

Review

# Alterations of calcium-regulatory proteins in heart failure

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## 1. Introduction

During the last decade there was accumulating evidence that alterations of excitation-contraction (EC) coupling may play a critical role in the pathophysiology of myocardial failure. EC-coupling comprises processes involved in calcium activation of contractile proteins and the subsequent removal of calcium facilitating relaxation (for review see [1]). The initial event is depolarisation of the membrane which opens voltage-gated, dihydropyridine-sensitive sarcolemmal calcium channels (dihydropyridine receptors) allowing an influx of calcium into the myocyte. There is a close proximity between one or a few sarcolemmal calcium channels and one or a few calcium channels of the sarcoplasmic reticulum (ryanodine receptors). This may enable calcium influx through one opening of a single sarcolemmal calcium channel to increase a local pool of calcium sufficiently to open adjacent ryanodine receptors to release calcium. This local, punctate, increase of calcium is termed calcium spark according to its visualization by confocal microscopy [2]. It is the sum of these local calcium releases which generate a global cytoplasmic increase in calcium which causes activation of contractile proteins. The global increase in calcium is immediately followed by calcium removal resulting in subsequent deactivation of the contractile machinery. Calcium removal from the cytosol occurs by activity of the sarcoplasmic reticulum calcium pump and by exchange of calcium for sodium by the sarcolemmal Na<sup>+</sup>-Ca<sup>2+</sup>-exchanger [1].

Defective EC-coupling in heart failure may result from altered density or function of proteins relevant for calcium homeostasis. Knowledge of these alterations is a pre-

requisite for understanding the pathophysiology of myocardial failure and for the development of new strategies to treat patients with heart failure. This review focuses on expression and function of calcium-regulatory proteins in failing myocardium from animal models of heart failure and from failing human hearts.

## 2. Sarcolemmal L-type calcium channels

Calcium entry through voltage-gated L-type calcium channels is the key event causing the transition from the resting state of the myocardium to contraction [3]. Sarcolemmal calcium channels are multimeric protein complexes comprised of five subunits termed  $\alpha_1$ ,  $\alpha_2$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ , which are encoded by separate genes (for review see [4]). The  $\alpha_1$ -subunit contains the calcium conducting pore and the binding sites for calcium channel blockers. The  $\alpha_2/\beta$ -,  $\gamma$ -, and  $\delta$ -subunits have regulatory properties. The  $\gamma$ -subunit is only expressed in skeletal muscle. At least three genes code for the dihydropyridine-sensitive  $\alpha_1$ -subunits in skeletal, neuronal and cardiac/smooth muscle tissue. The cardiac and smooth muscle L-type calcium channel  $\alpha_1$ -subunits are splice variants of the same gene [4].

### 2.1. L-type calcium channels in animal models of heart failure

Several studies have examined changes in the density of L-type calcium channels in myocardium from cardiomyopathic hamsters, a genetic cardiomyopathy model with the prominent characteristics of abnormal calcium homeostasis

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(Table 1). In this model, alterations in the density of the channels seem to depend on age of the animal or stage of the disease (for review see [5]). When there is fully developed morphological and hemodynamic evidence of myopathy, there seems to be no appreciable difference between control and myopathic hamsters as measured by antagonist binding [6]. In contrast, the number of dihydropyridine receptors seems to be increased in younger cardiomyopathic animals before morphological evidence for the myopathy is present [7].

The density of L-type calcium channels, as evaluated by antagonist binding was shown to be decreased in moderate to severe stages of congestive heart failure in rats following myocardial infarction [8,9] and in mongrel dogs with myocardial failure following intracoronary microembolization (Table 1) [10]. Similarly, [<sup>3</sup>H]nitrendipine binding was decreased in a rabbit tachycardia heart failure model [11]. In contrast, dihydropyridine binding was not altered in a tachycardia failure dog model [12].

## 2.2. L-type calcium channels in failing human myocardium

Abundance of the L-type calcium channel  $\alpha_1$ -subunit in failing and nonfailing human myocardium has been studied by Northern blot analysis and by dihydropyridine binding. Takahashi et al. reported a significant decrease in mRNA levels encoding the dihydropyridine receptor as well as a decrease in dihydropyridine binding sites in failing human hearts with dilated and ischemic cardiomyopathy [13]. This is in contrast to findings by Rasmussen et al. which indicate that dihydropyridine binding sites are not significantly altered in the human ventricular tissue from hearts with end-stage dilated cardiomyopathy [14].

