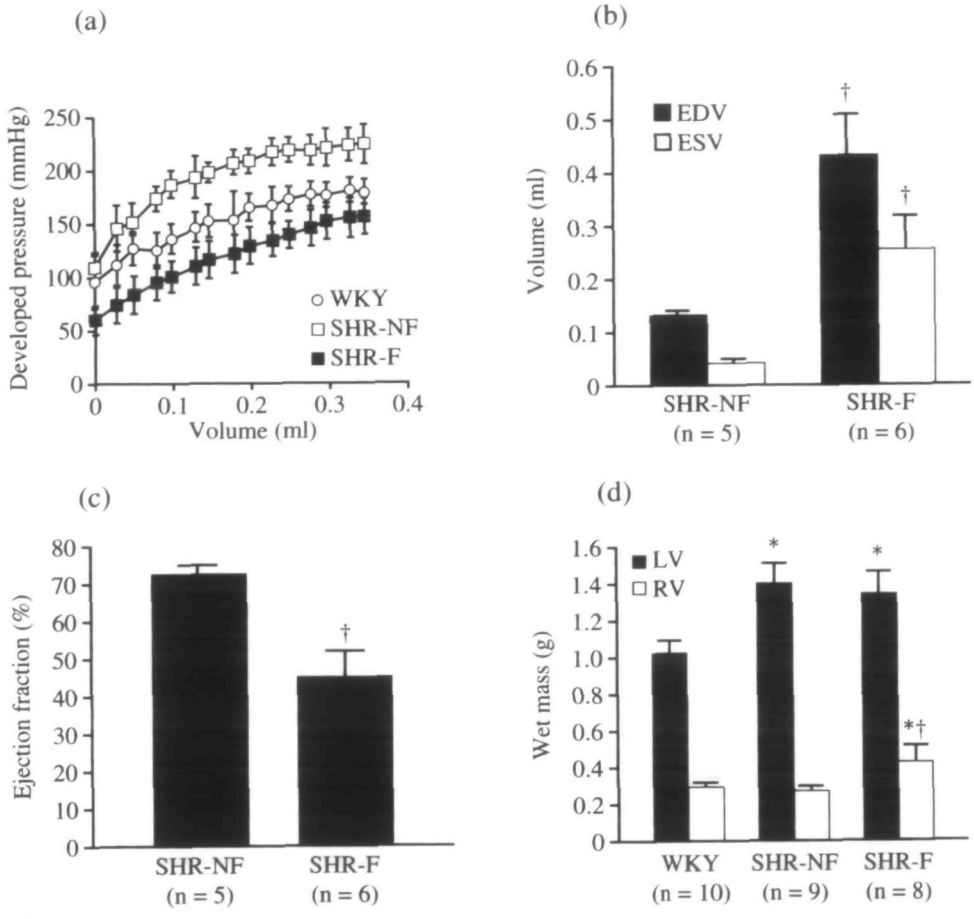

Figure 1 Depressed left ventricular (LV) function in hearts of aged spontaneously hypertensive rats (SHR). Baseline pressure–volume relationships (a) were measured in isolated perfused hearts of 8–13 age-matched Wistar Kyoto (WKY) rats, SHR without heart failure (SHR-NF), and SHR with heart failure (SHR-F) aged 18–20 months. Two-dimensional echocardiography (b,c) in intact, unanaesthetized SHR groups revealed increases in LV end-systolic volume (ESV) and end-diastolic volume (EDV) together with a decrease in LV ejection fraction in SHR-F. Ventricular wet mass (d) differed significantly between SHR groups as well as between SHR groups and WKY controls. All values are mean ± SE. *$P<0.05$ vs WKY, †$P<0.05$ vs SHR-NF. (Panel a reprinted from Brooks *et al.*[13] with permission. Panels b–d reflect data from Bing *et al.*[8].)

Historical perspective and current findings

Initial studies on ageing in the SHR model demonstrated that young adult and middle-aged SHR exhibited compensated cardiac hypertrophy, whereas depressed contractile function and increased fibrosis were observed in SHR of advanced age[5,6]. Careful observation of SHR, beginning when they were 18 months of age, allowed the identification of animals with clinical and echocardiographic evidence of heart failure. When studied within 1 week of identification, most of these rats exhibited pathological evidence of heart failure, including pleural/pericardial effusions, atrial thrombi, and right ventricular hypertrophy. Subsequent studies demonstrated that the transition to failure after the age of 18 months was characterized by an impairment in left ventricular (LV) function, ventricular dilatation, and reduced ejection fraction (Fig. 1(a–c)). Despite the prominent changes in function and the pathological evidence of failure, failing hearts showed no additional increase in LV mass (Fig. 1(d)).

To investigate the changes in mechanical properties of cardiac muscle during the transition to heart failure, we isolated papillary muscles from hearts of age-matched Wistar Kyoto (WKY) rats, SHR without signs of heart failure (SHR-NF), and SHR with heart failure (SHR-F) and studied them in vitro. Papillary muscle preparations have been shown to reflect properties of the ventricle from which they were obtained[8], and they provide a parallel fibred preparation that offers advantages for study of mechanical properties[14]. As shown in Fig. 2, papillary muscle from SHR-F displays increased fibrosis coupled with impaired contraction parameters. Increased stiffness correlates with a greater concentration of hydroxyproline in the LV free wall and an increase in histological fibrosis. Each of these factors is

