Myocardial phenotype changes in heart failure: cellular and subcellular adaptations and their functional significance

Gerd Hasenfuss, Hanjörg Just

Myocardial growth in response to mechanical and neurohumoral stimuli
In most cases heart failure is the fatal result of chronic pressure or volume overload due to valvar disease, arterial hypertension, and myocardial infarction. The myocardium responds to an increase in load—that is, in wall stress, by development of hypertrophy. This might be considered to be a compensatory response, as according to the law of Laplace, myocardial wall stress decreases with increasing wall thickness. Although in some way adaptive, part of this response is pathological in that it is associated with abnormalities in both diastolic and systolic function and by still unknown mechanisms, may ultimately lead to myocardial failure. With the development of failure the disease process that was initially localised at the heart generalises and many organs will be affected by activation of the renin-angiotensin system and the sympathetic nervous system. In the case of an aetiology other than pressure or volume overload, such as dilated cardiomyopathy, the initial pathological process in the myocardium is less well understood. With the development of failure, the consecutive changes seem to be rather uniform and independent of the aetiology.

Myocardial hypertrophy and failure involve quantitative and qualitative modifications of the genomic expression. In quantitative changes, there is an overall increase in the cardiac expressions of most genes, which finally leads to myocyte hypertrophy and fibroblast hyperplasia. Superimposed on this global response, qualitative changes in gene expression include a positive and negative modulation of cardiac specific genes and a shift in the expression of several isogenes towards a programme partially comparable with that expressed in the fetal heart (table).

Recently some of the mechanical stimuli, hormones, and growth factors that induce the quantitative and qualitative changes of gene expression as well as their subcellular pathways have been elucidated. Generally, the process of hypertrophy involves the expression of "immediate early genes" and "late responsive genes". The late responsive genes include the cardiac specific genes. The immediate early genes such as the protooncogenes c-myc, c-fos, c-jun, etc, the mRNA of which may be increased as early as several minutes after exposure to the stimulus, code for a rather complex group of oncoproteins that seem to induce and control gene expression in a rather non-specific way.

The pathological stimulus for altered gene expression has been investigated by several groups, who used neonatal and adult rat myocytes. They have shown that α1 receptor

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DCM, dilated cardiomyopathy; ICM, ischaemic cardiomyopathy; PCR, polymerase chain reaction; ANF, atrial natriuretic factor.

Medizinische Klinik
III, Universität
Freiburg, Germany
G Hasenfuss
H Just
Correspondence to:
Dr G Hasenfuss, Universität
Freiburg, Medizinische
Klinik III, Hugsetter
Strasse 55, 79106 Freiburg,
Germany.
stimulation results in an increase of mRNA that encodes immediate early and late responsive genes and in development of myocardial hypertrophy.34 35 Moderate development of hypertrophy has also been described after stimulation of β-adrenergic receptors.36 Furthermore, it was shown that different classes of peptide growth factors such as β1 transforming growth factor (TGFβ1) and fibroblast growth factors (FGFs) induce expression of gene programmes resembling those seen during hypertrophy caused by pressure overload.37 38

With rat neonatal cardiocytes, Komuro et al found that stretching the cells increased within minutes the total cell RNA content and mRNA concentrations of c-fos and skeletal α-actin, followed by activation of protein synthesis.39 40 These authors further suggested that activation of protein kinase C may be involved in stretch induced protein synthesis.41 More recently, Sadoshima and Izumo found that in neonatal rat myocytes angiotensin II (AII), through activation of the AT1 receptor, induces expression of many immediate early genes.42 Then myocyte hypertrophy and fibroblast hyperplasia developed. These authors also showed that activation of protein kinase C may be the dominant pathway for AII induced gene expression.43

As AII mediated induction of gene expression closely resembles the situation when mechanical stress is applied to myocytes it might be speculated that local production of growth factor AII may be involved in stress induced myocyte hypertrophy.44 In non-muscle tissues, such as fibroblasts, stretch has been shown to increase the expression of a number of growth factors, including FGFs.45 The expression of FGFs may be involved in the high rates of cell proliferation and hyperplasia that occur in hypertension.46