Unaltered levels of the  $\alpha_1$ -subunit of L-type calcium channels would be consistent with functional measurements indicating that calcium current densities, measured during basal conditions, are similar in isolated myocytes from failing hearts with dilated cardiomyopathy and from nonfailing hearts [15,16]. However, measurements by Piot et al. recently suggested that function of L-type calcium channels may be altered in human heart failure [17]. They observed that increasing frequencies augment calcium currents in myocytes from nonfailing hearts, whereas high frequency upregulation of calcium currents was attenuated

or lost in myocytes from hearts with reduced left ventricular function. These findings may indicate that quantitative or qualitative changes of subunits other than the  $\alpha_1$ -subunit of the multimeric L-type calcium channel may occur in the failing human heart.

## 3. Sarcoplasmic reticulum calcium release channels

Intracellular calcium release channels, located on the sarcoplasmic reticulum are among the largest proteins identified to date. Two different types of channels are known termed ryanodine receptor (RyR) and inositol 1,4,5-trisphosphate receptor (IP3R). In the cardiac sarcoplasmic reticulum, the density of the RyR is significantly higher than that of the IP3R, and the former is far more relevant for excitation-contraction coupling (for review see [18]). The calcium sensitive RyR which is located in the immediate vicinity of the L-type calcium channel is activated by a local increase in calcium subsequent to transsarcolemmal calcium influx [2]. Once activated, the channel opens and releases calcium for activation of contractile proteins [1,19,20]. This process is termed calcium-induced calcium release [3]. The RyR forms a tetrameric structure comprised of four monomers [20]. Molecular cloning analysis has revealed that two distinct genes encode the cardiac (RyR2) and skeletal muscle (RyR1) specific receptors [21,22]. A third type of ryanodine receptor (RyR3) exhibits functional properties distinct from those of RyR1 and RyR2 [23]. The tetrameric structure of RyR is stabilized by a channel-associated protein known as the FK506 binding protein (FKBP). The immunosuppressant drugs rapamycin and FK506 can dissociate FKBP from RyRs which increases the sensitivity of RyRs to agonists [18].

### 3.1. Ryanodine receptors in failing animal myocardium

In the cardiomyopathic hamster, Finkel et al. reported an increase in [<sup>3</sup>H]ryanodine binding to cardiac membrane fractions prepared from 1–2 months old animals [24]. In contrast, a decreased number of ryanodine receptors as measured by [<sup>3</sup>H]ryanodine binding and mRNA levels compared to control hamsters has been reported from measurements in crude membrane fractions [25].

Table 1  
Quantification of L-type calcium channels in animal models of heart failure

Animal model	Channel density	Method	Reference
Syrian hamster	n.s.	[ <sup>3</sup> H] nitrendipine binding	Finkel et al. Life Sciences 41;1987 [6]
idem	↑	[ <sup>3</sup> H] dihydropyridine binding	Wagner et al. Science 232;1986 [7]
Rat, infarction	↓	[ <sup>3</sup> H] nitrendipine binding	Dixon et al. Circ. Res. 66;1990 [8]
idem	↓	[ <sup>3</sup> H] PN 200110 binding	Gopalakrishnan et al. Am. J. Physiol. 261;1991 [9]
Dog, infarction	↓	[ <sup>3</sup> H] nitrendipine binding	Gengo et al. J. Mol. Cell. Cardiol. 24;1992 [10]
Rabbit, tachycardia pacing	↓	[ <sup>3</sup> H] nitrendipine binding	Colston et al. Cell. Calcium 16;1994 [11]
Dog, pacing tachycardia	n.s.	[ <sup>3</sup> H] dihydropyridine binding	Vatner et al. Circulation 90;1994 [12]

A decreased number and activity of RyRs has also been observed in two distinct types of dog failure models; a spontaneous occurring cardiomyopathy in dobermans, and a rapid ventricular pacing failure model of mongrels [26,27]. Consistently, Vatner et al. showed that in the dog pacing tachycardia failure model [<sup>3</sup>H]ryanodine binding was depressed as early as 1 day after pacing and remained at this depressed level up to 4–7 weeks of pacing when heart failure was manifest [12]. A decreased number of ryanodine receptors was also observed in chronic doxorubicin cardiomyopathy in rabbits [28].

Interestingly, in a recent study performed in hypertensive and failing rats, Gómez et al. suggested that calcium current density as well as density and function of RyRs are normal in this model. However, they showed that the relationship between calcium current density and the probability of evoking a spark was reduced indicating that calcium influx is less effective at inducing SR calcium release [29].

### 3.2. Ryanodine receptors in failing human myocardium

Several groups have studied mRNA expression of the RyR in human heart failure and results have not been consistent (Table 2). While Brillantes et al. observed decreased mRNA levels in ischemic but not in dilated cardiomyopathy, Go et al. described a reduction of RyR mRNA levels in both ischemic and dilated cardiomyopathy [30,31].

