Sarcomeric dysfunction in heart failure

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Sarcomeric dysfunction plays a central role in reduced cardiac pump function in heart failure. This review focuses on the alterations in sarcomeric proteins in diseased myocardium that range from altered isoform expression to post-translational protein changes such as proteolysis and phosphorylation. Recent studies in animal models of heart failure and human failing myocardium converge and indicate that sarcomeric dysfunction, including altered maximum force development, Ca2+ sensitivity, and increased passive stiffness, largely originates from altered protein phosphorylation, caused by neurohumoral-induced alterations in the kinase-phosphatase balance inside the cardiomyocytes. Novel therapies, which specifically target phosphorylation sites within sarcomeric proteins or the kinases and phosphatases involved, might improve cardiac function in heart failure.

1. Sarcomeric dysfunction

The failing heart is characterized by reduced contractility (systolic dysfunction) and/or impaired filling (diastolic dysfunction). A number of factors, including changes in cardiac structure (dilation and hypertrophy), apoptotic and necrotic cell death, maladaptive remodelling of the extracellular matrix, abnormal energy metabolism, impaired calcium handling, and neurohumoral disturbances have been implicated in the initiation and progression of heart failure.1–4 Recent studies revealed that alterations in sarcomeric function play a prominent role in reduced cardiac pump function.

Sarcomeric function is determined by the expression levels of multiple isoforms and by post-translational modifications of sarcomeric proteins. During muscle contraction a molecular interaction takes place between the thin (actin) and thick (myosin) filament of the sarcomeres, which is triggered by a rise in the intracellular calcium and is driven by the energy from ATP hydrolysis.5 The tropomyosin–troponin complex inhibits the actin–myosin interaction at low intracellular free calcium (Figure 1A). This inhibition is released when intracellular free calcium increases and binds to troponin C (Figure 1B). Alterations in sarcomeric protein composition under pathological conditions will influence contractile performance of the heart. Within the first part of this review, we discuss the functional role of individual sarcomeric protein isoforms and of post-translational protein modifications such as proteolysis and phosphorylation. In the second part, we highlight the major changes in sarcomeric function reported in failing myocardium and discuss the most likely underlying protein modifications.

2. Isoform composition and sarcomeric dysfunction

2.1 Myosin heavy chains

The thick filament is composed of myosin, which consists of two myosin heavy chains (MHC), and two pairs of myosin light chains (MLCs) (Figure 1). One of the major isoform changes which has been observed in hypertrophied and failing ventricular myocardium is the shift from the fast α-MHC to the slow β-MHC.6–8 The magnitude of the MHC shift largely depends on the amount of endogenous α-MHC present in ventricular tissue, which is species-dependent, being largest in small rodents and smallest in human.6–11 Hence, the functional significance of the shift in MHC composition in diseased human ventricles is still a matter of debate.8,10,12

The MHCs carry the site for ATP hydrolysis and are important determinants of the rate of energy consumption and the speed of contraction of the sarcomeres, which are closely related.13 In vitro studies have shown that the α-MHC isoform has a higher ATPase activity14 and a higher actin filament sliding velocity compared with the β-MHC isoform.15
consistent with the shorter action potential and contraction duration in the subepicardial myocardium. Thus, in human ventricular myocardium the change in MHC isoform composition during heart failure will be variable and this may have obscured significant effects on sarcomeric function. However, recent studies have shown that even a small shift will have a significant impact on cardiomyocyte contractility.11,27 In contrast to ventricular human tissue, human atria contain ~80% of α-MHC.12,24 In atrial fibrillation, the β-MHC expression increased almost two-fold, which coincided with a reduction in kinetics of force re-development.28 Overall, the shift towards the slow and more economical β-MHC isoform occurs in human diseased atria and to a lesser extent in diseased human ventricular myocardium. The MHC shift may be beneficial under pathological conditions, since less energy is required to maintain cardiac pump function at rest, though at the expense of reduced speed of contraction and power output.

