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Review Article

Cellular mechanisms of contractile dysfunction in human heart failure

C. H. Davies, S. E. Harding and P. A. Poole-Wilson

Department of Cardiac Medicine, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, U.K.

Introduction

Numerous cellular abnormalities of heart muscle are associated with ventricular dysfunction and the clinical syndrome of heart failure. Many of these changes have been described in animal models of heart failure where there is considerable species and model variation[1,2]. The increasing use of cardiac transplantation has provided access to fresh myocardial tissue from severely failing human hearts and this has generated a large volume of experimental data.

A classification of the cellular abnormalities encountered in the failing human heart is provided in Table 1. The relative contributions of each mechanism depend on the underlying aetiology of heart failure and different mechanisms may predominate at various times in the natural history of a particular disease.

Myocyte death

Heart failure in the setting of acute cardiogenic shock secondary to an extensive myocardial infarction is an example where a relatively well defined single mechanism is operative i.e. insufficient numbers of viable myocytes remain to sustain effective contraction[3]. Under these conditions a fairly close relationship exists between myocyte loss and functional impairment[4]. Myocyte loss is also prominent in more chronic forms of heart failure secondary to both ischaemia[5] and dilated cardiomyopathy[6], but here there is a weaker correlation between histological abnormalities and the severity of longstanding heart failure[7]. This implies that functional abnormalities among the remaining viable myocytes and in the interstitium are also important in the development of chronic myocardial failure.

Key Words: Heart failure, myocytes, E-C coupling

Correspondence: Dr C. H. Davies, Department of Cardiac Medicine, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, U.K.

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Table 1 Possible mechanisms in human heart failure

Myocyte death
Ventricular dilatation
Incoordinate contraction
Disruption of the extracellular matrix
Structural changes in the cardiac myocytes
Functional abnormalities in the cardiac mycoytes

Disruption of the extracellular matrix

The role of the extracellular matrix in maintaining the physical structure of the heart and in the transmission of mechanical force to the circulation dictates that alterations in matrix composition have potentially important consequences for myocardial function (reviewed in[8]). There is an increase in the collagen content of failing myocardium[5,9], due to increases in types I and III collagen with a proportionately greater increase in type I[9]. Alterations in collagen architecture (but not increases in collagen volume) have been shown to correlate with abnormalities of passive relaxation in aortic valve disease [10], but in general attempts to correlate the degree of histological fibrosis with functional abnormalities in heart failure have yielded inconsistent results[11–13].

Structural change in cardiac myocytes

Changes in myocyte shape and size

Myocyte hypertrophy is an almost universal finding in chronic heart failure[5,6,11,14,15], whether attributed to increases in cell length[15], width[16] or both[11]. Whilst originating as a compensatory response to increased haemodynamic load, there is clear evidence of its deleterious long-term effects from mortality studies[17,18]. In parallel with this there is slippage between adjacent myocytes resulting in a distortion of ventricular geometry[5], eventually resulting in the dilatation characteristic of systolic dysfunction[19,20]. The degree of dilatation frequently exceeds the capacity to hypertrophy with resultant wall thinning[7]. Although some

authors have demonstrated only weak correlations between cell size and myocardial function[11,21], we have recently demonstrated significant correlations between ventricular hypertrophy and abnormalities of relaxation in isolated ventricular myocytes[16]. We have also found that myocytes of normal size selected from hypertrophied hearts still demonstrated these relaxation abnormalities, suggesting that physical cell size alone is not responsible for the functional abnormalities.

There has been a considerable amount of animal research directed at identifying the mechanisms involved in coupling increased haemodynamic load to the production of myocyte hypertrophy. Interest has centred on changes in gene expression secondary to increases in cell loading and the actions of peptide growth factors (reviewed in[22,23]). However, there is a paucity of data from human studies on these mechanisms due to the non-availability of appropriate human tissue. The elevated levels of myotrophin[24] and endothelin[25] detectable in human heart failure provide evidence of possible stimuli to the hypertrophic process in man.