In three studies a radioligand binding assay was used. Go et al. in a small number of samples (and without any statistical analysis) observed that high affinity binding sites for [<sup>3</sup>H]ryanodine were decreased by about 30% in left ventricular myocardium from failing human hearts [31]. Schumacher et al. observed no differences in [<sup>3</sup>H]ryanodine binding between failing and nonfailing hearts [36]. Finally, Sainte Beuve observed an increase of RyRs in failing hearts [33].

At the level of the protein, no change in RyR levels

between failing and nonfailing hearts were consistently observed in three different studies [33–35]. From their findings of unchanged protein levels but increased [<sup>3</sup>H]ryanodine binding in failing hearts, Sainte Beuve et al. suggested that ryanodine binding properties may be affected in failing myocardium which may reflect altered channel activity [33].

Altered function of RyRs was also suggested from D'Agnoletto et al. [37]. They found that caffeine threshold of the ryanodine receptor was increased, suggesting impaired gating mechanism of the calcium release channel in dilated cardiomyopathy [37]. Furthermore, Nimer et al. reported differences in response to ryanodine between failing and nonfailing myocardium which may also reflect altered function of the RyRs [38]. In contrast, Holmberg and Williams who studied activity of single RyR under voltage-clamp conditions reported normal basal properties of the RyR from failing human hearts [39].

### 3.3. IP3R in failing myocardium

Like the RyR, the IP3R has a tetrameric structure (for reviews see [18]). Three different isoforms have been identified so far. It was shown that IP3Rs are expressed in myocytes and that activation by the second messenger inositol 1,4,5-trisphosphate (IP3) results in calcium release from the sarcoplasmic reticulum [40]. IP3 is generated by phosphoinositide hydrolysis following activation of G-protein coupled  $\alpha$ -receptors, AII-receptors and endothelin-receptors. This type of activation of intracellular calcium release is referred to as pharmacomechanical coupling [18].

It was recently observed that IP3R mRNA levels are significantly increased in failing human myocardium [31]. Although in the study by Go et al. [<sup>3</sup>H]IP3 binding sites were not significantly increased in failing compared to nonfailing myocardium in a small number of samples, it was emphasized that the number of IP3Rs increases significantly relative to that of ryanodine receptors in the failing human myocardium [31]. The functional relevance of this

Table 2  
Quantification of SR-Ca<sup>2+</sup>-release channels (RyR) in human heart failure

Quantity	Disease	Method	Reference
28% ↓	ICM	Northern blot	Brillantes et al. Circ. Res. 71;1992 [30]
n.s.	DCM	Northern blot	idem
31% ↓	DCM, ICM	Northern blot	Go et al. J. Clin. Invest. 95;1995 [31]
Inverse Relation With ANF	DCM	Northern blot	Arai et al. Circ. Res. 72;1993 [32]
30% ↓	DCM	Northern blot	Sainte Beuve et al. J. Mol. Cell. Cardiol. 29;1997 [33]
n.s.	ICM	Northern blot	idem
n.s.	DCM	Western blot	Meyer et al. Circulation 92;1995 [34]
n.s.	ICM	Western blot	Schillinger et al. Mol. Cell. Biochem. 160/161; 1996 [35]
n.s.	DCM, ICM	Western blot	Sainte Beuve et al. J. Mol. Cell. Cardiol. 29;1997 [33]
70–114% ↑	DCM, ICM	[ <sup>3</sup> H] ryanodine binding	idem
n.s.	DCM, ICM	[ <sup>3</sup> H] ryanodine binding	Schumacher et al. Naunyn-Schmiedeberg Arch. Pharm. 353;1995 [36]

ANF = atrial natriuretic factor; DCM = dilated cardiomyopathy; ICM = ischemic cardiomyopathy; RyR = ryanodine receptor; n.s. = no significant change versus nonfailing human myocardium.

alteration is unknown. It was speculated that IP3Rs could be involved in regulation of diastolic tone and signaling pathways in the heart (for review see [18]).

#### 4. Sarcoplasmic reticulum calcium storage proteins

Calsequestrin and calreticulin are located within the lumen of the SR [41–43]. Calsequestrin, a high-capacity moderate affinity calcium-binding protein is primarily responsible for the calcium storage capacity of the SR in cardiac muscle [41]. Two distinct isoforms of calsequestrin have been identified. The skeletal muscle isoform expressed in both fast- and slow-twitch skeletal muscle, and the cardiac isoform, expressed predominantly in the heart [44,45].