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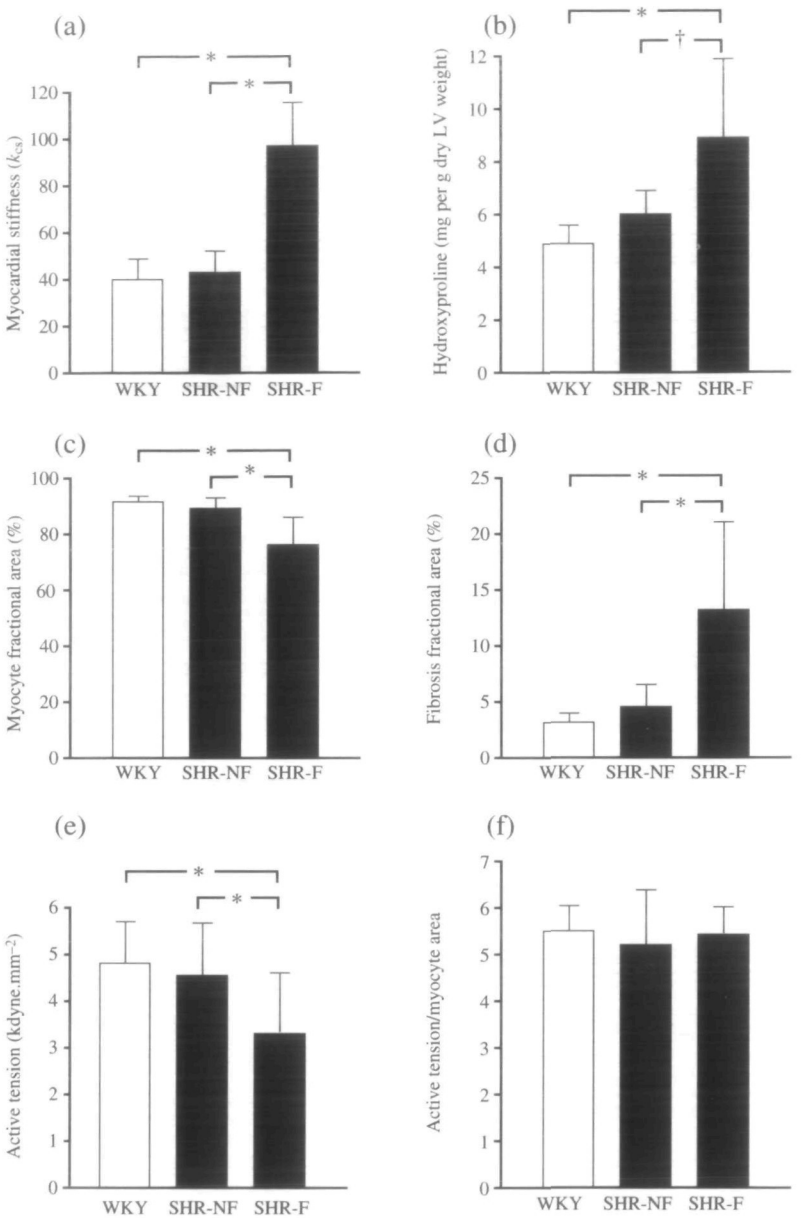

Figure 2 Changes in papillary muscle structure and function during the transition from stable hypertrophy to heart failure. Central segment stiffness (a), hydroxyproline concentration in the LV free wall (b), myocyte fractional area (c), fibrosis fractional area (d), and active tension (e) in papillary muscle preparations from SHR-F were all significantly different from these parameters in both WKY rat and SHR-NF preparations (7–12 per group). However, no significant difference occurred in measurements of active tension normalized to myocyte fractional area (f). Values are mean ± SD. *$P<0{\cdot}05$, †$P<0{\cdot}01$. k_{cs}=central segment myocardial stiffness constant; other abbreviations as in Fig. 1. (Adapted from Conrad *et al.*[10] with permission.)

related inversely to myocyte fractional area, suggesting that myocytes are lost and replaced with extracellular matrix (Fig. 2(a–d)). This altered structural profile of papillary muscle is coupled with a depression in active tension (Fig. 2(e)). When active tension is normalized to myocyte fractional area, however, the decrement in function disappears (Fig. 2(f)), suggesting that myocyte loss coupled with replacement fibrosis contributes substantially to the reduction in active tension exhibited by the papillary muscles of SHR-F. Other studies using the SHR model have focused on mechanisms relating to myocyte function and extracellular matrix regulation.

Myocyte mechanisms in progression to heart failure

To determine whether intrinsic properties of the muscle differ, calcium sensitivity and maximum calcium activated force were measured in chemically skinned

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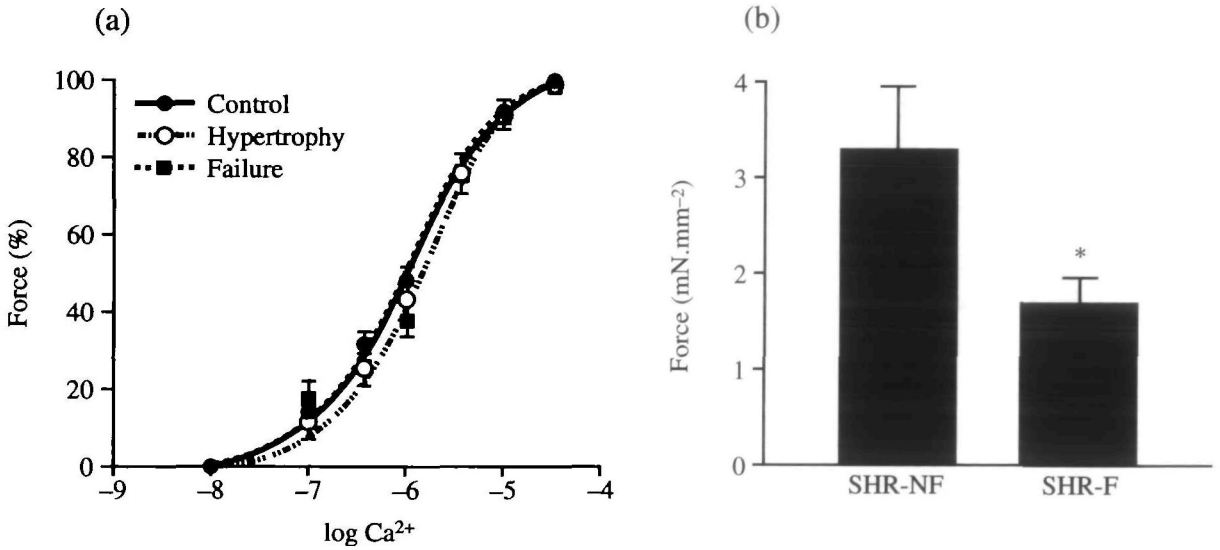

Figure 3 Function and calcium sensitivity in chemically skinned left ventricular papillary muscles from failing and non-failing hearts of SHR and controls. The relation of force to calcium concentration (a) revealed no difference between groups in calcium sensitivity. However, at maximal calcium activation (b), muscles of SHR-F developed significantly less force than did those of SHR-NF (*$P<0{\cdot}05$). Abbreviations as in Fig. 1. (Adapted from Perreault *et al.*[12] with permission.)