This hypothesis is supported by molecular analyses showing mRNA expression of all elements of the renin-angiotensin system in peripheral organs including the heart.44 45 Moreover, enhanced myocardial mRNA expression for angiotensinogen and angiotensin converting enzyme (ACE) was found in several models of cardiac hypertrophy.46 47 Enhanced expression of ACE mRNA was also found in human hearts with end stage ischaemic or dilated cardiomyopathy.48 As well as AII induced myocyte hypertrophy and fibroblast hyperplasia, interstitial fibrosis may also be mediated by an angiotensin induced rise in circulating aldosterone.49

Modulation of cardiac specific gene expression in the failing heart

CONTRACTILE PROTEINS

The key element of development of myocardial force or shortening is the crossbridge cycle, in which the myosin crossbridge head attaches to actin, rotates in a manner that develops force or causes shortening with thick and thin filaments sliding past each other, and then detaches from the actin filament to start another crossbridge cycle. Alterations in the number of the crossbridges activated or the characteristics of the individual crossbridge cycle may have profound effects on the contractile performance of the heart muscle.

The total number of crossbridges available for activation may be reduced due to replacement of contractile proteins by connective tissue. This situation may be the prominent finding in ischaemic cardiomyopathy after a myocardial infarction. A substitution of contractile material by connective tissue may be of some relevance in other causes of heart failure as well. Hitzel et al in a morphometric study in control hearts described a non-muscle tissue content of the myocardium of 4%.26 Non-muscle tissue content was increased to 23% in dilated cardiomyopathy and 25% in pressure overloaded myocardium. Accordingly, when the myosin content was quantified biochemically, a decrease of 20% was found in dilated cardiomyopathy.50 From these data it was concluded that the decrease in contractile protein content is less important in the contractile deficit of the failing heart than is disturbed crossbridge activation due to altered excitation-contraction coupling processes.51

Profound alterations to the characteristics of the individual crossbridge cycle have been found in animal models of myocardial hypertrophy.51 52 In these studies reduced activity of myosin ATPase or myofibrillar ATPase, maximum shortening velocity, and increased economy of isometric force development occurred in the hypertrophied myocardium. The increased economy has been interpreted as prolonged attachment time of the crossbridges and reduced crossbridge cycling rate.53 54 The changes within the crossbridge behaviour have been attributed to changes in the myosin isoform from V1 to V10 the V10 isoform consists of a myosin heavy chain and the V1 isoform of the β myosin heavy chain.55 In the hypertrophied and failing human heart, similar changes in the crossbridge cycle to those found in hypertrophied hearts of small mammals have been found, although an α/β myosin heavy chain heterogeneity does not seem to play an important part in the human ventricular myocardium (fig 1).56 57 58 Normal human ventricular myocardium primarily consists of one myosin isoform, V1 (β myosin heavy chain), and no significant isoform shift occurs in hypertro-

**Figure 1** Average crossbridge force-time integral in human, rabbit, and rat myocardium. Differences in crossbridge force-time integral may reflect differences in crossbridge attachment time and are positively related to economy of contraction and negatively related to myofibrillar ATPase activity, shortening velocity, and crossbridge cycling rate. Reproduced with permission.59

*p < 0.01 v control*
phied or in failing human myocardium. As no shift in the myosin heavy chain isoforms is found in the human ventricular myocardium alterations of crossbridge behaviour may be related to changes in the light chains or in thin filament regulatory systems.

The alterations to the behaviour of the individual crossbridge cycle found in the hypertrophied and failing myocardium may have two different consequences to myocardial function: (a) prolonged crossbridge attachment may be beneficial for economy of myocardial contraction, as a greater force-time integral is produced per unit of high energy phosphate hydrolysed; (b) prolonged crossbridge attachment may result in reduced rates of relaxation and reduced shortening velocity, and may prevent the myocardium from developing high power output. The relevance of altered crossbridge behaviour to systolic or diastolic dysfunction of the failing heart is as yet unknown. It should be mentioned that alterations in crossbridge behaviour similar to those found in the hypertrophied and failing myocardium, occur with ageing in patients without heart failure.