2.2 Myosin light chains

Apart from the shift in MHC, changes may occur in the expression pattern of MLCs within the heart. In particular, isoform changes have been reported for MLC-1 (or essential MLC), both in atrial and ventricular tissue. MLC-1 not only interacts with MHC, but also binds to actin with its N-terminus. It exists in two forms, a ventricular (VLC-1) and an atrial (ALC-1) form.29 The latter is expressed in the entire heart muscle during foetal and early life and is subsequently replaced by the ventricular form. In the ventricles of patients with hypertrophic obstructive cardiomyopathy relatively high amounts of ALC-1 were found, which correlated with the maximal rate of force development.30 Apart from its positive effect on dynamics of contraction, replacement of VLC-1 by ALC-1 increased isometric force development.29 Morano et al.31 hypothesized that MLC-1 tethers MHC to actin and thereby restrains cross-bridge cycling and reduces force generation. As actin-binding affinity of ALC-1 is less than of VLC-1, improved binding affinity of ALC-1 may be explained by weakening of the interaction between actin and MLC-1. However, although up-regulation of ALC-1 may represent a compensatory mechanism to improve cardiac function, it is not a consistent protein alteration in sarcomeric function as increased systolic force generation and rates of contraction and relaxation were observed in

From experiments in permeabilized cardiac preparations from small mammals and humans it followed that the β-MHC is 3–5 times more economical,11,16,17 but is associated with reduced power output18 and shortening velocity17,18 compared with the α-MHC isoform. Consequently, in rats a shift from α- to β-MHC coincided with significant reductions in ATPase activity,7,19 tension cost,19 and power output.18 Evidence that a shift in MHC may be of pathophysiological relevance for human sarcomeric dysfunction was already provided in 1962 by Alpert and Gordon20 who reported reduced myofibrillar ATPase activity in human congestive heart failure. However, subsequent human studies failed to unequivocally link this observation with an MHC isoform shift in failing ventricular myocardium. First, no differences were found in functional properties of myosin isolated from non-failing and failing hearts,10,21,22 indicating that protein alterations other than a change in myosin composition are responsible for the reduction in myofilament ATPase activity.20,23 Moreover, various expression levels of α-MHC (ranging from 0 to 30%) have been reported in the ventricles of different individuals.8,10,12,24 This may be due to age-dependent changes in MHC composition and heterogeneous expression of MHC isoforms within the ventricular wall.25,26 Both in human25 and rat26 ventricular tissue, a regional difference in MHC expression has been observed, characterized by a higher expression of the fast α-MHC in the subepicardial than in the subendocardial layer,
hearts from transgenic rats harbouring minigenes encoding the N-terminal domain of MLC-1.34 Thus, although endogenous heterogeneous expression of MLCs may be of minor relevance in failing human myocardium, up-regulation of the MLC-1 N-terminal fragment might provide a therapeutic tool to enhance cardiac performance.

2.3 Troponin T
Anderson et al.35 have proposed that re-expression of foetal troponin T (cTnT) may also contribute to the reduced ATPase activity in human heart failure. A significant inverse negative relationship was found between (re-)expression of foetal cTnT and myofibrillar ATPase activity in human ventricular tissue from normal and end-stage failing hearts. However, this observation has not been confirmed by others, and similar to the expression of α-MHC, the expression levels of foetal cTnT is variable among individuals.36–38 While Anderson et al.35 observed re-expression of foetal cTnT in all end-stage failing human hearts, Solaro et al.36 only observed this foetal cTnT isoform in one out of 10 failing heart samples. Similarly, we have observed foetal cTnT in only one out of 24 patients with end-stage heart failure.38 Mesnard-Rouiller et al.37 found expression of foetal cTnT in half of the failing ventricles and suggested that re-expression of foetal cTnT isoforms is not a common characteristic of heart failure and most likely depends on other factors such as intensity and duration of the elevation of wall stress.

The consequences of foetal cTnT on sarcomeric contraction were studied upon exchange of endogenous cTnT with foetal cTnT in rat cardiomyocytes.39 No differences were found in myofilament force development at maximal and submaximal Ca2+ concentrations at a sarcomere length of 2.2 μm between cardiomyocytes exchanged with troponin complex containing adult or foetal cTnT.39 Akella et al.40 observed a decrease in Ca2+-responsiveness at low (1.9 μm), but not at high (2.4 μm) sarcomere length in skinned cardiac trabeculae from diabetic rats which coincided with alterations in cTnT composition. More recently, it was shown by Gomes et al.41 that foetal cTnT modulates Ca2+-sensitivity in the presence of foetal (skeletal) Tnl. Hence, the effect of foetal cTnT on sarcomeric function seems to be dependent on sarcomere length and protein background in the heart.