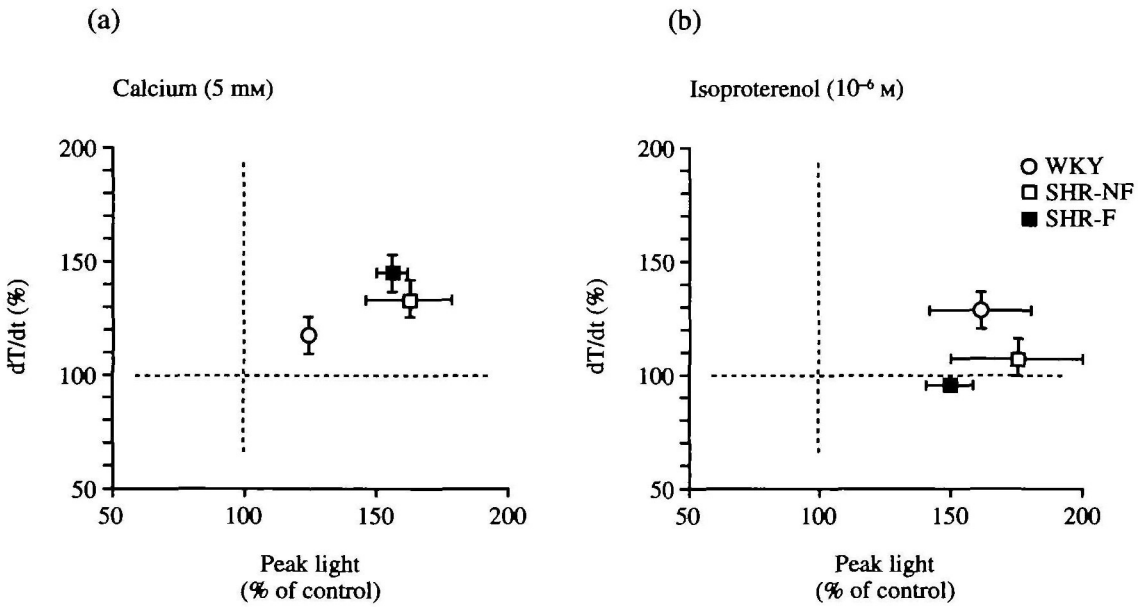

Figure 4 Changes in inotropism associated with heart failure in SHR. Papillary muscle preparations from SHR-F demonstrated increases in dT/dt and peak light (related to intracellular calcium concentration) in response to stimulation with 5 mM of calcium (a). Conversely, the response of SHR-F papillary muscle to 10^{-6} M of isoproterenol (b) was absent or negative, despite an increase in peak light. Control values (1·25 mM of calcium) represent 100%. Abbreviations as in Fig. 1. (Adapted from Bing *et al.*[11] with permission.)

papillary muscle preparations. Although there was no difference in calcium sensitivity, muscles from SHR-F developed less force than those of SHR-NF at maximal calcium activation (Fig. 3). Interpreted with the data from Fig. 2, these findings support the concept that myocyte loss may be responsible, in part, for the impaired muscle function observed in failing hearts, while individual myocyte function may be preserved.

The functional reserve of the heart, or its capacity to respond to a challenge, is a key property affecting survival of the organism as well as quality of life. There is evidence that the adaptive capacity of the heart may diminish with advancing age (reviewed in Lakatta[15]). To examine the response to inotropic stimulation in papillary muscle from failing hearts, papillary muscle mechanics and intracellular calcium concentrations

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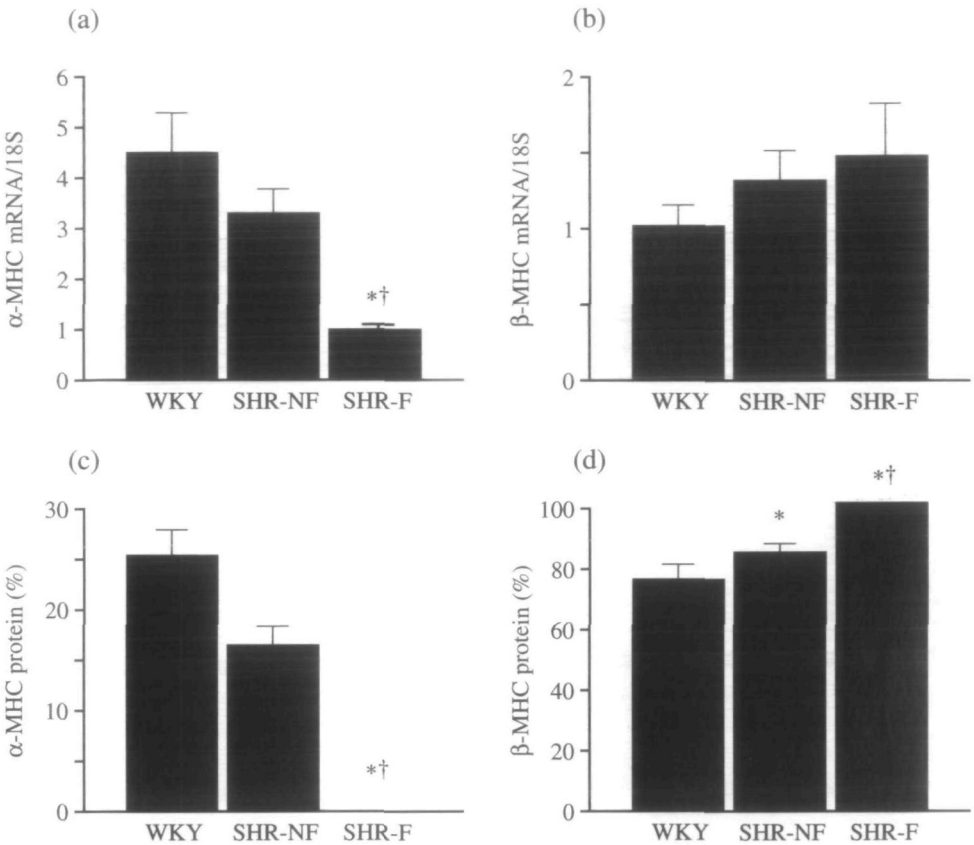