**EXCITATION-CONTRACTION COUPLING**

From experiments performed in isolated human myocardium, there is considerable evidence that alteration of the excitation-contraction coupling processes may be of importance in the failing human heart. Excitation-contraction coupling comprises all processes involved in calcium turnover and calcium activation of contractile proteins. Calcium, which enters the cell through the L type voltage gated calcium channel during the action potential, acts on the sarcoplasmic reticulum to release a larger amount of calcium through the ryanodine sensitive calcium release channel. The subsequent calcium binding to the regulatory protein troponin C facilitates actomyosin interaction. Calcium is removed from the cytosol predominantly by Ca\(^{2+}\) ATPases of the sarcoplasmic reticulum. Also, calcium is removed by the sarcolemmal Na\(^+\)– Ca\(^{2+}\) exchanger and by the sarcolemmal Ca\(^{2+}\) ATPase, which, however, seems to be of minor relevance for calcium homeostasis of the myocyte.

Recently considerable work has been performed in analysing the function of the various components of the excitation-contraction coupling system in the human heart. It is still unclear whether or not altered density or function of the L type sarcolemmal calcium channel may be related to the failing human heart. Dihydropyridine binding studies and functional measurements by voltage clamp techniques indicated unaltered density and properties of this protein. Molecular biology measurements recently indicated that mRNA expression of the dihydropyridine receptor is significantly reduced in hearts with dilated or ischaemic cardiomyopathy. This study also suggested a decreased number of dihydropyridine binding sites in the failing myocardium.

Reduced mRNA expression of the ryanodine receptor (sarcoplasmic reticulum calcium release channel) has been found in ischaemic cardiomyopathy, whereas no significant changes in expression have been described in dilated cardiomyopathy. Functional measurements with a modified voltage-clamp technique of isolated sarcoplasmic reticulum calcium release channels suggested normal function in failing human myocardium from dilated cardiomyopathic hearts. Another study that used caffeine stimulation, however, indicated altered gating properties of this channel in dilated cardiomyopathy.

There are several studies that show that expression of sarcoplasmic reticulum Ca\(^{2+}\) ATPase is reduced at the levels of mRNA and protein in failing human myocardium from hearts with ischaemic and dilated cardiomyopathy. According, reduced sarcoplasmic reticulum calcium reuptake in the failing human heart was suggested from Ca\(^{2+}\) uptake measurements in homogenates from human myocardium. This was not found in another study. The different results of the two studies may be related to experimental differences, as the first study was performed in ventricular homogenates and the second in highly purified vesicles of the sarcoplasmic reticulum. The activity of the sarcoplasmic reticulum Ca\(^{2+}\) ATPase is controlled by the regulator protein phospholamban. No data are available on phospholamban protein concentrations in the failing human heart; however, recent mRNA measurements indicated that phospholamban may decrease in parallel with sarcoplasmic reticulum Ca\(^{2+}\) ATPase in the failing myocardium. Taken together, there is considerable evidence that calcium transport capacity of the sarcoplasmic reticulum may be impaired in the failing human heart, which might be significant pathophysio-logically.

From the total amount of calcium cycling during a contraction-relaxation cycle, most is believed to be released from the sarcoplasmic reticulum and absorbed by the sarcoplasmic reticulum and a little seems to cross the sarcolemmal membrane. This might be different under some pathological conditions when calcium removal by the sarcoplasmic reticulum is impaired. Reinecke et al showed that at the level of the mRNA and protein, expression of Na\(^+\)–Ca\(^{2+}\) exchanger is increased, and that there is a significant increase in the ratio of Na\(^+\)–Ca\(^{2+}\) exchanger to sarcoplasmic reticulum Ca\(^{2+}\)-ATPase protein concentrations in the failing human heart. This may indicate that under some pathological conditions the relevance of sarcolemmal calcium transport mechanisms increase relative to sarcoplasmic reticulum calcium handling.