In contrast to the certainty as to the occurrence of hypertrophy in heart failure, is the controversy surrounding whether there might be progression to hyperplasia and myocyte proliferation under certain conditions[26]. Despite some evidence to the contrary[27], the established view has been that the adult cardiac myocyte is terminally differentiated and thus incapable of mitotic division[28,29]. This view has recently been challenged by careful morphometric studies demonstrating an increase in the number of myocytes in addition to the well recognised increases in myocyte size[30,31]. This appears to occur not during the early phases of adaptation to increased load but in association with the latter stags of dilatation and myocardial failure. These findings await further confirmation, but they raise important questions about a potential role for hyperplasia in the transition from hypertrophy as a beneficial adaptation to the one that is a cause of mortality.

Ultrastructural change

The most prominent feature is one of loss of myofibrils[6,13,32,33]. This loss of contractile units provides an obvious mechanism for the depression of systolic function and has been shown to correlate with both ejection fraction[13] and prognosis[33]. In addition Scholtz *et al.* have characterized a proliferation of T tubules, a diversity of nuclear shapes and the occurrence of numerous small mitochondria[32] occurring in a severe form in 30% of the myocytes of patients with dilated cardiomyopathy. Immunofluorescence has identified increased amounts of the cytoskeletal components desmin, tubulin and vinculin[6], disruption of which could result in malalignment of the contractile proteins and inefficiency of force transmission.

Recent experimental work on isolated cardiac myocytes from hypertrophied cat myocardium has suggested that microtubular proliferation might contribute to contractile dysfunction[34]. Unfortunately, the methodological constraints imposed by the fragility of microtubules makes it unlikely that this intriguing possibility can be investigated in human tissues in the near future. Sarcomere length appears to remain constant in studies of failing human heart[15,35,36], suggesting that sarcomere replication must form an integral part of changes in cell length.

Functional changes in ventricular myocytes

Although the structural changes outlined above are clearly important in the pathogenesis of chronic heart failure, there are additional functional changes that are present in myocytes which appear morphologically intact. There has been previous uncertainty as to whether impaired contractility could be demonstrated in isolated preparations, with some studies demonstrating a reduction in contractility in isometric myocardial preparations[37–40], whilst others have been unable to demonstrate a difference[41–46]. Those studies employing higher stimulation rates and physiological temperatures have generally been those in which a depression of contractility has been demonstrated, although results in patients with ischaemic heart disease have been less consistent[47]. Our findings with isotonically contracting isolated ventricular myocytes from patients with ischaemic cardiomyopathy have demonstrated no depression of contraction amplitude at lower stimulation rates[36], but a 50% depression of contractility emerging at physiological stimulation rates[48]. This frequency-dependent impairment of function is consistent with clinical evidence demonstrating that a reduced cardiac output in heart failure is only apparent at higher heart rates[49]. With the demonstration of impaired myocardial function in heart failure at the level of individual myocytes the question arises as to which components in the excitation–contraction process are at fault.

Physiology of excitation–contraction coupling

The proposed mechanisms responsible for excitation–contraction (EC) coupling have been comprehensively reviewed[50,51]. The finding that depressed contractility in heart failure is dependent on stimulation rate has focused interest on those sub-cellular mechanisms thought to be responsible for frequency-dependent behaviour in the myocardium: the L-type Ca^{2+} channel, the sarcoplasmic reticulum and the Na^{+}-Ca^{2+} exchanger[52–55] (Figs 1 and 2).

Abnormalities of excitation–contraction coupling in heart failure

Before setting out the abnormalities associated with heart failure, it is important to bear in mind the limitations of some of the biochemical techniques

