Review

Altered contractile function in heart failure

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Abstract

The syndrome of congestive heart failure (CHF) is an entity of ever increasing clinical significance. CHF is characterized by a steady decrease in cardiac pump function which is eventually lethal. The mechanisms that underlie the decline in cardiac function are incompletely understood. End-stage CHF often involves the general loss of functional myocytes, a hyperplasia of the extracellular matrix, ventricular chamber remodeling, and decreased myocyte function. This review article focuses on the latter aspect of CHF, mechanisms of decreased myocyte function. Recent data from studies on human myocardial tissue obtained in the setting of cardiac transplantation or from studies that employed experimental animal models of CHF have suggested depressed myocyte function. The mechanisms that may be involved in the decline of myocyte contractile function include alterations in (i) calcium handling, (ii) myofilament function, and (iii) the cytoskeleton. At present, however, it is not known how or to what degree these alterations in cellular processes contribute to the decline of in vivo cardiac pump function in CHF. Accurate knowledge regarding the cellular processes that participate in the development of CHF is critical to the development of innovative strategies aimed to combat CHF. © 1998 Elsevier Science B.V.

Keywords: Congestive heart failure; Ventricular hypertrophy; Sarcomere length; Myofibrillar function; Animal models; Calcium handling; Isolated cardiac trabeculae; Isolated cardiocytes

1. General features of CHF

There is a large incidence of congestive heart failure (CHF) in the general population [1]. CHF, a clinical syndrome, is a long term outcome of a number of cardiovascular diseases, such as ischemic heart disease, hypertension, valvular diseases, and idiopathic cardiomyopathy [1,2]. In its advanced stages, CHF has a poor prognosis despite its modern clinical management [3].

During the development of CHF there is a continuous decline in cardiac pump function [2,4]. Several of the potential mechanisms that lead to CHF are illustrated in Fig. 1. These processes, described in brief below, lead to both a gradual increase in end-diastolic ventricular pressure (LVEDP) and decrease in end-systolic pressure (LVESP). Eventually, the decline in cardiac pump function results in the clinical syndrome of CHF.

In an apparent attempt to compensate for the decreased pump function, several cardiovascular regulatory mechanisms are activated. Thus, in CHF we see a substantial activation of the sympathetic nervous system, which results in high plasma levels of catecholamines, endothelin, and activation of the renin–angiotensin–aldosterone system. Prolonged exposure to these hormones, however, is damaging to the heart. For example, high levels of circulating catecholamines cause a reduction in the density and activity of β-adrenergic receptors, adenylate cyclase and stimulatory G proteins in the heart. In this way a lethal vicious cycle is initiated, whereby the neurohormonal mechanisms invoked to sustain cardiac pump function...
cause damage to the heart, thereby reducing cardiac function even further [2].

Ventricular hypertrophy (VH) is also observed in most cases of CHF in humans [4] and in animal models [4–8]. This remodeling response probably occurs to maintain cardiac pump function. It is generally assumed that myocytes are terminally differentiated cells. Therefore, and on the basis of data obtained from animal models of CHF and VH, it has been suggested that remodeling mainly involves growth of individual myocytes [5,9]. Recently, however, it has been suggested that myocyte proliferation, possibly via the process of non-mitotic division, may also play a role in animal models [10–12] and in humans [13]. It should be noted, however, that CHF is also associated with a general loss of myocytes, possibly via the process of apoptosis [12,14,15]. Because of this, myocyte proliferation is difficult to measure and thus whether such occurs in CHF or VH is highly controversial. Finally, the mechanisms that control the remodeling response of the cardiac myocyte remain largely unknown [4,8].

Marked alterations in content and isoform distribution of collagen in cardiac interstitium have also been found in human CHF [13,16,17] and animal models of CHF [18,19]. The increase in collagen content appears to be accounted for mainly by an increase in the collagen isoform type I [17]. The extracellular matrix functions both to maintain the general structure of the heart and to transmit the contractile force generated by the cardiac myocytes to the ventricular chamber so as to generate ventricular pressure. Because of this, alterations in the extracellular matrix may have a potentially large impact on ventricular function and this non-myocyte remodeling process may, therefore, play a significant role in the decline of cardiac pump function in CHF [16,19]. In particular diastolic function may be affected, as has been suggested in humans [20]. Based on data obtained from chronically instrumented dogs it has been suggested that this aspect of ventricular dysfunction is largely responsible for the intolerance to exercise that is observed clinically in CHF and VH [21].