In conclusion, most isoform changes might be an intrinsic part of the so-called ‘fetal’ (hypertrophic) program yet their expression appears to be highly variable within human ventricular tissue. Minor shifts in MHC and MLC composition do not explain sarcomeric dysfunction in heart failure, but are of compensatory nature.

3. Proteolysis and sarcomeric dysfunction
3.1 Troponin I
There is ample evidence that proteolytic activity is enhanced after an acute ischaemic insult. Degradation products of several sarcomeric proteins42–47 have been observed, which may subsequently induce sarcomeric dysfunction. One of the main proteins thought to be responsible for impaired cardiac function upon ischaemia/reperfusion is cardiac troponin I (cTnI) (Figure 1).42–45 In rodents, McDonough et al.48 showed that with moderate ischaemia/reperfusion, cTnI is cleaved at its C-terminus, which results in a truncated cTnI product (cTnI1–192, in human cTnI1–193). More recently, it was postulated that degradation of cTnI might also impair cardiomyocyte function and contribute to reduced pump function in heart failure.49 Myocardial infarction in pigs induced a reduction in the maximal force generating capacity of single permeabilized cardiomyocytes isolated from remodelled non-infarcted left ventricular tissue, in which minor degradation of cTnl (4%) was observed.49 In addition, independent of ischaemia, cTnI degradation has been demonstrated in human myocardium from coronary artery disease patients with different degrees of heart failure.45,50,51 To investigate if truncated cTnI may contribute to depressed cardiac pump function in human ischaemic cardiomyopathy and heart failure, we recently investigated the direct functional effects of cTnI1–192 in human cardiomyocytes. Force measurements were performed in non-failing human cardiomyocytes permeabilized with Triton-X 100 and exchanged with troponin complex containing either full length (cTnIFL) or truncated cTnI.52 Surprisingly, truncated cTnI did not significantly alter maximal force development (Figure 2A). Likewise, passive force was not different between cells containing cTnIFL and cTnI1–192. However, myofilament Ca2+-sensitivity was significantly higher in cTnI1–192 exchanged preparations compared with cTnIFL cells (Figure 2B). This implicates that in humans truncation of cTnI may limit relaxation of the heart muscle, while systolic function would benefit from the increase in Ca2+-responsiveness of the myofilaments.

4. Role of protein phosphorylation in sarcomeric dysfunction
4.1 Protein kinase A-mediated phosphorylation
Not only truncation of cTnI, but also its phosphorylation status is a prominent determinant of sarcomeric function, both in health and disease. Upon β-adrenergic stimulation, protein kinase A (PKA)-mediated cTnI phosphorylation at serines 23/24 is associated with a decrease in myofilament Ca2+-sensitivity32,53,54 and contributes to an acceleration of cardiac relaxation.55,56 Since β-adrenergic signalling is reduced in heart failure due to down-regulation and desensitization of β-adrenoceptors,57–59 PKA-mediated cTnI phosphorylation might be less pronounced in failing myocardium. In agreement with reduced β-adrenergic signalling, reduced phosphorylation levels of cTnl have been reported in failing human myocardium compared with non-failing donor hearts.60–62 More specifically, phosphorylation at PKA sites 23/24 was significantly reduced in end-stage failing compared with donor human myocardium.51,63 At the functional level, reduced PKA-mediated cTnI phosphorylation would result in an increase in Ca2+-sensitivity of the myofilaments, as was observed in single permeabilized human cardiomyocytes isolated from end-stage failing hearts.32,54,61,63 In comparison with cells from non-failing donor myocardium, Ca2+-sensitivity was significantly higher in cardiomyocytes from patients with idiopathic or ischaemic cardiomyopathy (Figure 3A). This sarcomeric defect was normalized upon treatment of cardiomyocytes with the catalytic subunit of PKA (Figure 3B), as the reduction in pCa50 was larger in cardiomyocytes from failing compared with donor hearts.32
Recently, Messer et al.\textsuperscript{62} have shown that altered cTnI phosphorylation most likely underlies the increased Ca\textsuperscript{2+}-responsiveness as isolated thin filaments from failing human hearts displayed higher Ca\textsuperscript{2+}-responsiveness compared with filaments from donor myocardium in an in vitro motility assay. One should be careful when using non-failing donor myocardium as ‘normal’, because of the high blood catecholamine levels at the time of tissue procurement. The high level of cTnI phosphorylation and relatively small effect of PKA on myofilament Ca\textsuperscript{2+}-responsiveness might reflect over-stimulation of the β-adrenergic pathway in donors and thereby augment the difference between healthy and failing samples. However, a similar increase in myofilament Ca\textsuperscript{2+}-responsiveness has been observed in several animal models of heart failure.\textsuperscript{49,64–66}