Calreticulin is a major calcium binding protein of non-muscle endoplasmic reticulum membranes (for review see [43]). In addition to its apparent calcium storage role, evidence is accumulating which suggests that calreticulin has other regulatory functions within the cell. It may be involved in regulation of DNA-synthesis and protein-synthesis [43,46].

##### 4.1. Calsequestrin in failing myocardium

Calsequestrin expression has been shown to be unchanged during ontogenic development and aging as well as in hypertrophied myocardium due to pressure overload and hyper- or hypothyrosis [47,48].

Studies in failing human myocardium consistently showed unchanged mRNA and protein levels as compared to nonfailing myocardium [13,32,34,35,49]. Similarly, calreticulin protein levels were shown to be unchanged in the failing human hearts [34].

#### 5. Sarcoplasmic reticulum calcium pump and phospholamban

Calcium transport into the SR occurs by SR-Ca<sup>2+</sup>-ATPase, which transports two calcium ions per molecule of high-energy phosphate hydrolyzed against a high ion gradient from a free intracellular calcium between 100 nM and 10 μM to a free calcium in the SR ~ 1 mM [41,50]. This pump, together with the Na<sup>+</sup>-Ca<sup>2+</sup>-exchanger and the sarcolemmal calcium-ATPase, eliminates calcium from the cytosol in order to facilitate relaxation of the myocardium [1,50]. Moreover, SR-Ca<sup>2+</sup>-ATPase is crucial for calcium accumulation within the SR and thus, for the availability of calcium for systolic release through the RyRs. Sarco-endoplasmic reticulum Ca<sup>2+</sup>-ATPases are encoded by three genes and five different isoforms are expressed: the adult fast-twitch skeletal muscle isoform (SERCA1a), its alterna-

tively spliced neonatal isoform (SERCA1b), the cardiac/slow-twitch skeletal muscle isoform (SERCA2a), its alternative spliced smooth muscle/nonmuscle isoform (SERCA2b), and an isoform expressed in a broad variety of muscle and nonmuscle tissues (SERCA3) (for review see [51]). No isoform shift has been detected in the failing heart [51].

The SR-Ca<sup>2+</sup>-ATPase is regulated by phospholamban [41,52,53]. Dephosphorylated phospholamban is an inhibitor of the SR-Ca<sup>2+</sup>-ATPase activity. The inhibition has been suggested to involve direct protein-protein interaction followed by conformational changes in the SR-Ca<sup>2+</sup>-ATPase resulting in a decrease in the affinity of the calcium pump for calcium [53–55]. It has been recognized that phosphorylation of phospholamban by calcium/calmodulin-dependent protein kinase (CaM kinase; Thr 17) and by protein kinase A (Ser 16) results in stimulation of SR-Ca<sup>2+</sup>-ATPase [52,54,56]. The molecular mechanism of SR-Ca<sup>2+</sup>-ATPase stimulation by phospholamban phosphorylation is thought to involve primarily an increase in the affinity of the SR-Ca<sup>2+</sup>-ATPase for calcium, although an increase in V<sub>max</sub> may also occur [56–58]. Furthermore, direct phosphorylation of SR-Ca<sup>2+</sup>-ATPase (Ser 38) by CaM kinase was suggested to stimulate SR-Ca<sup>2+</sup>-ATPase activity and calcium transport though an increase in V<sub>max</sub> without any apparent change in the affinity of the ATPase for calcium [59,60]. However, more recently it was shown by Odermatt et al. that V<sub>max</sub> of the SR-Ca<sup>2+</sup>-ATPase is not altered by CaM kinase-dependent phosphorylation or by interaction with phospholamban. Both CaM kinase- and protein kinase A-dependent phosphorylation shifted the K<sub>0.5</sub> for calcium-dependence of calcium transport [61]. There are no isoforms of phospholamban, and the same protein is expressed in cardiac and slow twitch skeletal muscle [62].

##### 5.1. SR-Ca<sup>2+</sup>-ATPase in failing animal myocardium

Abundance of SR-Ca<sup>2+</sup>-ATPase was studied in different animal models of myocardial failure (Table 3). Kuo et al. showed decreased gene expression of SR-Ca<sup>2+</sup>-ATPase in Syrian hamsters with hereditary cardiomyopathy [63]. Alteration in gene expression preceded any noticeable myocyte damage in this model.

In a rat model of myocardial infarction induced by occluding the left coronary artery for 4, 8 and 16 weeks, SR-Ca<sup>2+</sup>-ATPase mRNA- and protein levels decreased continuously with increasing severity of congestive heart failure. Interestingly, SR-Ca<sup>2+</sup>-ATPase activity was found to be more depressed than expected from the reduction in protein levels [64].