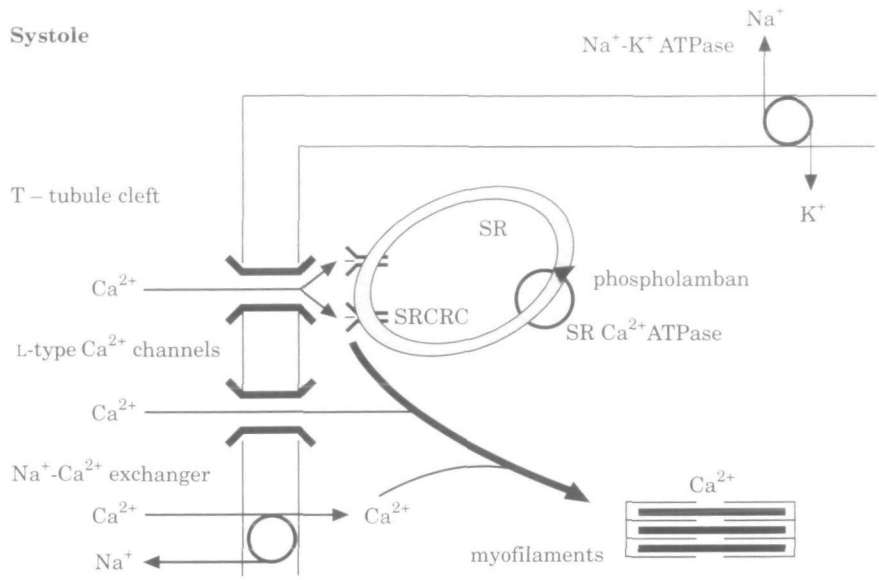

Figure 1 **Physiology of excitation–contraction coupling — systole. Membrane depolarization opens sarcolemmal L-type Ca^{2+} channels. The local rise in Ca^{2+} around the sarcoplasmic reticulum (SR) Ca^{2+} release channels (ryanodine receptors-SRCRC) triggers Ca^{2+} release from the SR. This combines with smaller contributions from the Na^+-Ca^{2+} exchanger (working in Ca^{2+} influx mode) and the L-type trigger current itself to form the Ca^{2+} transient which interacts with the myofilaments to produce contraction.**

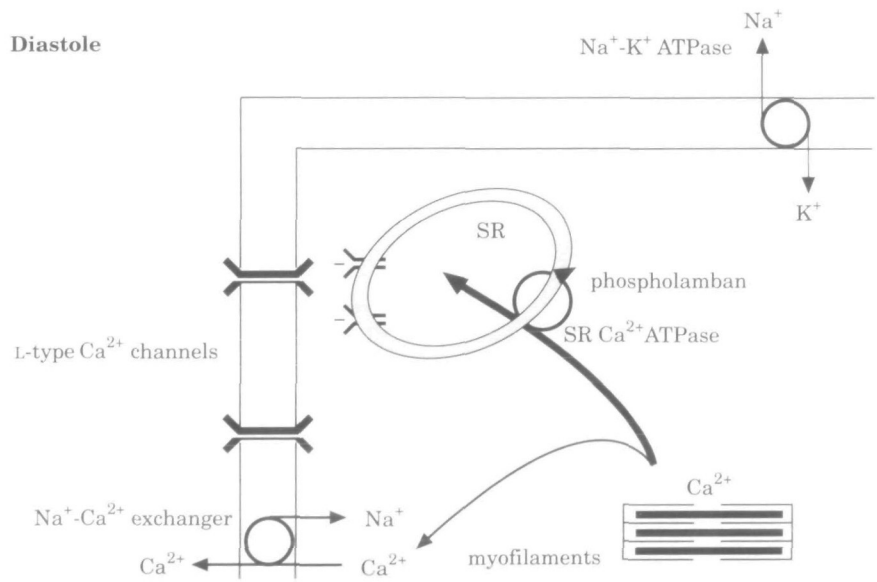

Figure 2 **Physiology of excitation–contraction coupling — diastole. Ca^{2+} dissociates from the myofilaments and is removed from the cytosol via uptake into the SR via the SR Ca^{2+} ATPase (SERCA2a) and by the Na^+-Ca^{2+} exchanger working in Ca^{2+} efflux mode. The negligible contribution from the sarcolemmal Ca^{2+} ATPase is omitted for clarity.**

involved. In particular the fact that changes in the concentration or activity of a particular substance must be interpreted with reference to a standard which is not itself subject to change in heart failure (typically per mg of total protein). Recent work by Böhm's group has challenged the tacit assumption that normalization per mg of total protein provides satisfactory standardization[56]. Levels of the G protein $G_{i\alpha}$ were found to be elevated by 139% in dilated cardiomyopathy and by 58% in ischaemic cardiomyopathy when referenced to