Recent data from studies that employed experimental animal models of VH [6,7,22–24] and CHF [25–27] have consistently demonstrated depressed myocyte function. Studies that employed human myocardial tissue obtained in the setting of cardiac transplantation have demonstrated depressed contractile function, albeit only at higher rates of stimulation [28–34].

The mechanisms of the decreased myocyte contractile function in CHF or VH is presently unknown but likely involves alterations in (i) calcium handling, (ii) myofilament function, and (iii) the cytoskeleton (see Fig. 1). At present, however, it is not known how, or to what degree, these alterations in cellular processes contribute to the decline of in vivo cardiac pump function that is seen clinically in the syndrome of CHF.

2. Calcium handling in CHF

The basic mechanisms involved in myocardial excitation–contraction coupling have been extensively reviewed [35,36]. It has been suggested that altered myocyte calcium handling plays an important role in the development of CHF, both in humans and in animal models of CHF or VH [37,38]. This hypothesis is consistent with alterations that have been found in some of the key proteins that are involved in the uptake and release of calcium in CHF and VH (see Table 1).

2.1. Sarcoplasmic reticulum

Calcium transport into the sarcoplasmic reticulum (SR) occurs via the action of the SR Ca\(^{2+}\)-pump [35,36]. This process requires the hydrolysis of one molecule of ATP for two calcium ions that are pumped against a large concentration gradient into the lumen of the SR. In human CHF, a reduction has been noted in the enzyme activity of the sarcoplasmic reticulum (SR) Ca\(^{2+}\)-ATPase [29,39–43], and similar results have also been obtained in animal models of VH [44–48] and CHF [49–53]. It should be noted, however, that although mRNA levels have been consistently reported to be down-regulated in human and animal CHF (or VH), there have been several reports that showed no change in SR Ca\(^{2+}\)-ATPase protein levels in human CHF (see Table 1).

The Ca\(^{2+}\)-ATPase is regulated by phospholamban, a system in which phosphorylation of phospholamban by a variety of protein kinases relieves the inhibitory action of this protein [35,36]. Phospholamban mRNA levels have
Table 1
Alterations in key proteins involved in calcium homeostasis in human CHF and animal VH and CHF

<table>
<thead>
<tr>
<th></th>
<th>Human CHF</th>
<th>Animal VH</th>
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<tbody>
<tr>
<td><strong>SR Ca ATPase</strong></td>
<td></td>
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<tr>
<td>mRNA</td>
<td>↓ [39,43,54,55,67,152,153]</td>
<td>↓ [45–47,56]</td>
<td>↓ [50,51,147]</td>
</tr>
<tr>
<td>Protein</td>
<td>↓ [28,57,63,153]</td>
<td>↓ [44,45,47,48,68]</td>
<td>↓ [49–51]</td>
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<tr>
<td>Function</td>
<td>↓ [29,39–43]</td>
<td>↓ [44–48]</td>
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<tr>
<td><strong>Phospholamban</strong></td>
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</tr>
<tr>
<td>mRNA</td>
<td>↓ [39,43,54,55]</td>
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<tr>
<td>Protein</td>
<td>↓ [57]</td>
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<td>Function</td>
<td>↓ [65]</td>
<td>↓ [53]</td>
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<tr>
<td><strong>Calsequestrin</strong></td>
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<td>mRNA</td>
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<tr>
<td><strong>SR Ca Release Channel</strong></td>
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<tr>
<td>mRNA</td>
<td>↓ [55,58,59]</td>
<td>↓ [45,46]</td>
<td></td>
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<tr>
<td>Protein</td>
<td>↓ [58]</td>
<td>↓ [60]</td>
<td>↓ [61]</td>
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<tr>
<td>Function</td>
<td>↓ [62,64]</td>
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<tr>
<td><strong>SL L-type Ca Channel</strong></td>
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<tr>
<td>mRNA</td>
<td>↓ [67]</td>
<td>↓ [67]</td>
<td>↓ [155]</td>
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<tr>
<td>Protein</td>
<td>↓ [69]</td>
<td>↓ [154]</td>
<td>↓ [61,156,157]</td>
</tr>
<tr>
<td>Function</td>
<td>↓ [83,84]</td>
<td>↓ [159–161]</td>
<td>↓ [90,159]</td>
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<tr>
<td><strong>SL Na⁺ / Ca²⁺ exchanger</strong></td>
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<td>mRNA</td>
<td>↑ [153,162]</td>
<td>↑ [71]</td>
<td>↑ [147]</td>
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<tr>
<td>Protein</td>
<td>↑ [70,153,162]</td>
<td>↑ [71]</td>
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<tr>
<td>Function</td>
<td>↑ [70,162]</td>
<td>↓ [163]</td>
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<td><strong>Calcium transient</strong></td>
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<tr>
<td>Amplitude</td>
<td>↓ [83,84]</td>
<td>↓ [85–89]</td>
<td>↓ [85,89,90]</td>
</tr>
<tr>
<td>Diastolic levels</td>
<td>↑ [84]</td>
<td>↑ [79–82,85–88,146]</td>
<td>↑ [90,78,85,82]</td>
</tr>
<tr>
<td>Duration</td>
<td>↑ [37,77,84]</td>
<td></td>
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CHF: congestive heart failure; Human CHF: data obtained using explanted hearts; Animal CHF: data obtained using experimental models of CHF; Animal VH: data obtained using experimental models of ventricular hypertrophy; SR: sarcoplasmic reticulum; mRNA: messenger RNA levels as detected by Northern blot analysis; Protein: protein levels as detected either by Western blot analysis or by receptor binding studies; Function: measured either as transporter gating mechanism or as measurement of calcium flux, either directly or by using myothermal measurements. ↓: significant decrease; ↑: significant increase; ↔: no change.