To minimize variable receptor stimulation at the time of biopsy procurement, we recently conducted a series of experiments on single cardiomyocytes from pigs with a myocardial infarction or sham-operated animals isolated from transmural needle biopsies, which were instantly frozen in liquid nitrogen. Biopsies were taken from remote left ventricular tissue 3 weeks after myocardial infarction induced by ligation of the left circumflex coronary artery or from sham-operated animals. Consistent with previous observations (Figure 3),\textsuperscript{49} Ca\textsuperscript{2+}-responsiveness was significantly higher in cells from infarct compared with sham animals, while the shift upon PKA was smaller in sham than in post-infarct remodelled myocardium (unpublished data). These data clearly show that alterations in β-adrenergic signalling and the concomitant reduction in PKA-mediated cTnI phosphorylation impair sarcomeric function. The increased Ca\textsuperscript{2+} sensitivity of the myofilaments might contribute to diastolic dysfunction via impaired relaxation of failing myocardium.

4.2 Protein kinase C and D

Apart from the β-adrenergic pathway, other signalling routes might be involved in the alterations in phosphorylation and function of sarcomeric proteins in heart failure.
Noteworthy, overall phosphorylation status of cTnl, determined on ProQ Diamond stained gels, did not significantly differ between sham-operated and MI pigs (unpublished data), while the shift in Ca\(^{2+}\) sensitivity upon PKA treatment was larger in infarct compared with sham animals (Figure 4B). This implies that, whereas PKA-mediated cTnl phosphorylation is down-regulated in infarct animals, cTnl phosphorylation by other kinases should be increased. One of the most likely candidates is protein kinase C (PKC). Its activity and expression levels are increased in heart failure and myocardial infarction compared with sham-operated animals (A). Similar to the observations in human heart failure, Ca\(^{2+}\) sensitivity was significantly higher (A) and the PKA-mediated reduction in Ca\(^{2+}\) sensitivity was larger (B) in cells from myocardial infarction compared with sham animals (Modified from van der Velden et al. Circ Res 2004;95:e85-e95, with permission).

Figure 4 Isometric force measurements were performed in single Triton-permeabilized cardiomyocytes isolated from remote left ventricular tissue from pigs 3 weeks after myocardial infarction. Maximal force development (F\(_{\text{max}}\)) was significantly lower in myocardial infarction compared with sham-operated animals (A). A similar to the observations in human heart failure, Ca\(^{2+}\) sensitivity was significantly higher (A) and the PKA-mediated reduction in Ca\(^{2+}\) sensitivity was larger (B) in cells from myocardial infarction compared with sham animals (Modified from van der Velden et al. Circ Res 2004;95:e85-e95, with permission).

Maximal force generating capacity can be attributed to altered troponin phosphorylation, while changes in maximal force generating capacity most likely rely on the permissive action of other sarcomeric proteins.

Whether PKC- and PKD-mediated phosphorylation and a concurrent reduction in Ca\(^{2+}\)-responsiveness is detrimental for cardiac function or represents an alternative mechanism to preserve positivelusitropy during exercise and compensates for reduced PKA-mediated cardiac relaxation requires further investigation.