Figure 5 Change in relative proportions of myosin heavy chain (MHC) isoforms in left ventricles of SHR during the transition to heart failure. Normal ageing and pressure overload hypertrophy are associated with a shift from α- to predominantly β-MHC isoforms. Northern blot data for α- and β-MHC mRNA (a, b) were normalized relative to that obtained for 18S ribosomal RNA; the α-MHC value for the left ventricle of SHR-F and the β-MHC value for the left ventricle of WKY were arbitrarily set at 1·00, and other values were adjusted to correspond. Protein composition of the left ventricle (c, d) reflects the altered α- and β-MHC mRNA expression. *$P<0·05$ vs WKY, †$P<0·05$ vs SHR-NF. Abbreviations as in Fig. 1. (Panels a and b adapted from Boluyt *et al.*[20] with permission; panels c and d reflect data from Bing *et al.*[11].)

(aquorin method) were measured during exposure to 5 mM of calcium or 10^{-6} M of isoproterenol and compared with controls (1·25 mM of calcium). In response to calcium stimulation, muscles from failing hearts exhibited an increase in inotropism correlated to the increase in intracellular calcium concentration (Fig. 4(a)). In contrast, the response to β-adrenergic receptor stimulation was impaired in the muscles from failing hearts. Papillary muscle from SHR-F rats displayed an absent or negative inotropic response to isoproterenol stimulation, despite increases in intracellular calcium concentration (Fig. 4(b)). This suggests that in addition to previously noted impairments in cardiac muscle function, muscle from failing hearts is less responsive to β-adrenergic stimulation even though the response to calcium appears to be intact. Many factors may be responsible for this absent inotropism; among them are changes in β-adrenergic receptor dynamics, which become altered during normal ageing[15].

One factor that may contribute to the absent inotropic response to isoproterenol stimulation in the failing heart is a reduction in the proportion of α-myosin heavy chain (α-MHC) protein. Cardiac muscle myosin in the rodent is composed of a mixture of α- and β-MHC isoforms, with differing contraction kinetics and energetics. In young adult rats, the α-MHC isoform predominates, accounting for more than 80% of the total ventricular MHC protein. Both normal ageing and pressure overload hypertrophy are associated with a shift from α-MHC (faster kinetics, less economical) to the β-MHC isoform (slower kinetics, increased energetic economy). These effects can be observed in the levels of MHC protein and mRNA measured in the left ventricle of WKY rats and SHR-NF (Fig. 5). In addition to the ageing and hypertrophic effects that probably account for the levels of α- and β-MHC in SHR-NF, failure exacerbates the shift from α- to β-MHC and results in the virtual disappearance of α-MHC mRNA and protein. It may be that some minimum level of α-MHC protein is essential for an optimal inotropic response to isoproterenol. This reduction of α-MHC protein during the transition to heart failure may then account, at least in

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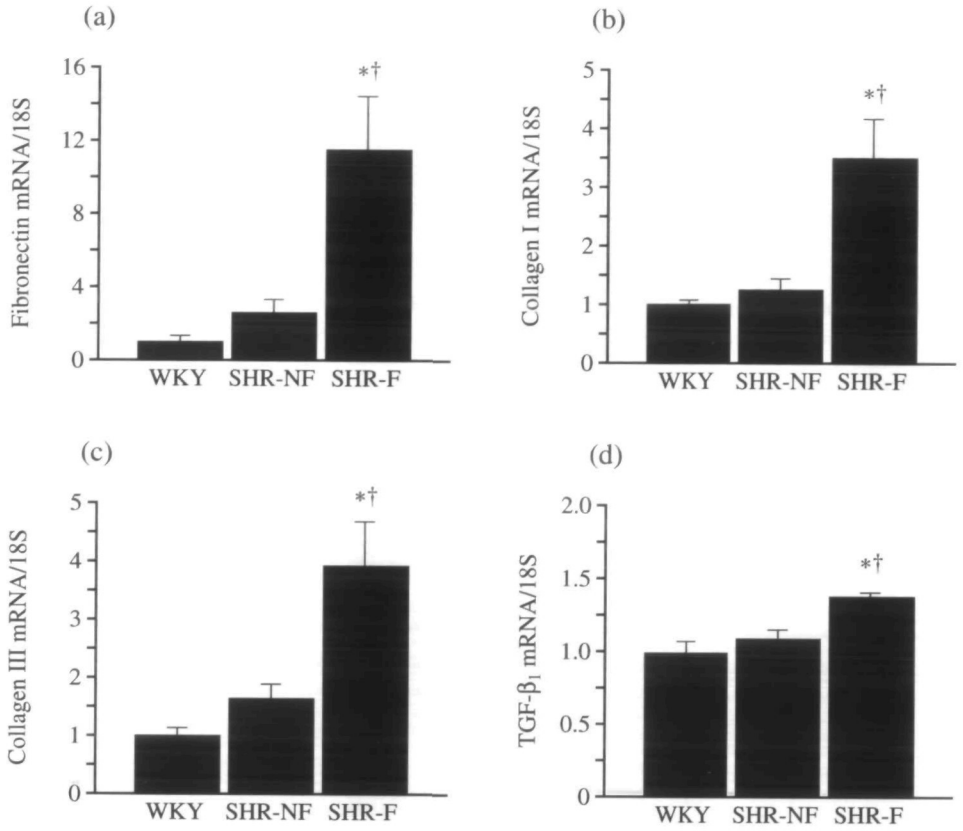

Figure 6 Levels of mRNA encoding extracellular matrix components in the left ventricles of SHR-NF, SHR-F, and WKY rats. Marked increases in mRNA expression of fibronectin (a), type I-α_1 collagen (b), and type III-α_1 collagen occur during the transition to heart failure; such increases may contribute to tissue stiffness and impaired LV function. Up-regulation of extracellular matrix gene expression correlates with a significant increase in expression of transforming growth factor-β_1 mRNA (d). Values are arbitrary in these Northern blot analyses: the WKY value was set at 1·00 and the other values were adjusted to correspond. *P<0·05 vs WKY, †P<0·05 vs SHR-NF. Abbreviations as in Fig. 1.

part, for the inability of the papillary muscle from failing heart to respond to isoproterenol stimulation (Fig. 4(b)).