been shown to be decreased in human CHF [39,43,54,55] and animal VH [45,56]. However, measurement of protein levels has mostly yielded no changes in human CHF (see Table 1). Indeed, when phospholamban levels were normalized to calsequestrin levels in the study by Meyer et al. [57], significant differences between failing and non-failing human myocardium could no longer be detected. It should be noted, however, that the reduction of beta-adrenergic responsiveness that is seen in CHF [2] should result in a decreased level of phospholamban phosphorylation in vivo, thus resulting in a depressed SR Ca²⁺-ATPase even in the presence of normal levels of phospholamban in the failing heart.

Calcium is released from the SR via a calcium sensitive SR calcium release channel [35,36]. The channel tightly binds ryanodine and is, therefore, also referred to as the ryanodine receptor (RYR). The level of RYR mRNA has been shown to be reduced in both human CHF [38,55,58,59] and animal VH [45,46]. Likewise, RYR protein levels have been reported to be decreased in animal VH [60] and animal CHF [61]. Results in human CHF, however, have been inconsistent. Thus, Go et al. reported a 30% decrease in ryanodine binding sites in a small number of samples obtained from human CHF [58], while Nimer et al. found no change in the receptor density in human CHF [62]. Two recent studies [57,63] employing Western blotting tech-
niques also failed to detect a decrease in RYR protein in human CHF. D’Agnolo et al. [64] described an altered gating mechanism in human CHF, and Nimer et al. [62] reported differences in response to ryanodine between failing and nonfailing human myocardium. In contrast, Holmberg et al. [65] described normal basic properties of isolated human CHF cardiac calcium release channels in lipid membranes, although these data were not compared to data from nonfailing human hearts. Likewise, Cory et al. found decreased calcium channel release function in canine CHF [53]. Thus, although gene expression of the calcium release channel (RYR) may be unchanged, its gating behavior might be impaired in failing myocardium.

Calsequestrin is a calcium binding protein that is located within the lumen of the sarcoplasmic reticulum and is primarily responsible for the calcium storage capacity of this organelle [35,36]. Most studies have indicated no significant changes in either human or animal models of CHF at either the mRNA or the protein level (see Table 1) [38,46,55,57,63,66–68].

### 2.2. Sarcolemma

Calcium enters the cardiac myocyte during the plateau phase of the action potential via the L-type calcium channels [35,36]. The channel tightly binds the calcium channel blocking agents of the dihydropyridine family. In human CHF, Takahashi et al. [67] have shown decreased levels of mRNA coding for the calcium channel as well as decreased levels of dihydropyridine receptors. In contrast, Rasmussen et al. [69] reported no differences in dihydropyridine receptors between failing and nonfailing human myocardium. Similar conflicting results have been obtained in animal models of CHF or VH (see Table 1). Likewise, measurements of calcium current carried by the channel either in human CHF or in animal models of CHF or VH have yielded conflicting results. Thus, whether the function of L-type calcium channel is altered in the failing heart is, at present, still unknown.