Table 2 Abnormalities of the Ca^{2+} transient in human heart failure

Study	Preparation	Temperature	Indicator	Load	Contraction in CHF	Peak Ca^{2+}	Time for Ca^{2+} decay
Gwathmey[78]	Papillary	30 °C	aequorin	M	↓	↔	↑
Hasenfuss[79]	Papillary	37 °C	aequorin	M	↓	↓	↑
Vahl[46]	Papillary	37 °C	fura-2	M	↔	↔	↑
				T	↓	↑	↑
Beuckelmann[64]	Myocyte	35 °C	fura-2	T	*	↓	↑

↑=increased; ↓=decreased; ↔=unchanged; M=isometric; T=isotonic; *=not measured; CHF=heart failure.

total protein, but to be increased by 135% and 155% respectively when normalized to the sarcolemmal membrane marker of ^{3}H-ouabain binding sites (although this itself is not without its limitations — see below). The implication is that non-viable or non-myocyte tissue is diluting the sample in ischaemic but not dilated cardiomyopathy. Similar concerns have arisen concerning the use of β-actin as a standard in studies examining changes in mRNA levels in heart failure.

Na^+-K^+ ATPase

Several investigators have described a reduction in ^{3}H-ouabain binding[57–60] itself in heart failure, although the reduction was not statistically significant in one study[61] and was absent in one[45]. The discrepancies in these findings are perhaps not surprising in view of the known limitations of ligand binding techniques that occur due to non-specific binding and the presence of receptors on cells other than myocytes. There is no evidence for either a depression in the levels of Na^+-K^+ ATPase mRNA or a change in the relative abundance of the three isoforms in man[60,61]. The presence of an endogenous ouabain in heart failure[62] suggests that functional Na^+-K^+ ATPase inhibition may occur in the presence of undiminished enzyme concentrations. The confusion surrounding Na^+ homeostasis in heart failure is compounded by observations that increases in intracellular Na^+ normalize the abnormal force-frequency relationship[38,63].

Action potential duration

Action potential duration is prolonged in heart failure[64,65], and this appears to be secondary to reductions in both I_{to} (the transient outward current) and I_{k1} (the inward rectifier current)[65,66] but not to changes in the L-type Ca^{2+} current (see below). This has two important consequences, firstly the prolonged membrane depolarization increases Ca^{2+} flux into the myocyte via the Na^+-Ca^{2+} exchanger[50] whilst allowing a shorter period of time for the exchanger to operate in Ca^{2+} efflux mode. Secondly, any inhomogeneity of prolongation may predispose to the development of re-entry and arrhythmias.

L-type Ca^{2+} channel

No functional abnormality has been detected in the L-type Ca^{2+} (I_{ca}) current in isolated myocytes from failing hearts[67,68], although I_{ca} may contribute a proportionately greater amount to the calcium transient[69]. The assumptions made in using capacitance to correct for changes in cell surface area have been questioned[70] but there is evidence to support a correlation between electrical capacitance and cell size (K. MacLeod; personal communication). These Ca^{2+} current studies are in agreement with descriptions of an unaltered number of dihydropyridine (DHP) binding sites[71] but are in contrast to the finding of a reduction in DHP binding and receptor mRNA by Takahashi *et al.*[72]. Nevertheless, the balance of functional evidence would seem to suggest that L-type channel conductance is not impaired in heart failure.

Sarcoplasmic reticulum Ca^{2+} release channels

This is an area of controversy with some groups reporting a reduction in the ryanodine sensitive release channel mRNA in ischaemic[73] but not dilated cardiomyopathy[73,74] with others describing reductions in both ischaemic and dilated cardiomyopathy[75]. It has been suggested that these reductions are associated with upregulation of the inositol 1,4,5,-triphosphate ($ins(1,4,5)P_3$) triggered Ca^{2+} release channel[75]. However, the role played by the $ins(1,4,5)P_3$ channel in the physiology of human EC coupling is uncertain and the significance of this observation awaits confirmation. The sensitivity of mRNA techniques in general must be weighed against the fact that alterations in translation rates and protein turnover mean that changes in mRNA do not necessarily equate to changes in protein levels or to altered function. Single channel recordings have been unable to detect differences between ryanodine-sensitive release channel function in failing human heart compared with non-failing sheep hearts[76].