Extracellular matrix regulation

As discussed earlier, hypertrophied and, particularly, failing hearts of SHR are characterized by an accumulation of extracellular matrix proteins and a corresponding increase in cardiac muscle stiffness (Fig. 2)[5,10,16]. Since fibronectin and collagen types I and III are major components of the interstitial fibrillar network, up-regulation of genes encoding these components in fibroblasts (or other matrix producing cells) may account, in part, for the increase in fibrosis observed during the transition to failure and may thus contribute to the decline in contractile performance[16]. Of particular interest is the fibronectin mRNA splicing variant encoding the extra type III segments EIIIA and EIIIB. These isoforms are expressed during wound healing[17], embryogenesis[18], and pressure overload hypertrophy[19]. Therefore, an investigation of the relative levels of mRNA encoding these extracellular matrix components in the hearts of WKY rats, SHR-NF, and SHR-F was conducted.

A marked increase in collagen and fibronectin mRNA levels was observed during the transition to ventricular failure in SHR (Fig. 6(a–c))[20]. The increase in fibronectin mRNA could be attributed primarily to an increase in EIIIA-containing transcripts (Fig. 7). This suggests that the onset of failure involves events that up-regulate fibronectin and collagen gene expression in a manner that resembles developmental and wound healing paradigms. Since increases in fibrillar collagen in the interstitium augment tissue stiffness, increases in fibronectin and collagen gene expression may contribute to impaired function. Although it is impossible at this point to separate cause and effect, the implications of a degenerative feedback interaction between function and stiffness are ominous.

What are the molecular signalling events that initiate the up-regulation of extracellular matrix genes? Among the candidates, transforming growth factor-β_1 (TGF-β_1) stands out. There is considerable evidence from studies of extracardiac tissues that TGF-β_1 plays a key role in regulating many aspects of the remodelling process, including the up-regulation of extracellular matrix genes. In the failing hearts of SHR that exhibited markedly increased levels of fibronectin and collagen

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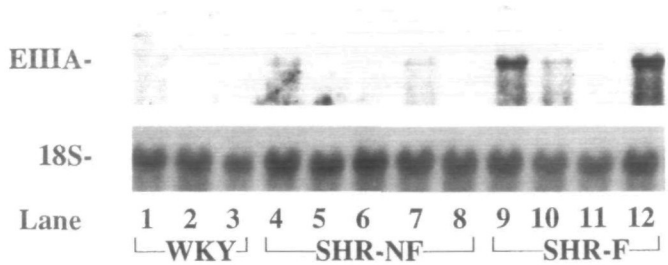

Figure 7 Levels of extra type III segment (EIIIA) fibronectin mRNA in the left ventricle of SHR-NF, SHR-F, and WKY rats, assessed by autoradiography. The Northern blot was probed with an oligonucleotide specific for the EIIIA segment and subsequently stripped and probed for 18S ribosomal RNA. Betascope analysis indicates a 3-fold to 5-fold increase in levels of EIIIA in SHR-F compared to SHR-NF and WKY rats. Quantitation must be interpreted with caution since the latter values were barely distinguishable from the background signal. Abbreviations as in Fig. 1. (Adapted from Boluyt *et al.*[20] with permission.)

gene expression, a small but significant increase in TGF-β_1 mRNA levels was observed in both ventricles (Fig. 6(d)). The up-regulation of TGF-β_1 gene expression observed in these failing hearts is consistent with a stimulatory role for this growth factor that may lead to accumulation of extracellular matrix. Active TGF-β_1 increases fibronectin and collagen gene expression in a variety of cell types and stimulates their incorporation into extracellular matrix[21]. Villareal and Dillman[19] also noted a transient increase in TGF-β_1 mRNA abundance that preceded the elevation in fibronectin and collagen mRNA levels after experimental aortic constriction in rats. In contrast to the transient, sequential increase in expression of extracellular matrix components after aortic constriction[19], the elevated levels of TGF-β_1, fibronectin, and collagens were evident simultaneously in hearts of all rats exhibiting signs of failure, suggesting that increased expression of these genes is sustained in the failing heart.

To assess more precisely the location of the mRNAs encoding extracellular matrix components in the ventricle, Conrad *et al.* used in situ hybridization to detect type I-α_1 collagen mRNA[22]. Fibrosis detected by Masson trichrome staining (Fig. 8(a)) was much more pronounced in ventricles of SHR-F than in either WKY rats or SHR-NF[22]. Fibrosis was focal in nature, and was particularly evident around vessels. In situ hybridization suggested that collagen mRNA was localized in both perivascular areas and interstitium (Fig. 8(b)). This pattern is reminiscent of that observed after administration of angiotensin II (Ang II) or aldosterone[23] and is consistent with the hypothesis that circulating factors as well as substances produced locally by various cell types in the heart (i.e. myocytes, fibroblasts, endothelial cells, and vascular smooth muscle cells) play a role in the development of fibrosis during heart failure.

There is mounting evidence that the renin-angiotensin-aldosterone system is involved in cardiac hypertrophy and perhaps the accumulation of extracellular matrix as well[16,24,25]. This hypothesis is supported by the up-regulation of extracellular matrix gene expression in both ventricles during the transition to failure[20]. Furthermore, treatment of SHR with captopril initiated at the age of 12 months completely prevented the development of heart failure[26,27] and markedly altered the pattern of gene expression. Captopril-treated rats, for example, had higher levels of α-MHC mRNA than age-matched SHR-F, SHR-NF, or WKY rats (Brooks and Boluyt, unpublished observations). Chronic captopril treatment prevented or reversed accumulation of extracellular matrix as well as increases in TGF-β_1 and type III collagen mRNA levels associated with the transition to failure[28]. Finally, late interventional treatment of SHR-F with captopril led to a reappearance of α-MHC mRNA and a decrease in TGF-β_1 mRNA, but no improvement in papillary muscle function (unpublished observations).