The sarcolemmal Na⁺/Ca²⁺ exchanger transports three sodium ions for one calcium ion [35,36]. The energy for this transport is indirectly derived from ATP hydrolysis via the sodium gradient that is established by the sarcolemmal Na⁺/K⁺ pump. It is the major calcium extrusion mechanism of the cardiac myocyte and may contribute significantly to myocardial relaxation. Most studies indicate that both mRNA and protein levels of the sarcolemmal Na⁺/Ca²⁺ are increased in either human CHF or animal models of CHF or VH (see Table 1). Likewise, the functional capacity of the Na⁺/Ca²⁺ exchanger to transport calcium was shown to be increased in human CHF [39,70]. It is of interest that in rat pressure overload-induced hypertrophy, expression of cardiac Na⁺/Ca²⁺ exchanger is increased which results in increased Na⁺/Ca²⁺ exchanger activity, in a manner that is very similar to the early phase of postnatal development in the rat [71]. This suggests a common underlying mechanism in the control of Na⁺/Ca²⁺ exchanger expression in the immature and the hypertrophied myocardium in the rat. Furthermore, this is similar to the reduced expression of SR Ca²⁺-ATPase and reexpression of fetal isoforms of myosin in CHF and VH in this species [4,72–75]. Whether a similar mechanism is operating in human CHF remains to be established, however. The functional consequence of an increased activity of the Na⁺/Ca²⁺ exchanger in CHF may be to compensate for the reduction in SR Ca²⁺-ATPase activity. This would aid in myocardial relaxation, albeit at the cost of reduced calcium release from the SR during systole. This would be particularly evident at higher rates of stimulation, and thus lead to a blunted force–frequency relationship [29,76].

### 2.3. Calcium transient

Attempts to directly measure intracellular calcium in CHF or VH have yielded conflicting results (see Table 1). That is, studies using the photo protein aequorin on isolated myocardium from patients with end-stage CHF [37,77], or from animals with experimental CHF [78] or VH [79–82] have consistently revealed prolongation of the intracellular calcium transient. Systolic calcium concentration, on the other hand, was found to be unaltered, while diastolic calcium concentration was either unchanged or elevated. However, it should be noted that most of these studies report uncalibrated light signals and are performed at unphysiologically low experimental temperatures. Both these factors may have affected the obtained results, as has been discussed by Pieske et al. [29]. On the other hand, studies using fluorescent calcium indicators in single isolated myocytes obtained from the hearts of either patients with end-stage CHF [83,84], or from animals with experimental VH [85–89] or experimental CHF [85,89,90] have revealed a decreased amplitude of the intracellular calcium transient. Furthermore, most of these studies also showed an increase in the duration of the intracellular calcium transient. The uncertainties regarding the details of alterations in calcium handling in CHF may be due to inherent difficulties with either the aequorin or the single myocyte technique. That is, calibration of an aequorin light signal is fraught with uncertainty. This is because the indicator’s response to [Ca²⁺] is highly non-linear [91,92]. Moreover, aequorin is relatively insensitive at the low levels of [Cu²⁺] that prevail during diastole. Calibration of aequorin critically depends on an accurate estimate of the total amount of aequorin that is introduced into the muscle preparation [91,92]. Micro-injection of aequorin into a surface cell may inflict local cell damage and thus potentially alter the very calcium transient one attempts to measure. In addition, in this method, a limited number of surface cells are loaded with the calcium indicator, while the contractile performance of the preparation is obtained from the entire population of myocytes present in the
Fig. 2. Impact of uncontrolled sarcomere shortening on the intracellular calcium transient in an isolated, electrically stimulated rat cardiac trabecula. Intracellular calcium was assessed by Fura-2 that was introduced as a free salt by micropipette impalement and iontophoresis [98]. Panel C, force development and Panel D 340/380 Fura-2 fluorescence ratio. Thin lines represent data from a regular twitch; thick lines represent data from a sarcomere length (SL) that was held constant by means of an iterative computer feedback system [99]. In the regular twitches, SL decreased during the twitch because of damage-end compliance. A shows the muscle length waveform (thick line) that was required to prevent shortening of the central sarcomeres to within ~0.01 μm (SL clamp). Application of a SL clamp resulted in increases in peak force development and twitch duration, as well as a significant decrease in the 340/380 fluorescence ratio. Reproduced with permission by the American Physiological Society from Janssen and De Tombe [94].