In left ventricular myocardium from rats with ascending aortic banding, a decrease in SR-Ca<sup>2+</sup>-ATPase mRNA levels by the polymerase chain reaction occurred in failing

Table 3  
Quantification of SR-Ca<sup>2+</sup>-ATPase in animal models of heart failure

Animal model	Alteration	Method	Reference
Syrian hamster	↓	Northern blot	Kuo et al. Biochim. Biophys. Acta 1138;1992 [63]
Rat, infarction	↓	Northern blot	Zarain-Herzberg et al. Mol. Cell. Biochem. 163/164; 1996 [64]
Rat, infarction	↓	Western blot	idem
Rat, aortic banding	↓	PCR	Feldman et al. Circ. Res. 73;1993 [65]
Rat, aged SHR	n.s.	Northern blot	Boluyt et al. Circ. Res. 75;1994 [66]
Guinea pig, aortic banding	↓	Western blot	Kiss et al. Circ. Res. 77;1995 [67]
Dog, pacing tachycardia	n.s.	PCR	Williams et al. Circ. Res. 75;1994 [68]
Dog, pacing tachycardia	↓	Western blot	O'Rourke et al. Circulation in press 1997 [69]

animals after 20 weeks of banding but not in nonfailing hypertrophied hearts [65]. From this data it was suggested that the decrease in SR-Ca<sup>2+</sup>-ATPase mRNA levels may be a marker of the transition from compensated hypertrophy to failure in this model [65]. Of note, in another rat failure model no decrease in mRNA levels was found during the transition from compensated hypertrophy to failure [66]. In this study, the transition to failure was associated with significant alterations in expression of genes encoding extracellular matrix.

A decrease in SR-Ca<sup>2+</sup>-ATPase protein levels was observed in failing guinea pig hearts following 8 weeks of banding of the descending thoracic aorta as compared to an age matched banded group without clinical signs of heart failure [67].

Expression of calcium cycling proteins has also been studied in left ventricular endocardial biopsies from dogs at baseline and at the onset of pacing tachycardia-induced heart failure. SR-Ca<sup>2+</sup>-ATPase mRNA levels did not significantly change from baseline, despite development of heart failure [68]. In contrast, more recently a significant decrease in SR-Ca<sup>2+</sup>-ATPase protein levels was observed in dogs with pacing-tachycardia failure [69]. The latter finding is consistent with measurements from Cory et al. showing a decreased activity of the SR calcium pump in

mongrel dogs with pacing-induced heart failure and in Doberman Pinscher dogs with dilated cardiomyopathy [70].

### 5.2. SR-Ca<sup>2+</sup>-ATPase in failing human myocardium

In all studies on SR-Ca<sup>2+</sup>-ATPase mRNA levels published by now it has been reported that mRNA levels of SR-Ca<sup>2+</sup>-ATPase are reduced in the failing compared to the nonfailing human heart (Table 4). Consistently, several studies indicated that SR calcium uptake or SR-Ca<sup>2+</sup>-ATPase activity are reduced in the failing human myocardium [75–77]. This, however, was not observed by Movsesian et al. [78]. At the level of the protein findings have been controversial (Table 4). Interestingly, a significant correlation between SR-Ca<sup>2+</sup>-ATPase protein levels and myocardial function, which was assessed by the force-frequency relation, has been found [76]. This analysis indicated that a wide variation exists in protein levels of SR-Ca<sup>2+</sup>-ATPase within the group of failing hearts (protein levels differed by a factor of four) and that this variation in protein levels matches with differences in myocardial function. In other words, in a subgroup of failing hearts SR-Ca<sup>2+</sup>-ATPase protein levels are similar to that in nonfailing hearts and this is associated with preserved myocardial systolic function by force-frequency relation [76].

Table 4  
Quantification of SR-Ca<sup>2+</sup>-ATPase in human heart failure

Quantity	Disease	Method	Reference
45% ↓	DCM, ICM	Northern blot	Mercadier et al. J. Clin. Invest. 85;1990 [71]
50% ↓	DCM, ICM	Northern blot	Takahashi et al. Circ. Res. 71;1992 [72]
Inverse Relation With ANF	DCM	Northern blot	Arai et al. Circ Res 72;1993 [32]
50% ↓	DCM, ICM	Northern blot	Studer et al. Circ. Res. 75;1994 [73]
50–60% ↓	DCM, ICM	Northern blot	Linck et al. Cardiovasc. Res. 31;1996 [74]
54% ↓	DCM	Northern blot	Schwinger et al. Circulation 92;1995 [75]
40% ↓	DCM, ICM	Western blot	Studer et al. Circ. Res. 75;1994 [73]
36% ↓	DCM, ICM	Western blot	Hasenfuss et al. Circ. Res. 75;1994 [76]
33% ↓	DCM	Western blot	Meyer et al. Circulation 92;1995 [34]
n.s.	DCM	Western blot	Movsesian et al. Circulation 90;1994 [49]
n.s.	DCM	Western blot	Schwinger et al. Circulation 92;1995 [75]
n.s.	DCM, ICM	Western blot	Linck et al. Cardiovasc. Res. 31;1996 [74]