Ca^{2+} transient

There is general agreement on the prolonged diastolic decay of the transient in heart failure (Table 2) and this is in accord with the prolonged relaxation noted in isolated ventricular myocytes[16] from failing hearts and

Table 3 Functional abnormalities of the sarcoplasmic reticulum (SR)

Study	Method	SR function in heart failure
Harigaya[130]	Spectrophotometric	↓
Lentz[131]	$^{45}Ca^{2+}$ uptake	↓
Limas[132]	$^{45}Ca^{2+}$ uptake	↓
D'Agnolo[99]	Caffeine contractures	↓
Denvir[100]	Caffeine contractures	↓
Moravec[74]	Electron probe microanalysis	↓
Beukelmann[92]	Ca^{2+} transient decay in zero extracellular Na^+	↓
Beukelmann[64]	Ca^{2+} transient decay with high intracellular Na^+	↓
Movsesian[82]	$^{45}Ca^{2+}$ uptake	↔

↓ = reduced; ↔ = unchanged.

the frequent occurrence of diastolic dysfunction in clinical practice[77]. There remains uncertainty concerning the magnitude of the peak Ca^{2+} transient with the finding of Gwathmey's *et al.* that this was unchanged in heart failure[78] contrasting with reports of a depressed transient by Hasenfuss *et al.*[79] and Beukelmann *et al.*[64]. Some of these apparent discrepancies may relate to the use of right ventricular samples and the lower temperatures used by Gwathmey's group[79]. But this observation does not explain the findings of Vahl *et al.*[46], in particular the increase in the calcium transient in preparations from failing hearts under isotonic conditions, which is in direct contradiction to the findings of Beukelmann *et al.*[64] in isolated cells. One possible explanation for these discrepancies lies in the technical limitations associated with recording Ca^{2+} transients from multicellular preparations where the outer layers of myocytes may contribute disproportionately to the Ca^{2+} signal whilst damaged myocytes may generate a Ca^{2+} signal but may not contribute to the work of contraction. Unfortunately, the technical difficulties in applying external load to cardiac myocytes[80,81] have meant that there is no available data on the effects of mechanical loading on the Ca^{2+} transient in isolated myocytes from failing human hearts. There thus remains no clear consensus surrounding this central event in excitation–contraction coupling and further careful studies are needed under both isometric and isotonic conditions

Ca^{2+} uptake and storage

As outlined above, there is clear evidence of prolonged diastolic Ca^{2+} decay and attention has focused on abnormalities of uptake into sarcoplasmic reticulum (SR). The majority of functional studies have demonstrated reduced Ca^{2+} uptake or storage (Table 3), the only exception being the study of Movsesian *et al.*[82]. Studies of the mRNA of the SR Ca^{2+} ATPase (SERCA2a) have uniformly demonstrated depressed levels in failing heart[72,74,83–85]. There are, however, conflicting estimates of SERCA2a protein, with some studies demonstrating depressed levels[84,86] whilst Movsesian *et al.* were unable to confirm this[87].

Phospholamban inhibits SERCA2a at two separate sites, with this inhibition being attenuated by phosphorylation[88]. Although levels of phospholamban mRNA are depressed in heart failure[74,89], findings with respect to protein levels have been conflicting[86,87,90] and there has been no demonstration of functional impairment[91]. There is no evidence of a disturbance of the SR Ca^{2+} storage protein calsequesterin[72,74,85,87].