5. Sarcomeric dysfunction in heart failure
5.1 Increased vs. decreased Ca\(^{2+}\) sensitivity

Opposite to the increased myofilament Ca\(^{2+}\) sensitivity observed in human end-stage failing myocardium (Figure 3) and in several animal models of cardiac disease (Figure 4), a decrease in myofilament Ca\(^{2+}\) sensitivity was reported in rodent models of heart failure resulting from chronic pressure overload (aortic banding) and myocardial infarction. One possible explanation for these contrasting observations might be the level of neurohumoral stimulation present at the time of tissue procurement. An intricate balance exists between kinase and phosphatase activities within the cardiomyocyte as was shown recently by Braz et al. They reported that both PKA and PKD may alter phosphorylation status of proteins indirectly via phosphorylation of protein phosphatase inhibitor-1 (I-1). Opposite to PKA, which suppresses PP-1 activity, PKC enhances PP-1 activity via phosphorylation of I-1. This illustrates the delicate balance between kinases and phosphatases within a cell. An increase in PKC from its effect on maximal force, PKC has been shown to reduce myofilament Ca\(^{2+}\) sensitivity in rodent and human myocardium. The possible involvement of PKC-mediated protein phosphorylation in sarcomeric function in failing myocardium was shown recently in rat models of end-stage heart failure resulting from chronic pressure overload (aortic banding) and myocardial infarction. In both models increased expression and activation of PKC\(_\alpha\) were observed in the late, but not in the early phase of heart failure. Maximal force generating capacity and Ca\(^{2+}\) sensitivity of permeabilized cardiomyocytes were significantly reduced in end-stage failing animals compared with age-matched controls, and both parameters increased upon treatment with protein phosphatase 1 (PP-1). In contrast, PKC\(_\alpha\) did not significantly alter cardiomyocyte function of failing cardiomyocytes, while it reduced both maximal force and its Ca\(^{2+}\) sensitivity in cells from the control group. In a previous study, the same group performed experiments where in failing cardiomyocytes the endogenous Tn-complex was exchanged by unphosphorylated troponin complex, while control cells were exchanged with troponin complex extracted from failing hearts. Upon exchange, Ca\(^{2+}\) sensitivity of failing cardiomyocytes was restored towards the value observed in controls, while failing troponin complex induced a significant reduction in Ca\(^{2+}\) sensitivity in control cells. However, troponin exchange did not affect maximal tension, indicating that PKC-mediated phosphorylation of troponin is not involved in the reduced force generating capacity. Overall, the data confirm that altered myofilament Ca\(^{2+}\) sensitivity can be attributed to altered troponin phosphorylation, while changes in maximal force generating capacity most likely rely on the permissive action of other sarcomeric proteins.

Figure 3 A) Representative tritium-stained micrographs of control (left) and MI myocardial sections. B) The functional consequences of PKC-mediated protein phosphorylation have been investigated in rodent models and indicated a central role for cTnl and cTnT in reducing maximal myofilament force development. Apart from its effect on maximal force, PKC has been shown to reduce myofilament Ca\(^{2+}\) sensitivity in rodent and human myocardium. The possible involvement of PKC-mediated protein phosphorylation in sarcomeric function in failing myocardium was shown recently in rat models of end-stage heart failure resulting from chronic pressure overload (aortic banding) and myocardial infarction. In both models increased expression and activation of PKC\(_\alpha\) were observed in the late, but not in the early phase of heart failure. Maximal force generating capacity and Ca\(^{2+}\) sensitivity of permeabilized cardiomyocytes were significantly reduced in end-stage failing animals compared with age-matched controls, and both parameters increased upon treatment with protein phosphatase 1 (PP-1). In contrast, PKC\(_\alpha\) did not significantly alter cardiomyocyte function of failing cardiomyocytes, while it reduced both maximal force and its Ca\(^{2+}\) sensitivity in cells from the control group. In a previous study, the same group performed experiments where in failing cardiomyocytes the endogenous Tn-complex was exchanged by unphosphorylated troponin complex, while control cells were exchanged with troponin complex extracted from failing hearts. Upon exchange, Ca\(^{2+}\) sensitivity of failing cardiomyocytes was restored towards the value observed in controls, while failing troponin complex induced a significant reduction in Ca\(^{2+}\) sensitivity in control cells. However, troponin exchange did not affect maximal tension, indicating that PKC-mediated phosphorylation of troponin is not involved in the reduced force generating capacity. Overall, the data confirm that altered myofilament Ca\(^{2+}\) sensitivity can be attributed to altered troponin phosphorylation, while changes in maximal force generating capacity most likely rely on the permissive action of other sarcomeric proteins.
and a decrease in PKA-mediated phosphorylation of I-1 would enhance PP-1 activity and thereby induce hypophosphorylation of sarcomeric proteins. Apart from differences in neurohumoral status when tissue is retrieved from the heart, diverse alterations in the signalling pathways known to alter sarcomeric protein phosphorylation upon neurohumoral stimulation most likely underlie diverse functional properties of the sarcomeres. Already in 1991, Bristow et al. have shown different alterations in the β-adrenergic pathway in hearts with ischaemic heart disease (ISHD) and IDC. Analysis of sarcomeric protein phosphorylation on Pro-Q Diamond stained gradient gels (Figure 5A) revealed significant differences between left ventricular myocardial tissue from end-stage failing patients with IDC and ISHD (Figure 5B). Phosphorylation of cTnI was significantly higher in non-failing donor compared with end-stage failing myocardium. In addition, myosin binding protein C, which is phosphorylated upon β-adrenergic stimulation is lower in failing compared with donor hearts. Noteworthy, MLC-2 phosphorylation was significantly higher in ISHD compared with donor and IDC myocardium, and statistical analysis revealed significant different phosphorylation of cTnI between ISHD and IDC samples. In line with a higher level of cTnI phosphorylation in ISHD samples, myofilament Ca²⁺ sensitivity was significantly lower in ISHD compared with IDC myocardium. These data provide evidence that diverse alterations in sarcomeric protein composition and function in failing hearts are related to underlying cause or phenotype.