Considerations for future research

CELL LOSS AND APOPTOSIS

Normal ageing in the rat is associated with evidence consistent with myocyte loss[29,30]. A number of observations indicate the possibility that cell loss may be a factor leading to the demise of compensated function during the transition to failure (Fig. 2). Apoptosis is one mechanism proposed to account for myocyte loss during the transition from compensated hypertrophy to failure[31]. Apoptosis is a process by which cells commit themselves to a suicidal multi-step chain of events. Apoptosis or programmed cell death can be initiated in various cell types by an assortment of pathways, including growth signals that are for some reason incongruous[32]. Recent observations suggest that apoptosis, as evidenced by fragmented DNA in the nucleus of myocytes detected by end-labelling immunocytochemistry, appears with the transition from stable hypertrophy to heart failure in SHR (Li, Lakatta, and Bing, unpublished observations).

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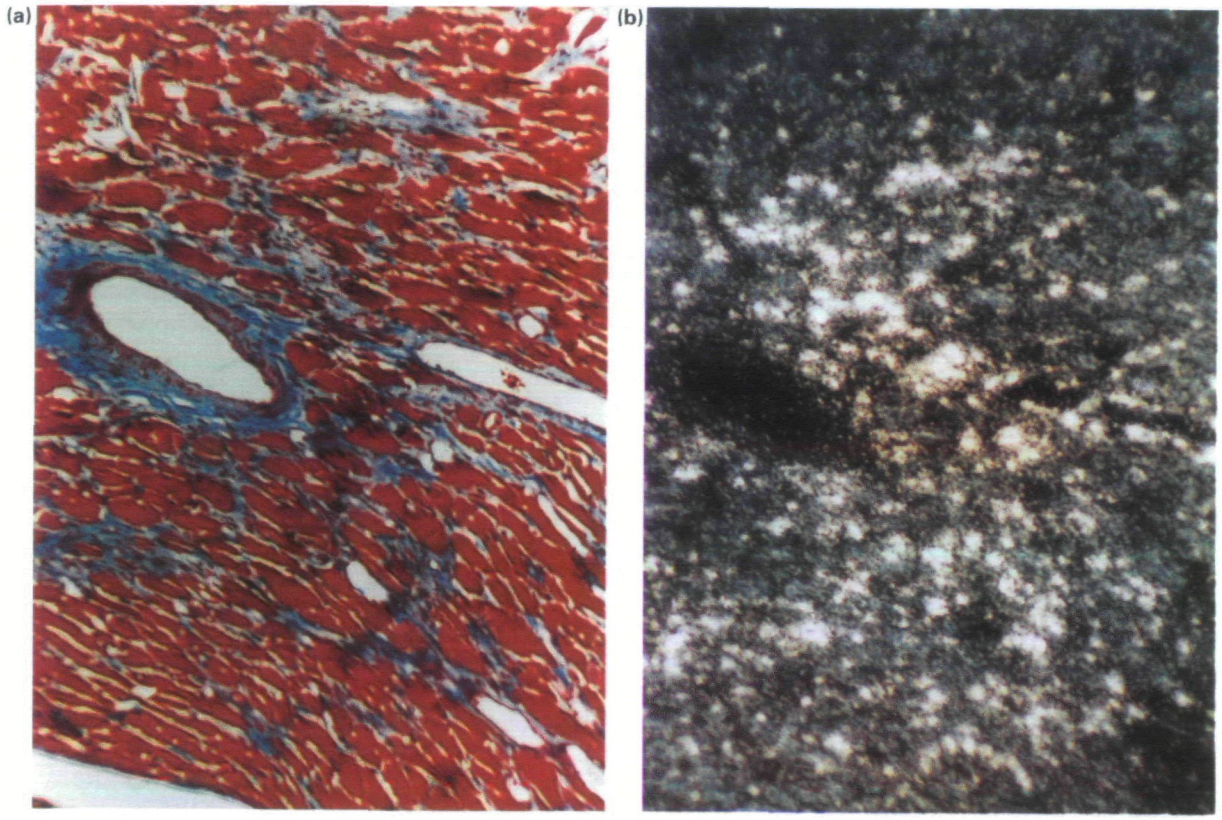

Figure 8 Sections from the myocardium of a 19-month-old spontaneously hypertensive rat with heart failure. Masson trichrome staining (a) demonstrates extracellular matrix (blue) between myocytes (red) and around a blood vessel. Dark-field microscopic view (b) of a serial section of the same myocardial area hybridized with an antisense probe for type I-α_1 collagen. Note that the signal (white dots) emanates primarily from the interstitium. × 200.

While the current knowledge base regarding apoptosis is derived almost exclusively from studies of non-muscle cells, evidence for apoptotic myocyte death has recently emerged from studies of cardiomyocytes in culture. DNA fragmentation and expression of Fas antigen mRNA, which is sometimes associated with apoptosis, was observed in neonatal cardiomyocytes cultured in hypoxic conditions for several days[33]. Since it has been proposed that hypoxia secondary to reduced coronary blood flow reserve occurs with advancing age and hypertrophy, an intriguing possibility is that hypoxia-induced apoptosis may contribute to myocyte loss observed in the heart during ageing and hypertrophy[29,30,34,35].

Because apoptosis could have profound effects over time, while affecting only a minuscule proportion of heart cells at any given point, determining whether or not it occurs in vivo will be problematic. Clues may be obtained by a search for expression of genes associated with apoptotic processes. In this regard, determination of the expression of Fas antigen, *bcl-2*, c-*myc*, *p53*, *WAF1* or other genes that have been associated with apoptosis[32] might be a useful first step. Given the post-mitotic nature of cardiac myocytes, the significance of apoptosis for cardiac ageing and its associated diseases cannot be overstated.