**Functional alterations in the failing human myocardium**

With the photoprotein aequorin, Gwathmey et al showed that at a low temperature (30°C) and stimulation rate (0.3 Hz) isometric contractions and Ca\(^{2+}\) intermediaries of muscles from failing hearts were considerably pro-
longed and that Ca$^{2+}$ intermediaries exhibited two distinct components.84 Surprisingly, in these experiments twitch tension and peak systolic calcium concentrations were not different between failing and non-failing hearts.85-86 When experiments were performed at physiological temperature and rates of stimulation (\(\geq 1\) Hz), twitch tension was significantly reduced in the failing myocardium.50 Heat measurements performed in the same study indicated that reduced tension results from a decreased number of contractile proteins activated as a consequence of a reduced amount of calcium cycling.80 Accordingly, intracellular calcium measurements in isolated myocytes with Fura-2 showed a pronounced reduction of systolic calcium concentrations and increased diastolic calcium concentrations in the failing human myocardium.69

Further experiments on the force-frequency relation in failing and non-failing human myocardium clearly indicated that discrepant results regarding the development of contractile force in the failing human myocardium may be related to experimental conditions. These experiments showed that with increasing rate of stimulation, twitch tension rises significantly in the non-failing human myocardium, whereas frequency potentiation of contractile force is absent or inverse in the failing heart (fig 2).61-69

Therefore, intracellular calcium and contractile force are assumed to be similar at low stimulation rates in both the failing and non-failing myocardium but are significantly reduced in the failing myocardium at higher rates of stimulation. It is important to note that even in the failing myocardium, which shows a pronounced decline in force development at higher stimulation rates, only small changes of diastolic tension were found.70 The potential clinical relevance of the altered force-frequency relation in the failing myocardium has been outlined in two studies. It was shown that during right atrial or ventricular pacing the maximum rate of rise in pressure increases at higher heart rates only in patients with normal left ventricular function but does not change in patients with dilated cardiomyopathy.71 72 Furthermore, it was shown that the cardiac index increases at higher right ventricular pacing rates in patients with normal left ventricular function, and declines in patients with failing dilated cardiomyopathy (fig 3).

As the altered force-frequency relation of the failing human heart may be of pathophysiological and therapeutic relevance further studies were conducted to evaluate the underlying subcellular defects. With the photoprotein aequorin, we recently showed that in the non-failing myocardium the frequency dependent increase in contractile force is associated with increasing intracellular calcium concentration and that the inverse force-frequency relation of the failing myocardium goes parallel with a frequency dependent decline of the intracellular calcium signal.73 From these findings, there is considerable evidence that disturbed calcium release is an important defect underlying the altered force-frequency relation of the failing human heart. Disturbed calcium release could result from a decreased amount of trigger Ca$^{2+}$ entering the cell through the L type Ca$^{2+}$ channels or from alterations of theryanodine sensitive sarcoplasmic reticulum calcium release channel. Alternatively, calcium release may be reduced due to decreased sarcoplasmic reticulum calcium uptake, and thus, sarcoplasmic reticulum calcium depletion. From the studies discussed above, there is considerable evidence supporting the second hypothesis. Furthermore, we showed recently that there is a close correlation between the frequency dependent changes in contractile force and the protein concentrations of sarcoplasmic reticulum Ca$^{2+}$ ATPase.74

It should be pointed out that uptake of cal-
calcium into the sarcoplasmic reticulum could also be impaired as a consequence of lack of energy in the failing myocardium, which may result from disturbed mitochondrial function. This, however, was not obvious from myothermal measurements that indicated unaltered efficiency of metabolic recovery processes in the failing human myocardium.

If an insufficient capacity of the sarcoplasmic reticulum to accumulate calcium at higher rates of stimulation is the cause of reduced systolic calcium release, and thus reduced tension development, one would expect diastolic calcium to rise. This in turn would result in diastolic activation of contractile proteins, and thus would cause a rise in diastolic tension. As even at the highest stimulation frequency the rise of diastolic tension was inconsistent in the failing human myocardium, alternative mechanisms to remove calcium from the cytosol and prevent diastolic activation of contractile proteins must exist. An alternative to calcium removal by the sarcoplasmic reticulum is that calcium could be removed from the cytosol by mitochondria, which have the potential to accumulate high amounts of calcium and which have been suggested to be involved in the control of intracellular calcium homeostasis.