5.2 Reduction in maximal force generating capacity

Reduced maximal force has been observed in diverse models of cardiomyopathy (Figure 4A). In rat with pressure-overload and infarction-induced cardiomyopathy, the reduction in F_max amounted to 35 and 42%, respectively. As reduced F_max was only partly reversed by PP-1 (15%), and as described earlier, may not directly involve the troponin complex, alternative signalling routes, and other sarcomeric proteins may be of relevance. Moreover, depressed cardiomyocyte force development was also observed in enzymatically isolated preparations from failing rat hearts, in which the isolation procedure most likely reduced phosphorylation status of most sarcomeric proteins. Therefore, part of the reduction in maximal force might at least in part be related to altered isoform composition and/or proteolysis of sarcomeric proteins. A recent study in transgenic mice by Vahebi et al. indicated in which p38 MAPK (mitogen activated protein kinase) was constitutively active in the heart, revealed a possible role for tropomyosin dephosphorylation in the depression of maximal force of the sarcomeres. Activation of p38 MAPK, as occurs in pressure overload-induced hypertrophy, has been shown to exert a negative effect on cardiomyocyte contractility without altering Ca²⁺-handling. The study in transgenic mice indicated that apart from its role in remodelling and apoptosis, activated p38 MAPK leads to sarcomeric dysfunction, possibly via activation of phosphatases and a subsequent dephosphorylation of tropomyosin. The level of tropomyosin phosphorylation appears to be species-dependent, being relatively high in mice and lower in human myocardium (Figure 5A). However, similar to isoform changes in MHC, small changes in phosphorylation may exert a significant effect on sarcomeric function. Therefore, the (patho)physiological role of tropomyosin phosphorylation for sarcomeric function requires further investigation.

In conclusion, depressed force development cannot be explained by a single protein alteration, though seems to be the result of complex interactions between various sarcomeric proteins.

5.3 Increased cardiomyocyte stiffening

Subtle, though functionally important changes in protein phosphorylation, induced by kinases and phosphatases other than PKA, may have been obscured in failing human myocardium in comparison with donor hearts. Separation of patients with heart failure into subgroups, based on severity, cause or phenotype, represents a powerful approach to reveal the causes and functional implications of alterations in sarcomeric function in human heart failure. Comparison of patients with diastolic (DHF) and systolic heart failure (SHF) revealed an increased passive force
development in cardiomyocytes from DHF compared with SHF patients (Figure 6). A significant positive correlation was present between in vivo left ventricular end-diastolic pressure (LVEDP) and $F_{\text{pas}}$, indicating that cardiomyocyte stiffening contributes to high filling pressures in DHF. Increased cardiomyocyte passive force was corrected to values observed in hearts with preserved ejection fraction and normal LVEDP upon incubation with PKA (Figure 6), indicative for hypophosphorylation of sarcomeric proteins. The hypophosphorylated sarcomeric protein, possibly titin, could be a specific myocardial target for drug therapy to lower LVEDP in DHF.