Another approach is the use of isolated single myocytes. However, single myocytes that survive the isolation procedure may not be representative of the entire heart. In addition, measurement of force from intact single isolated myocytes has not been possible until now due to irreversible damage inflicted onto a single myocyte during attachment to the measuring device. Lack of attachment of the myocytes also allows for substantial sarcomere shortening during the contraction. This is a major limitation of the use of single isolated myocytes, because sarcomere shortening in itself significantly alters the intracellular calcium transient [94–96] (see below). Furthermore, calibration of a fluorescent calcium indicator is only possible when (i) the indicator is present exclusively in the cytosol and (ii) the in-situ dissociation constant is accurately known [92,97,98]. None of the studies regarding calcium handling in VH or CHF reported to date that used fluorescent indicators in isolated myocytes have fulfilled all of these conditions. Hence, whether the calcium transient is altered in VH and CHF remains a controversial issue at present.

We have recently begun to address some aspects of these limitations with the ultimate goal to apply the newly developed technique to the study of calcium handling in CHF and VH [94]. In our study, we used a recently developed iontophoresis technique that allows for the introduction of the free salt of a fluorescent indicator into thin cardiac trabeculae [98], thereby circumventing the intracellular compartmentalization of the calcium indicator. This is a prerequisite for obtaining calibrated calcium transient data. To address the impact of sarcomere shortening on the fluorescence ratio, a computer controlled iterative muscle loading system was used to control sarcomere length during the electrically stimulated contraction [99]. Fig. 2 shows results obtained in a typical cardiac trabeculae [94]. Sarcomere shortening of approximately 150 nm (trace B) caused a decrease in peak force development (trace C), and an increase in peak intracellular calcium (trace D). The impact of sarcomere shortening during the twitch proved to be directly proportional to the amount of sarcomere shortening (Fig. 3). Hence, these data clearly indicate that comparison of calcium transient data between a control group and a CHF or VH group is fraught with muscle preparation. Some of these limitations have been overcome by the use of a macro-injection technique [93]. Nevertheless, this technique is potentially hampered by inhomogeneous measurement of the parameters at interest. The main advantage of the aequorin technique, nevertheless, is its high temporal resolution and the fact that it allows for the simultaneous measurement of force and calcium [91].

Fig. 3. Percent decrease in 340/380 fluorescence ratio in the SL clamp twitch plotted as function of the extent of uncontrolled sarcomere shortening during a regular twitch for seven individual isolated rat cardiac trabeculae. There is a direct relationship between the extent of uncontrolled sarcomere shortening and the magnitude of the impact of sarcomere shortening on the measured calcium transient by Fura-2. Solid line, linear regression fit to the data (r² = 0.92, p < 0.01). Reproduced with permission by the American Physiological Society from Janssen and De Tombe [94].
uncertainty. That is, the measured calcium transient in a population of cardiac myocytes obtained from CHF or VH hearts may be reduced in amplitude simply because these myocytes also exhibit reduced sarcomere shortening patterns. Hence, further studies are required in which the above reviewed limitations are carefully avoided to resolve this important aspect of contraction–excitation coupling in the setting of CHF and VH. Experiments in our laboratory are currently underway to apply this technique to the measurement of calcium handling in experimental CHF and VH in rats. In addition, future development of improved methods to allow for the attachment to intact single isolated myocytes [100,101], in combination with computer guided sarcomere length control [99] and cytosolic calcium signals [94,98], would greatly aid in achieving this goal.