ANF = atrial natriuretic factor; DCM = dilated cardiomyopathy; ICM = ischemic cardiomyopathy; n.s. = no significant change versus nonfailing human myocardium.

Table 5  
Quantification of phospholamban in human heart failure

Quantity	Disease	Method	Reference
↓	DCM	PCR	Feldman et al. Circulation 83;1991 [79]
27–35% ↓	DCM, ICM	Northern blot	Linck et al. Cardiovasc. Res. 31;1996 [74]
Inverse Relation With ANF	DCM, ICM	Northern blot	Arai et al. Circ. Res. 72;1993 [32]
46% ↓	DCM	Northern blot	Schwinger et al. Circulation 92;1995 [75]
n.s.	DCM	Western blot	Movsesian et al. Circulation 90;1994 [49]
18% ↓	DCM	Western blot	Meyer et al. Circulation 92;1995 [34]
n.s.	DCM, ICM	Western blot	Linck et al. Cardiovasc. Res. 31;1996 [74]
n.s.	DCM	Western blot	Schwinger et al. Circulation 92;1995 [75]

ANF = atrial natriuretic factor; DCM = dilated cardiomyopathy; ICM = ischemic cardiomyopathy; n.s. = no significant change versus nonfailing human myocardium; PCR = polymerase chain reaction.

### 5.3. Phospholamban in heart failure

There is little data available on phospholamban expression in animal models of myocardial failure. In left ventricular endomyocardial biopsies from dogs with pacing tachycardia-induced heart failure no change in phospholamban mRNA levels was observed at the onset of clinical heart failure compared to baseline [68]. In contrast, in the guinea pig failure model due to descending thoracic aorta banding, a decrease in phospholamban protein levels was associated with the development of overt clinical heart failure [67].

Previous reports on alterations in mRNA and protein levels in failing versus nonfailing human hearts are given in Table 5. While a decrease in phospholamban mRNA levels has been consistently observed, only one study showed a small decrease in phospholamban protein levels relative to total protein in failing dilated cardiomyopathy [34]. However, when phospholamban was normalized to caldesquestrin, no difference existed between failing and nonfailing myocardium [34].

Interestingly, in the study by Meyer et al. SR-Ca<sup>2+</sup>-ATPase protein levels were decreased to a greater proportion than protein levels of phospholamban in the failing myocardium [34]. If we assume that the stoichiometry of phospholamban to SR-Ca<sup>2+</sup>-ATPase determines the level of SR-Ca<sup>2+</sup>-ATPase inhibition, this finding may indicate that in the basal low phosphorylated state inhibition of SR-Ca<sup>2+</sup>-ATPase is more pronounced in the failing compared to nonfailing human myocardium [80]. This could be one possibility to explain the finding of reduced activity of SR-Ca<sup>2+</sup>-ATPase in failing compared to nonfailing human myocardium [75].

## 6. Sarcolemmal calcium pump

The sarcolemmal calcium pump is not homologous with the SR-Ca<sup>2+</sup>-ATPase and has a different ATP to calcium stoichiometry [81]. Rate of calcium transport by this pump seems to be small compared to that of SR-Ca<sup>2+</sup>-ATPase, and therefore, this pump is not considered to quantitatively

contribute to beat to beat calcium elimination and myocardial relaxation [82]. Data on expression or function of the sarcolemmal calcium pump in human myocardium are not available.

## 7. Sarcolemmal sodium-calcium exchanger

The Na<sup>+</sup>-Ca<sup>2+</sup>-exchanger is the dominant myocardial calcium efflux mechanism which contributes significantly to relaxation. The Na<sup>+</sup>-Ca<sup>2+</sup>-exchanger extrudes one calcium ion for three sodium ions using the electrochemical sodium gradient (for review see [83] and [84]). In this mode, it produces a net movement of charge resulting in a net inward current. The Na<sup>+</sup>-Ca<sup>2+</sup>-exchanger is also voltage dependent and can reverse its mode during the action potential (for review see [83] and [85]). Under experimental conditions with high intracellular sodium levels, the Na<sup>+</sup>-Ca<sup>2+</sup>-exchanger can promote calcium influx sufficiently to induce excitation-contraction coupling [86]. There are at least three different genes coding for different Na<sup>+</sup>-Ca<sup>2+</sup>-exchanger molecules and a number of splice variants has been described (for review see [83]). Whether or not an isoform shift occurs in hypertrophied or failing myocardium is not known.