6. Future perspectives

The question if depressed cardiomyocyte contractility is involved in heart failure has been positively answered. Overall, there seems to be general consensus that sarcomeric dysfunction in heart failure results from altered protein phosphorylation, which is the result of complex changes in kinase and phosphatase expression and activity. The balance between kinases and phosphatases in the cardiomyocyte are humoral and heart rate-dependent and as a consequence the activities of kinases and phosphatases vary in time. Apart from temporal changes, spatial changes occur, as complex interactions have been shown between kinases and phosphatases regulating calcium homeostasis within cardiomyocytes. Such complex signalling may also apply to the myofilaments. Moreover, within the complex pattern of sarcomeric protein phosphorylation each protein and its phosphorylation status influences the behaviour of other sarcomeric proteins. As sarcomeric function most likely reflects differences in phosphorylation status and heart rate at the time of tissue procurement, investigation of the functional properties of the sarcomeres should be performed in cardiac tissue, which is obtained under standardized conditions. The use of cather biopsies has proved to be a major leap forward in unravelling sarcomeric dysfunction in human myocardium. Linkage of in vivo haemodynamic data with cardiomyocyte force measurements revealed that stiffening of the sarcomeres contributes to impaired filling of the heart in DHF patients. To obtain insight in dynamic signalling cardiac samples could be retrieved upon receptor stimulation. This approach allows determination of the direct relation between functional, structural, and protein characteristics at the cellular level with in vivo haemodynamics measured at the time of tissue harvesting.

The sarcomeric proteome will be even more complex than described in the present review, since other signalling routes, apart from the β-adrenergic pathway, may be triggered under pathological conditions and affect sarcomeric function. Only recently, Yuan et al. discovered novel phosphorylation sites within the N-terminus of MyBP-C, which were differentially phosphorylated upon stunning in canine and rat myocardium. Apart from phosphorylation, post-translational modifications resulting from oxidative stress might impair sarcomeric function. Moreover, mutant sarcomeric proteins as found in inherited cardiomyopathies further complicate analysis of causality between protein alterations and function of the sarcomere. Over the past years knowledge on mutated sarcomeric proteins present in cardiomyopathies increased swiftly. However, the exact consequences of these mutations on cardiomyocyte function in human cardiac tissue are still unclear and knowledge concerning additional (mal)adaptive changes in sarcomeric proteins is scarce. Hence, it remains to be elucidated if and to what extent altered sarcomeric protein expression and/or post-translational changes impair sarcomeric function in inherited cardiomyopathies.

The combination of sarcomeric force measurements with proteomic analysis (i.e. functional proteomics) will reveal (novel) post-translational modifications involved in cardiomyocyte dysfunction in heart failure. The use of transgenic animal models and protein exchange experiments in cardiac preparations are required to define the specific role of post-translational protein modifications and mutant sarcomeric proteins in cardiac function.

7. Clinical implications

The recently obtained data in human myocardium (Figures 5 and 6) indicate that divergent disturbance of receptor-signalling cascades depend on underlying cause and phenotype. Diverse alterations in signalling pathways might alter the responsiveness of patients to drug therapy and therefore the current strategy of treating heart failure should be re-evaluated. Large randomized, double-blind, placebo-controlled multicentre trials have shown that treatment of heart failure patients (classified according to the New York Heart Association into class II–IV) with neurohumoral receptor blockers, such as ACE-inhibitors and beta-blockers, reduces both morbidity and mortality. However, it remains to be investigated if and to what extent reversal of sarcomeric dysfunction contributes to the beneficial effects of beta-blocker and ACE-inhibitor therapy in different patient groups.

Interestingly, exercise in mice with a myocardial infarction reversed depressed sarcomeric function to values observed in controls. The beneficial effects appeared to be the result of improved β-adrenergic signalling. Future studies should investigate if the combination of currently used neurohumoral blockers with exercise yield added benefit. Novel therapy may include drugs targeted to mediators down-stream of the adrenergic and angiotensin receptors. Cardiac performance may be improved by targeting a specific myofilament protein to directly modulate...
sarcomeric function. Further exploration of the complex signal-routing defects underlying defects in sarcomeric function is required in order to develop more precise, individualized therapy in heart failure patients.

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