3. Myofilament function CHF

The contractile dysfunction that is seen in CHF and VH may also be caused by a decrease in the force that is generated by the contractile proteins for any given level of intracellular calcium. Heart failure is associated with changes in the distribution and content of myocardial contractile proteins both in human CHF [72,73,102–105] and in animal VH [4,72–74,106] and animal CHF [107]. It appears that these changes are of functional significance, since decreased myocardial myofibrillar ATPase activity has been demonstrated in the failing human heart [108,109], and in the hearts of animal models of VH [4,74]. In small animal models of VH and CHF, this has been shown to be associated with a marked shift in the isomyosin expression from the V1 to the V3 isoform [4,72–74]. It is known that the V3 form of myosin has a lower ATPase activity [110] and that expression of this isoform is correlated with a reduction in the maximum velocity of sarcomere shortening [111]. However, it should be noted that an isoform shift of V1 myosin to V3 myosin is not seen in diseased myocardium of larger species, such as human, since cardiac myosin in these species is already in the V3 form under control conditions [73,75]. This is consistent with the observation that the ATPase activity of purified cardiac myosin is not altered in human heart failure [75,108]. Therefore, it is likely that alterations in other contractile proteins play a role in determining the contractile properties of failing myocardium [112]. Several observations support this hypothesis. Increased expression of a fetal cardiac isoform of Troponin-T has been found in human CHF [103]. It has been shown that shifts in Troponin-T isoform expression are associated with changes in the calcium responsiveness of the myofilaments during development [113]. Likewise, altered Troponin-I isoform expression has also been demonstrated in the developing heart [114], although isoform shifts in this contractile protein have not been found in human CHF [115]. In rats, the fetal isoform of Tn-I is associated with a reduction of the impact of acidic pH on the calcium responsiveness of the cardiac myofibril [116] and a reduction of the sensitivity to phosphorylation by protein kinases [112]. Recently, it has been suggested that the amount of myocardial Troponin-I is reduced in experimental CHF [107] and experimental diabetes [117], but increased in experimental VH [106]. The functional consequence of these changes in contractile protein isoforms remains unclear, however. Finally, changes in some of the myosin accessory proteins have been found in CHF. Thus in human CHF, Margossian et al. [104] reported a marked decrease in the myosin light chain content, apparently due to increased levels of neutral protease in these hearts, while others [105] have demonstrated an increase in the atrial form of myosin light chain-1. Morano et al. [118] have recently shown in isolated human myocardium, using synthetic peptide fragments of myosin light chain and monoclonal antibodies, that alterations in the myosin light chain–actin interaction can have a profound effect upon force development and calcium responsiveness. Likewise, myosin light chain 2 extraction in skinned myocardium has been shown to result in decreased velocity of shortening as well as decreased calcium responsiveness [119]. Thus, changes in myosin light chain in heart failure may be of functional consequence for contractile activation.

Previous studies using multicellular preparations from several laboratories have indicated unaltered myofibrillar calcium responsiveness and maximum force development in either human CHF [64,120] or experimental CHF or VH [121]. Recently, Schwinger et al. [122] have proposed that the failing human cardiac myofilament has lost its ability to alter calcium sensitivity upon a change in length. More recent follow-up studies, however, have failed to confirm these observations [77,123,124]. Interpretation of these mechanical studies of the cardiac myofibril is limited, however, since sarcomere length was not measured in those studies during rest and during contractile activation. Kentish et al. [125] have shown that continuous measurement and control of sarcomere length is essential in the study of myofibrillar calcium responsiveness. That is, these investigators found a marked sarcomere length dependence of maximum force development, calcium responsiveness, and steepness of the developed force-free calcium concentration relation in skinned rat cardiac trabeculae. On the other hand, when sarcomere length was not controlled during activation, ambiguous data were obtained. We have recently employed a laser diffraction technique to measure and control sarcomere length during active force development in thin, chemically permeabilized cardiac trabeculae obtained from rats that were suffering from CHF secondary to chronic myocardial infarction [126]. Using this experimental model we found a preserved Frank–Starling mechanism — consistent with the findings by others in human CHF [77,123,124] — a reduced level of maximum tension development, and no change in myofilament cal-
calcium sensitivity under conditions of strict sarcomere length control [125].

Studies employing multicellular preparations, however, are limited due to the fact that CHF or VH may lead to alterations at the myofilament level that are not uniformly distributed. Furthermore, it is difficult to exclude the influence of changes in the extracellular matrix. It is for these reasons that investigators have recently begun to employ single isolated myocytes for the study of myofilament function in animal CHF [107,127,128] and animal VH [129], and in human CHF [130]. Following chemically permeabilization (skinning), a single isolated myocyte can be attached to micropipettes by glue such that force development can be measured at varying levels of calcium in the bathing medium [100,101,131]. Fig. 4 shows an example of a rat myocyte both during relaxing (panel A) and activating conditions (panel B). Since CHF invariably leads to myocyte hypertrophy it is necessary to obtain an accurate measure of cross-sectional area (panel C) to allow for the normalization of force development thus converting this parameter to tension (force per cross-sectional area) [129]. Unfortunately, application of the single cell technique has thus far yielded conflicting results. Fan et al. [129] have found a reduction in calcium sensitivity and maximum tension development in experimental right ventricular hypertrophy in the rat as is illustrated in Fig. 5.