### 7.1. Na<sup>+</sup>-Ca<sup>2+</sup>-exchanger in animal models of failure

During normal cardiac development, reciprocal changes in expression of sarcolemmal Na<sup>+</sup>-Ca<sup>2+</sup>-exchanger and SR-Ca<sup>2+</sup>-ATPase have been described [87]. In both rat and rabbit myocardium, exchanger mRNA levels peak near birth and decline postnatally [87,88].

Little data is available on alteration of the Na<sup>+</sup>-Ca<sup>2+</sup>-exchanger in animal models of hypertrophy and failure. In rat cardiac hypertrophy, a decreased activity as assessed by Na<sup>+</sup>-dependent <sup>45</sup>Ca<sup>2+</sup> transport was observed by Hanf et al. [89]. In contrast, from electrophysiological measurements in the cardiomyopathic Syrian hamster, enhanced activity of the Na<sup>+</sup>-Ca<sup>2+</sup>-exchanger was suggested [90].

Recent studies indicate that Na<sup>+</sup>-Ca<sup>2+</sup>-exchanger protein levels are significantly increased in failing my-

ocardium from dogs with pacing tachycardia-induced heart failure [69].

### 7.2. $\text{Na}^+\text{-Ca}^{2+}$ -exchanger in human heart failure

While preliminary data from pooled human hearts did not reveal significant changes of  $\text{Na}^+\text{-Ca}^{2+}$ -exchanger mRNA levels in failing hearts [91], Studer et al. showed that mRNA as well as protein levels are significantly increased in the failing human heart [73]. This finding was recently confirmed by Flesch et al. [92]. Accordingly, it was shown that  $\text{Na}^+\text{-Ca}^{2+}$ -exchange activity is increased in myocardium from failing hearts [93]. Functional relevance of increased  $\text{Na}^+\text{-Ca}^{2+}$ -exchanger expression is evident from a recent study showing that diastolic performance of failing human myocardium correlates with protein levels of  $\text{Na}^+\text{-Ca}^{2+}$ -exchanger [94].

## 8. Interpretation of the data available on calcium-regulatory proteins in heart failure

### 8.1. Comparison of different studies

A great number of functional studies have suggested that altered calcium handling may be of significant relevance for the pathophysiology of myocardial failure in various animal models as well as in human heart failure. However, studies on alterations of calcium-regulatory proteins have been rather controversial. At least five variables may underlie these discrepant findings. First, pathophysiology of myocardial failure clearly is different between the various heart failure models. Quite obviously, subcellular defects underlying myocardial failure in the inherited cardiomyopathy of the Syrian hamster or subsequent to doxorubicin toxicity may be different from models of pressure or volume overload or tachycardia pacing-induced heart failure. The second variable of note is the molecular level which is studied. Steady-state mRNA levels cannot necessarily be assumed to be representative of protein levels, in particular because both mRNA and protein synthesis or degradation may be altered in the failing heart [33,74,75]. Furthermore, protein levels do not necessarily change in parallel with ligand binding data. This was demonstrated in a recent study showing unchanged RyR protein levels in failing human myocardium but a two-fold increase in the number of high affinity [ $^3\text{H}$ ]ryanodine binding sites. It was suggested that this discrepancy may be due to regulatory factors which may modulate the RyR [33]. Along this line, alterations in protein function may occur without changes in protein levels or ligand binding data. This may result from altered stoichiometry of a calcium handling protein and a regulatory protein, i.e. changes of protein levels of the  $\text{SR-Ca}^{2+}\text{-ATPase}$  relative to its regulatory protein phospholamban [34]. Alternatively, alterations in function may result from isoform shifts or other modifications of