The other major pathway for Ca^{2+} removal from the cytosol involves the Na^+-Ca^{2+} exchanger[50] as the cytosolic Ca^{2+} ATPase contribution appears to be negligible[92]. There is recent evidence for upregulation of the exchanger both in terms of increases in mRNA and protein levels[93] in addition to an increase in the transport of Ca^{2+} across sarcolemmal membrane vesicles[94]. This appears to be functionally significant as inhibition of SERCA2a produces a greater prolongation of relaxation in myocytes from non-failing hearts than in those from patients with heart failure, implying that myocytes from failing hearts are better adapted to SR dysfunction[95]. Thus the weight of evidence, particularly from functional studies, would therefore seem to favour an abnormality of SR Ca^{2+} uptake secondary to an abnormality of SERCA2a with uncertainty surrounding the function of phospholamban.

Myofilament abnormalities

Myofilament Ca^{2+} sensitivity

Studies have been performed on skinned fibre preparations in an attempt to assess the function of the myofilaments separately from the controlling influence of the Ca^{2+} regulatory apparatus. The majority of these have failed to demonstrate an overall reduction in Ca^{2+} sensitivity in heart failure[96–100]. Two studies have shown an *increased* myofilament Ca^{2+} sensitivity in dilated cardiomyopathy, those of Wankerl *et al.*[35] and that of Schwinger *et al.*[101]. Schwinger *et al.* have proposed that whereas the Ca^{2+} sensitivity in non-failing myocardium is dependent upon fibre length this is not the case in failing hearts[101] resulting in a failure of the Frank–Starling mechanism. D'Agnolo *et al.* have not confirmed these findings[99]. The fact that no study has demonstrated reduced myofilament Ca^{2+} sensitivity implies that this is not a mechanism for the impaired systolic function in the failing heart.

Alterations in myofilament composition

The total myosin content and myofibrillar Mg-ATPase are reduced in the failing heart[102,103] and although this is in keeping with the histological descriptions of a reduced number of myofibrils[13,32,33], changes in thin filament composition may also play a role[104]. There is no evidence for any myosin isoform switch from the predominant V3 subtype[105], but there are two lines of evidence to suggest that abnormalities of myofilament composition may not be of primary importance in

Table 4 Alterations in unstimulated adenylate cyclase in the failing human heart

Author	Parameter	Change in heart failure
Böhm[121]	Basal adenylate cyclase activity	↓
Feldman[133]		↓
Bristow[122]		↓
Bristow[123]		↓
Bristow[126]		↔
Dennis[127]		↔
Danielsen[124]	Basal cAMP levels	↓
Von der Leyen[35]		↓
Böhm[90]		↓
Regitz[134,135]		↔
Brown[136]	Effect of phosphodiesterase inhibition on contraction	↓
Feldman[41]		↓
Böhm[42]		↓
Von der Leyen[137]		↓

↓=reduced; ↔=unchanged.

the pathogenesis of depressed systolic performance in heart failure. Firstly, the failure of skinned fibre preparations[35,96–100] to demonstrate the reduced force development seen in intact preparations[37,38,40,48] suggests that the major disturbance lies in the Ca^{2+} control mechanisms and not in the contractile units. Secondly, Hasenfuss *et al.*[47], on the basis of myothermal measurements, have demonstrated no reduction in the efficiency of EC coupling to mechanical work, again supporting evidence in favour of an abnormality of Ca^{2+} control over one of myofilament function.

Could myofilament abnormalities play a role in diastolic dysfunction? There is some evidence to support this from myothermal work demonstrating prolongation of the crossbridge force–time integral in myocardium from failing hearts[102]. Decreases in the atrial-like light chain 2 content of ventricular myocardium in dilated cardiomyopathy[106] have been postulated to slow crossbridge dissociation[107] as might the modest increases of the TnT_2 isoform of troponin T that have been reported[108]. The interest generated by these findings must be tempered by the fact that similar abnormalities of the crossbridge force–time interval are also found in elderly non-failing hearts[102] and thus the contribution of these abnormalities to diastolic dysfunctio' 'ains uncertain.