Fig. 4. Photomicrograph of single isolated rat myocyte attached to micropipettes by silicon glue in relaxing solution (A) and during calcium activation (B; pCa = 5.24). Average sarcomere length is estimated at 2.30 μm in A and 2.08 μm in B. C, method used to obtain the thickness of a myocyte by placing a small mirror under a 45° angle close to the cell by using a three-dimensional hydraulic manipulator. Reproduced with permission by the American Heart Association from Fan et al. [129].

Fig. 5. Line graphs showing the average relationship between active tension development and free calcium concentration in single isolated rat myocytes at sarcomere length 2.3 μm (A), and 2.0 μm (B). Hypertrophy was associated with a significant decrease in calcium responsiveness for tension development. Data were obtained from control animals (open circles) and animals with long-term right ventricular hypertrophy and signs of right heart failure (filled circles). Average data from seven animals. Reproduced with permission by the American Heart Association from Fan et al. [129].
Interestingly, despite the overall changes in calcium responsiveness, the response of the myofilaments to a change in sarcomere length was preserved, which is again consistent with the findings by others in human CHF [77, 123, 124]. The reduction in calcium responsiveness is consistent with a recent report by Li et al. [107] employing a rat model of CHF secondary to myocardial infarction. In contrast, Wolff et al. have found an increased calcium sensitivity in canine CHF [128] and human CHF [130]. Some of the different findings between these studies may be due to varying levels of contractile protein phosphorylation [107, 132]. Although it is difficult to control, this parameter plays a major role in determining the calcium sensitivity of the cardiac myofibril to calcium [112, 133]. One way to account for varying levels of contractile protein phosphorylation is by applying protein kinase A to saturate CHF preparations were no longer detectable. It is possible, however, that maximal phosphorylation masks by saturation potential intrinsic alterations in contractile function. For example, in contrast to the work of the group of Cooper [141, 142], a recent study by Bailey et al. [87] in which single isolated feline myocytes in the setting of experimental VH were also employed, failed to find an effect of colchicine (an agent that induced depolymerization of microtubular tubulin) on passive viscosity or contraction dynamics. This result is consistent with our own results on isolated rat cardiac trabeculae where we have been unable to demonstrate an effect of either colchicine or taxol (an agent that induces polymerization microtubular tubulin) on passive viscosity or contraction dynamics (Naya and de Tombe, unpublished observations). Thus, whether changes in the cytoskeleton composition impact on ventricular function in CHF or VH remains unclear at present.

5. Concluding remarks

This review has focused on the potential mechanisms of congestive heart failure (CHF) and ventricular hypertrophy (VH) at the level of the cardiac myocyte. It has been shown rather conclusively that calcium handling is altered in end-stage CHF and VH. This is particularly the case with regard to the calcium pumping capacity of the sarcoplasmic reticulum and the sarcolemmal Na⁺/Ca²⁺ exchanger. Recent reports have indicated that the changes in gene expression of the calcium handling proteins may occur very early, that is within days, during the development of CHF or VH in animal models [61, 146]. Furthermore, on a slightly longer time scale, that is weeks, there appears to be a gradual increase in the alteration of calcium handling protein levels described above [50, 52, 146, 147]. It should be pointed out, however, that calcium handling may be affected without any changes in the individual components involved in the cardiac excitation–contraction coupling processes. That is, Gomez et al. [89] have recently shown in an elegant study that the coupling between calcium influx via the L-type calcium channels and the quantal release of calcium by the SR (‘calcium sparks’) was reduced in an animal model of CHF. The intrinsic properties of calcium influx and SR calcium release processes, however, were not affected. Rather, it appeared that the subtle coupling between the sarcoplasmic calcium channels and SR calcium release channels and that between neighboring SR release channels, was disturbed leading to altered calcium handling. Whether this mechanism plays a role in human CHF remains to be established, however.