Furthermore, calcium could be buffered by different intracellular calcium binding proteins such as troponin C or calmodulin. Finally, calcium could be extruded into the extracellular space by sarclemmal transport mechanisms such as the sarcolemmal Ca\(^{2+}\) ATPase or the Na\(^+\)–Ca\(^{2+}\) exchanger. Recent findings from Reinecke et al that show increased expression of the Na\(^+\)–Ca\(^{2+}\) exchanger in the failing human myocardium suggest that the sarclemmal Na\(^+\)–Ca\(^{2+}\) exchanger may reflect an effective alternative mechanism to remove calcium from the cytosol. Of course, calcium extruded into the extracellular space is no longer available for activation of contractile proteins during systole. Also, for each calcium ion eliminated by the Na\(^+\)–Ca\(^{2+}\) exchanger three Na\(^+\) ions enter the cell. Increased calcium elimination by the Na\(^+\)–Ca\(^{2+}\) exchanger may, therefore, decrease the membrane potential, which may cause electrical instability and arrhythmias.

**Therapeutic interventions**
Which therapeutic implications may be derived from the cellular and subcellular changes in heart failure?

**DIURETICS AND VASODILATORS**
Reducing the load of the failing heart with diuretics or vasodilators may reduce the hypertrophic stimulus and myocardial oxygen consumption. Also, haemodynamics are influenced by those agents through their effects on preload and afterload. Accordingly, a reduction of left ventricular hypertrophy during long-term treatment with diuretics and vasodilators has been found in patients with pressure overload due to arterial hypertension.

**THE ACE INHIBITORS**
The ACE inhibitors reduce ventricular load and may reflect a more causal strategy to modulate the pathological changes of the myocardium in congestive heart failure. Accordingly, prevention of left ventricular dilatation after myocardial infarction, prevention of the development of heart failure in patients with left ventricular dysfunction, and improvement of prognosis with long-term ACE inhibitor treatment have been shown in recent large clinical trials.

**INOTROPIC AGENTS**
The benefit of long-term treatment with inotropic agents that increase cyclic AMP, such as catecholamines and phosphodiesterase inhibitors, is questionable. Moreover, several studies indicated increased mortality of patients with congestive heart failure under long-term treatment with inotropic agents.

The reasons for the unfavourable long-term effects of cyclic AMP increasing inotropic agents are speculative, but factors such as increased myocardial oxygen consumption, increased heart rate, and calcium overload of the myocyte may be relevant.

**DIGITALIS GLYCOSIDES**
In contrast with catecholamines and phosphodiesterase inhibitors, the cyclic AMP independent digitalis glycosides seem to have favourable effects on symptoms and haemodynamics during long-term treatment in patients with congestive heart failure. This might in part result from the decrease in sympathetic tone, increase in parasympathetic tone, reduction of heart rate, and increase in inotropic state.

**β-ADRENOCEPTOR BLOCKER**
β Adrenoceptor blockers have been shown to improve haemodynamics, exercise capacity, and symptoms in patients with congestive heart failure. Although in earlier studies beneficial effects of β blocker treatment were supposed to be related to upregulation of β adrenoceptors in the failing myocardium, it was shown more recently that the beneficial effects are present regardless of β receptor upregulation. The beneficial effects of β blockers may result from the protection of the myocardium from the increased sympathetic tone. Also, a reduction in heart rate may contribute to the beneficial effects of those agents. In most studies, higher resting heart rates seem to favour a beneficial response to β blocker treatment. Moreover it was shown that increased heart rate is most predictive of a favourable clinical response to β adrenoceptor blockade with metoprolol in patients with dilated cardiomyopathy.

**REDUCTION IN HEART RATE**
Reduction in heart rate itself may be a promising therapeutic approach in congestive heart failure. Reduction of heart rate in patients with impaired left ventricular function may be beneficial from four perspectives: (a) due to the inversion of the force-frequency relation,
intrinsic contractile force of the myocardium may increase; (b) myocardial oxygen consumption, which is proportional to heart rate, decreases; (c) time available for diastolic chamber filling is prolonged; and (d) time available for coronary perfusion is prolonged. Reduction in heart rate may be obtained by application of β-adrenergic blockers, digoxin, calcium channel blockers, or amiodarone. Also, a new class of heart rate reducing agents, the so called sinus node inhibitors, which act by direct effects on pacemaker currents, are now under clinical investigation.85

38 Sadoshima J, Isomou S. Molecular characterization of angiotension II-induced hypertrophy of cardiac myocytes and hyperplasia of cardiac fibroblasts. Critical role of...


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