the protein. Of note, in a study by Schwinger et al., although protein levels of  $\text{SR-Ca}^{2+}\text{-ATPase}$  were unchanged, activity of the pump and SR calcium uptake were decreased in the failing human heart [75]. The third important variable within one specific failure model is the time point at which measurements are performed: In the Syrian hamster cardiomyopathy model the number of L-type calcium channels seems to depend on the age of the animals [5]. In the pacing tachycardia dog failure model, [ $^3\text{H}$ ]ryanodine binding was decreased already after 1 day of pacing and remained depressed at similar levels when heart failure became manifest at 4 to 7 weeks [12]. In rats with congestive heart failure due to myocardial infarction,  $\text{SR-Ca}^{2+}\text{-ATPase}$  mRNA levels decreased continuously during 16 weeks after infarction [64]. Finally, in rats and guinea pigs with chronic aortic banding a decrease in  $\text{SR-Ca}^{2+}\text{-ATPase}$  mRNA- or protein levels only occurred in animals with overt clinical signs of heart failure [65,67]. The fourth variable is the nature of the preparation studied: Because of fractionating artefacts, differences between failing and nonfailing myocardium may depend on whether crude homogenates or partially purified membrane preparations are studied. Accordingly, in the study by Schwinger et al,  $\text{SR-Ca}^{2+}\text{-ATPase}$  activity and SR calcium uptake were decreased in crude membrane preparations but not in isolated SR vesicles from failing compared to nonfailing human hearts [75]. Furthermore, the region from which the sample is obtained may be of great relevance. This is obvious from a recent study showing that a transmural gradient of  $\text{SR-Ca}^{2+}\text{-ATPase}$  mRNA and protein levels exists in the failing human heart with lower levels in the subendocardial region [95]. The fifth variable, which is of critical relevance for studies in human heart failure, is the clinical situation of the patient: This includes etiology of the disease, duration of heart failure, course of the disease, accompanying diseases, and drug therapy. It seems mandatory that these factors are carefully considered when different studies are compared or when new studies are designed.

### 8.2. Functional relevance of altered calcium-regulatory proteins for the pathophysiology of human heart failure

The pathophysiology of heart failure includes altered function at the level of myocardium, chamber remodeling, neurohumoral activation as well as altered loading conditions. The contribution of each of these factors to the clinical syndrome of heart failure may vary depending on the etiology and severity of the disease as well as on therapeutic interventions. At the level of the myocardium, disturbed SR function seems to play a central role for the altered systolic and diastolic performance. Under physiological conditions calcium released from the SR is the dominant source for systolic activation of contractile proteins. Diastolic relaxation depends on calcium removal

from the cytosol by the SR and the sarcolemmal  $\text{Na}^+$ - $\text{Ca}^{2+}$ -exchanger. We assume that the crucial defect of EC-coupling is a decreased capacity of the SR to accumulate calcium. This may result from a decreased number of calcium pumps [34,35,73,76], an increased phospholamban inhibition of the pump [34] or a decreased activity of the pump by other mechanisms [75]. In addition, because SR- $\text{Ca}^{2+}$ -ATPase competes with  $\text{Na}^+$ - $\text{Ca}^{2+}$ -exchanger for calcium, increased  $\text{Na}^+$ - $\text{Ca}^{2+}$ -exchanger activity may contribute to decrease SR calcium accumulation [73,92,93]. The decreased capacity of the SR to accumulate calcium may be of minor relevance at low heart rates. However at higher heart rates — with a decreased time available for calcium transport — SR calcium accumulation may become inadequate, calcium release from the SR may decline and systolic activation of contractile proteins may decrease [96,97]. This is consistent with the findings of an altered force-frequency relation, a frequency-dependent decrease in calcium transients, and a decreased post-rest potentiation in the failing human heart [76,98–101]. Furthermore disturbed SR calcium loading at higher heart rates in failing compared to nonfailing human myocardium has been shown by studying rapid cooling contractures [102]. A second consequence of decreased SR function is diastolic calcium accumulation which may result in diastolic activation of contractile proteins and disturbed diastolic function. Because SR- $\text{Ca}^{2+}$ -ATPase and  $\text{Na}^+$ - $\text{Ca}^{2+}$ -exchanger work in concert regarding removal of calcium from the cytosol, increased activity of the  $\text{Na}^+$ - $\text{Ca}^{2+}$ -exchanger may compensate for decreased SR calcium transport and preserve diastolic function. Accordingly, it was shown that in failing human myocardium diastolic function is correlated with protein levels of  $\text{Na}^+$ - $\text{Ca}^{2+}$ -exchanger [94]. Of course, calcium eliminated across the sarcolemma by  $\text{Na}^+$ - $\text{Ca}^{2+}$ -exchanger is no longer available for systolic activation of contractile proteins. Furthermore, the exchange of one calcium ion for three sodium ions results in an inward current which may cause electrical instability. Therefore, alteration of calcium handling with increased activity of  $\text{Na}^+$ - $\text{Ca}^{2+}$ -exchanger relative to SR- $\text{Ca}^{2+}$ -ATPase may contribute to disturbed myocardial function as well as to the development of arrhythmias in patients with heart failure.

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