Abnormalities of energy utilization

In a small series, Bashore *et al.* have described reduced ATP levels in endomyocardial biopsies from failing hearts and correlated these with the depression of ejection fraction[12], while Schultheiss *et al.* have demonstrated dysfunction of the mitochondrial ADP/ATP carrier in dilated but not ischaemic cardiomyopathy[109]. In contrast, studies of ATP in heart failure using surgical biopsies (where the time between tissue harvest and freezing will have been shorter) have not demonstrated depression of ATP levels[110]. Some investigators have described a reduction in the ratio of phosphocreatine to ATP levels in dilated cardiomyopathy using ^{31}P MRI studies[111] but this finding has not been confirmed by others[112].

There are several reasons to doubt that a depressed level of ATP is a significant cause of contractile dysfunction in chronic heart failure. Firstly, there are technical difficulties in the measurement of ATP in endomyocardial biopsy specimens and measurement of total adenine nucleotides has failed to show reduced levels in heart failure[113]. Secondly, the Michaelis constants of both SERCA2a and myosin-ATPase are 1000 times less than cytosolic ATP levels[114,115] suggesting that neither of these enzymes' activity is ATP-limited. Thirdly, intracellular addition of ATP does not reverse the abnormalities in the Ca^{2+} transient seen in heart failure[64].

Abnormalities of the β-adrenoceptor signalling system

The well-recognized β-adrenoceptor down-regulation [45], increases in G_i protein[56,116] and up regulation of β-adrenoceptor kinase activity[117] in heart failure have been recently reviewed in detail[118] and will not be covered here. Although the importance of a depressed β-adrenoceptor axis in the impaired response to exercise is clear, recent interest has centred on a potential role for β-adrenoceptor desensitization in the pathogenesis of progressive heart failure. The SOLVD trial[119] demonstrated that elevation of catecholamine levels in patients with asymptomatic left ventricular dysfunction preceded the development of heart failure, whilst sequential measurements in patients with stable heart failure have revealed progressive increases in plasma noradrenaline[120]. Several studies have demonstrated that there is a reduction of both basal adenylate cyclase activity[121–123] and basal levels of cAMP itself[90,124,125] in heart failure, although these have not been universal findings[90,126,127]. However, studies examining the effects of phosphodiesterase inhibition on contraction (which provide a functional measurement of basal cAMP) have consistently demonstrated reduced effects in heart failure (Table 4). These observations have led to the hypothesis that reduced levels of basal cAMP might adversely affect basal myocyte contraction. Several authors have demonstrated that small (sub-inotropic) increases in cAMP can reverse the abnormal force–frequency relationship found in trabecular preparations from failing hearts[38,63,128] and we have recently demonstrated that isoprenaline can normalize the abnormal time course of contraction observed in myocytes from patients with heart failure[129]. Against this background it is somewhat surprising that cAMP analogues do not appear to normalize the abnormalities of the Ca^{2+} transient seen in heart failure[64] and that levels of phosphorylated phospholamban are unaltered[90].

Conclusions

Structural changes are clearly of fundamental importance in the pathogenesis of heart failure, both in terms of a reduction in the total number of myocytes and in a reduction in the myofibril content of those remaining. Changes in the extracellular matrix probably play an important role both in the process of remodelling and in determining passive diastolic function (although conclusive evidence for this in humans is still lacking). In addition to this there are important functional abnormalities of the remaining viable myocytes contributing to both systolic and diastolic dysfunction. The evidence favours disturbance of the Ca^{2+} regulatory mechanisms over myofilament abnormalities as the predominant contributor to these functional disturbances. There remains uncertainty concerning the abnormalities of the various subcellular organelles involved in Ca^{2+} homeostasis, but the evidence is most convincing for an abnormality of sarcoplasmic reticulum Ca^{2+} uptake, possibly with partial compensation by upregulation of the Na^{+}-Ca^{2+} exchanger. Whether this abnormality could be reversed by pharmacological intervention is unknown, nor is it known whether these changes are beneficial by reducing cardiac contraction and delaying cell death or harmful by contributing to diminished function of the heart as a pump. Future therapies for heart failure will need to be directed towards avoiding cell necrosis, promoting and controlling cell growth and possibly regulating increases in myocardial cell numbers.

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