In contrast to calcium handling, it is less clear whether contractile protein function is altered in heart failure. Changes have been noted in some of the contractile sub-

isolated myocytes which would allow for the measurement of the mechanical properties of the cytoskeleton in human CHF. It should be noted that alterations in microtubule content have not been unequivocally associated with changes in contractile activity. For example, in contrast to the work of the group of Cooper [141, 142], a recent study by Bailey et al. [87] in which single isolated feline myocytes in the setting of experimental VH were also employed, failed to find an effect of colchicine (an agent that induced depolymerization of microtubular tubulin) on passive viscosity or contraction dynamics. This result is consistent with our own results on isolated rat cardiac trabeculae where we have been unable to demonstrate an effect of either colchicine or taxol (an agent that induces polymerization microtubular tubulin) on passive viscosity or contraction dynamics (Naya and de Tombe, unpublished observations). Thus, whether changes in the cytoskeleton composition impact on ventricular function in CHF or VH remains unclear at present.

4. Cytoskeleton in CHF

Recent work on experimental hypertrophy has suggested a role for the microtubular network in shaping the response of the heart to the increased load [15, 141–143]. Tsutsui et al. reported an increase in the microtubule component of the cardiac myocyte in response to pressure overload that correlated with a depression of contractile function [142]. Similar observations have been made in experimental models of CHF [143]. Schaper et al. have demonstrated in human CHF a disproportionate increase in the relative abundance of cytoskeletal proteins [15]. A decrease in the myofilament protein Titin has also been observed [144, 145]. Recently, Tagawa et al. reported on measurements of myocyte cytoskeletal stiffness and viscosity in a cat model of VH using a novel technique of magnetic cytometry, a method by which magnetically induced force is applied directly to the cytoskeleton via ferromagnetic beads coated with a RGD peptide, which forms the integrin binding motif [141]. This technique should, in principle, be applicable to the study of human.
proteins, notably Troponin-T. However, due to the uncertainty of the sites and extent of contractile protein phosphorylation in vivo, it is not known at present whether contractile protein function is depressed in CHF or VH. Likewise, the cytoskeleton of the cardiac myocyte has been shown to be significantly altered in CHF and VH, but the functional consequence of this change is unclear. It should be remembered, however, that most studies have only correlated changes in all of these cellular processes with ventricular dysfunction. In particular in human CHF, one invariably deals with end-stage heart disease that is severe enough to warrant cardiac transplantation. Therefore, whether any of these mechanisms play a causal role in the development of heart failure is not known at present. Many of the studies that attempt to investigate this aspect by studying the time course of cellular changes during the development of experimental CHF in animals are hampered by the inherent variability of many of the experimental models that are employed. Thus, development of reproducible experimental models of CHF is of paramount importance to the study of the cellular causes of CHF. Some of the experimental models of CHF and VH have suggested that altered calcium handling may be the underlying cause of the decreased myocardial function that is seen at end-stage CHF. Consistent with this notion, we have recently observed alterations in twitch kinetics and twitch force development in isolated cardiac trabeculae obtained from rats at eight weeks following a large left ventricular myocardial infarct. Myofilament contractile function, however, was not different from control at that time (Daniels and de Tombe, unpublished observations). Since we have previously shown profound alterations in myofilament function at end stage CHF in this rat model [126], these observations suggest that during the development of CHF, alterations in calcium homeostasis precede any changes in contractile filament function. Clearly, future experiments aimed at studying the time course of calcium handling and myofilament function in precise detail are necessary to resolve this important issue.

It should be noted that there are some technical difficulties in studying the mechanism of CHF by using isolated myocardial tissue preparations. In particular, the problem of non-homogeneous effects of CHF or VH on the myocytes in the heart should be considered. That is, pathological specimens, multicellular preparations, and single cells are all selected from samples of the heart. There are few, if any, studies that have used a methodology that provides information on the heterogeneity of any abnormality within various regions of the heart. Hence, this limitation should be taken into account when one extrapolates findings from isolated myocardial tissue preparations to the whole heart.

Finally, accurate knowledge regarding the cellular processes that participate in the development of CHF is critical to the development of innovative strategies aimed to combat CHF [2,148,149]. Indeed, some pioneering studies have been published recently in which the ground work is laid for possible correction of the sarcoplasmic reticulum calcium pump defect via ‘gene therapy’ [150]. Likewise, a reduction in calcium sensitivity may be treated by the application of new generation calcium sensitizing agents [149,151]. Clearly, intense further investigations are required to enhance our understanding of the cellular processes that are involved in the development of heart failure.

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