Although indirect evidence for limited hyperplasia of myocytes in the ventricles has been reported[30,36], the

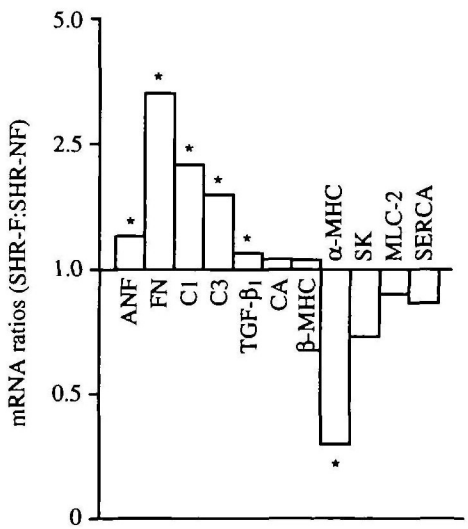

Figure 9 Summary of changes in relative gene expression during the transition from stable hypertrophy to heart failure in spontaneously hypertensive rats with failure (SHR-F) and without failure (SHR-NF). These ratios were derived from left ventricular tissue Northern blot data. ANF=atrial natriuretic factor; α-MHC=α-myosin heavy chain isoform; β-MHC=β-myosin heavy chain isoform; CA=cardiac α-actin; C1=type I-α_1 collagen; C3=type III-α_1 collagen; FN=fibronectin; MLC-2=myosin light chain-2; SERCA=cardiac sarco-endoplasmic reticulum calcium adenosine triphosphatase; SK=skeletal α-actin; TGF-β_1=transforming growth factor β_1. *P<0·05. (Data from Boluyt *et al.*[20].)

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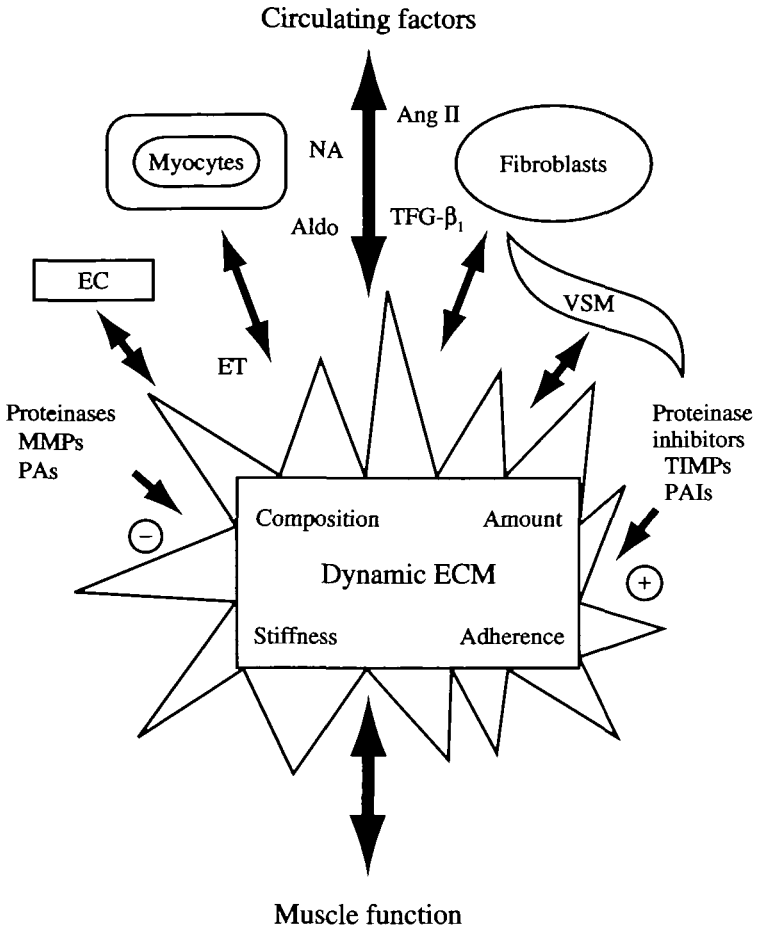

Figure 10 Interactions between the extracellular matrix (ECM) and other heart compartments. Changes that may be initiated during the transition from stable hypertrophy to heart failure contribute to an increase in fibrosis and/or impaired muscle function. Fibrosis increases muscle stiffness, causing or exacerbating deteriorating function; impaired function leads to an increase in wall stress and neurohumoral activation, which may augment the development of fibrosis. Circulating factors include neuroendocrine molecules and paracrine factors produced locally by myocytes and fibroblasts as well as by other cell types, e.g. endothelial cells (EC) and vascular smooth muscle cells (VSM). Endothelin (ET) may also be secreted by fibroblasts and may exert autocrine effects in response to angiotensin II (Ang II). Aldo=aldosterone; MMPs=matrix metalloproteinases; NA=noradrenaline; PAs=plasminogen activators; PAIs=plasminogen activator inhibitors; TGF-β_1=transforming growth factor β_1; TIMPs=tissue inhibitors of metalloproteinases.

net result of age-associated processes and heart disease seems to be that the senescent heart consists of fewer, larger myocytes[15]. Myocyte loss is also suggested by the decrease in myocyte fractional area observed in papillary muscles of SHR with heart failure[10]. Prevention of myocyte death or promotion of controlled myocyte hyperplasia may offer promise for the treatment of heart disease and heart failure.

EXTRACELLULAR MATRIX ACCUMULATION

Marked changes in gene expression have been observed to occur during the transition from stable hypertrophy to heart failure in the SHR model (Fig. 9). The pattern of change is consistent with the hypothesis that contractile protein may be replaced with extracellular matrix during the transition to failure and that this process is regulated, at least in part, pretranslationally. The data reviewed in Figs 1 and 2 show that fibrotic area increases and myocyte area decreases during the transition to failure. Considered together with the fact that LV mass does not increase in failing compared to non-failing SHR hearts, these findings support this hypothesis. At least one study of human heart failure due to idiopathic cardiomyopathy or coronary artery disease also provided evidence consistent with this hypothesis[37]. The extracellular matrix appears to play an extremely important and

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Table 2 Extracellular matrix-degrading enzymes and their inhibitors

Name	Other nomenclature	Substrates
Serine proteinases		
Plasminogen activators (PAs)/ plasmin*		Gelatins, fibronectins, laminin, proteoglycans
Elastases		Gelatins, elastin, proteoglycans
Cathepsin G		Gelatins, elastin; collagen types I, III†, IV‡
Matrix metalloproteinases (MMPs)		
MMP-1	Interstitial collagenase	Collagen types I, II, III, VII, X
MMP-2	72 kDa gelatinase	Collagen types IV, V, VII, X; gelatins, fibronectin
MMP-3	Stromelysin, transin, proteoglycanase, procollagen activating factor	Gelatins, fibronectin
MMP-7	Putative or punctuated metalloproteinase (PUMP-1)	Gelatins, fibronectins, proteolglycans
MMP-8	Polymorphonuclear collagenase	Collagen types I, II, III, VII, X
MMP-9	92 kDa gelatinase	Collagen types IV, V, VII, X; gelatins, fibronectins
MMP-10	Stromelysin-2, transin-2	Gelatins, fibronectins
MT-MMP	Membrane type MMP	Activates MMP-2
Inhibitors		
TIMP	Tissue inhibitor of metalloproteinase	MMPs
TIMP-2	Tissue inhibitor of metalloproteinase-2	MMPs
PAI-1	Plasminogen activator inhibitor-1	PA
PAI-2	Plasminogen activator inhibitor-2	PA
PN-1		PA

*PAs convert the ubiquitous plasma protein plasminogen into the active proteinase plasmin.
†Affect only telopeptide region.
‡Affect only non-helical region.

multi-faceted role in the transition to failure. It must be viewed as dynamic in nature, interacting with other components of the heart, modulating heart function, and in turn being modulated by changes in function (Fig. 10).

EXTRACELLULAR MATRIX DEGRADATION

Although the synthesis of extracellular matrix components is receiving increasing attention from investigators[16,24,25], the factors impacting matrix degradation are of equal importance and have only recently begun to be addressed. Much of what is known regarding degradation of the extracellular matrix derives from studies of tumour invasion, a process for which extracellular matrix degradation is an obligatory step[38]. Tissue repair or remodelling that occurs in the heart in response to alterations in loading factors or after a myocardial infarction requires migration of the cells involved in the repair/remodelling process. Both the type and the amount of extracellular matrix components can be modulated effectively by increasing or decreasing the activity of the specific proteinase cascades.

Proteinases involved in extracellular matrix degradation

Degradation of extracellular matrix proteins in the heart is primarily the domain of two families of enzymes: the serine proteinases, including the plasminogen activator (PA)/plasmin system, and the matrix metalloproteinases (MMPs, Table 2). The activity of each of these classes of proteinases is modulated in a number of ways, including transcriptional regulation, activation of latent forms of the enzymes, and suppression by specific inhibitors. The sheer number of proteinases involved in matrix degradation, their interactions, and their varied specificities results in a high degree of complexity that is magnified by the numerous regulatory mechanisms governing their actions.

A good example of coordinate regulation of extracellular matrix dynamics is the modulation in vitro of matrix synthesis and degradation by TGF-β_1. TGF-β_1 regulates not only the expression of matrix-producing genes, but also many features of the complex proteinase network that is responsible for matrix turnover. TGF-β_1 positively modulates expression of tissue inhibitor of metalloproteinase and the PA inhibitor PAI-1 while suppressing the expression of PA and MMPs[38]. The consequence of each of these influences is to favour accumulation of matrix.

Role in cardiac remodelling

Although little is known at present, the first glimpses of the role of proteinases in cardiac tissue remodelling are beginning to emerge. Expression of collagenase and a potential regulator of collagenolytic activity, interleukin-1α, both exhibit a temporal and spatial pattern of expression in the developing heart that suggests a role in tissue remodelling during development[39]. In a study of whole tissue extracts from adult rat hearts, zymography indicated activity of a 52 kDa collagenase[40,41], suggesting the presence of MMP-1. This activity could be potentiated 30-fold by incubation of the extract with either serine proteinase or oxidized glutathione. In the canine rapid-ventricular-

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pacing model of heart failure, the activity of the 92 kDa gelatinase (MMP-9) was augmented more than three-fold, while that of the normally predominant 72 kDa gelatinase (MMP-2) was unchanged[42]. Two preliminary reports indicate increases in collagenolytic activity in tissue explanted from patients with idiopathic dilated cardiomyopathy[43] and heart failure[44]. A small increase in membrane type MMP mRNA abundance was observed after 1 h of aortic constriction in the left ventricles of rats, suggesting an up-regulation of this key regulatory proteinase in migration and remodelling processes (unpublished observations). In contrast, LV dilatation resulting from volume overload in the rat is associated with a decrease in collagen protein fraction and an increase in collagenolytic activity[45]. The activities of MMP-1 and MMP-2, but not MMP-9, were decreased in hearts of human patients with hypertensive heart disease; however, of these three MMPs, only the activity of MMP-2 was reduced in hearts of patients with hypertrophic cardiomyopathy[46].

In studies of cultured cardiac fibroblasts, Ang II, aldosterone, and endothelin-1, but not endothelin-3, inhibited collagenase activity that was detected by zymography of the culture media[47,48]. When examined by an assay technique involving the degradation of ^{14}C-collagen, Ang II, but not aldosterone, inhibited collagenase activity[49]. When rat cardiac fibroblasts were co-cultured with bovine aortic endothelial cells, a marked increase in collagenolytic activity of the media was observed.[48]. The increase in collagenase activity stimulated by co-culture could not be attributed to Ang II, endothelin-1, or aldosterone[47,48], although an interaction of these factors was not ruled out.

Since the regulation of collagenase activity has many facets and the potential for interaction among the key regulators is great, much work will be required to elucidate the precise roles of these regulators in cardiac remodelling during heart failure. The SHR model of heart failure, with its definable transition from stable hypertrophy to overt failure, provides an opportunity to investigate mechanisms of matrix dynamics in an in vivo setting and, therefore, should afford continuing insight into the pathogenesis of heart failure.

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