Something old, something new: Changing views on the cellular mechanisms of heart failure

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See article by Fauconnier et al. [84] (pages 204–212) in this issue.

1. Introduction

Despite improvements in therapy, mortality in heart failure (HF) remains high, and there is a need for alternative and additional approaches [1,2]. The possibility of replacement and/or supporting the failing heart with new cardiomyocytes from stem cells, implanted or recruited locally, is one of the exciting possibilities under investigation [3]. Other approaches aim at improving the function of the failing myocytes. Although the temporal and causal relationship between the development of chronic HF and myocyte dysfunction remains unclear [4], there is little doubt that at the time of advanced HF, the isolated myocytes have reduced contractility, in particular under normal physiological stress, i.e. at physiological heart rates and in the response to adrenergic stimulation. The first trials with chronic inotropic therapy, however, had a very poor outcome, and the idea was abandoned for quite a long time [5]. However, new ideas to improve contractility of the myocytes are growing from recent insights into the cellular mechanisms underlying the contractile dysfunction and the link to signals for remodeling. In this editorial, we will briefly review the background of excitation–contraction coupling and changes in heart failure against which these views developed and discuss some future perspectives.

2. Excitation–contraction coupling in the normal heart

The process of excitation–contraction coupling has been reviewed extensively and is schematically illustrated in Fig. 1 [6,7]. Central in the link between membrane depolarization and contraction is the transient elevation of \([\text{Ca}^{2+}]_i\), or \([\text{Ca}^{2+}]_r\), transient.

During each cardiac cycle a certain amount of \(\text{Ca}^{2+}\) enters the cell through voltage-activated \(\text{Ca}^{2+}\) channels, with a small additional amount entering through the Na/Ca exchanger, depending on the \([\text{Na}^+]_i\). This \(\text{Ca}^{2+}\) acts as a trigger to activate the \(\text{Ca}^{2+}\) channel in the sarcoplasmic reticulum, the ryanodine receptor (RyR), and more \(\text{Ca}^{2+}\) is released from the sarcoplasmic reticulum, which is the major source of \(\text{Ca}^{2+}\) to activate the myofilaments, with a ratio of about 10:1 for release to influx [8,9]. As \(\text{Ca}^{2+}\) channels inactivate and RyRs close, \(\text{Ca}^{2+}\) is removed from the cytosol by re-uptake into the sarcoplasmic reticulum by the ATP-driven \(\text{Ca}^{2+}\) pump, SERCA, and by efflux through the Na/Ca exchanger. To maintain a steady state, the same amount of \(\text{Ca}^{2+}\) that entered via \(\text{Ca}^{2+}\) channels, and via reverse Na/Ca exchange, has to be removed from the myocyte within the same cardiac cycle. Most evidence indicates that the Na/Ca exchanger is the major extrusion pathway for maintaining this beat-to-beat balance [10,11].

Underlying these global events are local control processes in the dyadic cleft between the sarcolemma containing \(\text{Na}^+\) and \(\text{Ca}^{2+}\) channels, Na/Ca exchanger, and Na/K-ATPase, and the sarcoplasmic reticulum (SR) membrane containing the RyR. \(\text{Ca}^{2+}\) concentrations in this space, coined the ‘fuzzy’ space [12], can deviate substantially from the global cytosolic concentrations during periods of large
ion fluxes [13–15], and Na⁺ most likely as well (reviewed in [16,17]). Ca²⁺ influx during opening of the L-type Ca²⁺ channel can thus elevate [Ca²⁺] sufficiently for activation of the RyR. RyR gating is also dependent on the intra-SR or lumenal [Ca²⁺]. A higher [Ca²⁺]SR not only provides a larger driving force, but also increases the RyR open probability (reviewed in [18]).

Alterations in the amplitude of the [Ca²⁺] transient, and of the subsequent contraction, result from changes in the triggering of Ca²⁺ release and in the amount of Ca²⁺ available in the SR for release. Phosphorylation of several key proteins can physiologically regulate both these steps, the best-known example being β-adrenergic stimulation in which both the triggering L-type Ca²⁺ current and the available Ca²⁺ are increased [19].

3. Global changes of contractile function in HF

The cellular mechanisms responsible for the contractile deficit in end-stage heart failure have become rather well defined [20,21]. Ca²⁺ release from the sarcoplasmic reticulum is reduced, and the [Ca²⁺], transient is unable to fully activate myofilaments. This has been shown in tissue from human hearts at the time of transplantation [22–24], and the reduction in Ca²⁺ release was seen in several animal models of overt heart failure, including the dog with pacing-induced heart failure [25] and heart failure following combined aortic stenosis and insufficiency in the rabbit [26]. Animal models of heart failure after extensive myocardial infarction have shown more heterogeneous results, probably reflecting the heterogeneity of remodeling in this condition [27]. Data obtained in skinned myocytes isolated from human tissue indicate that the myofilament response is possibly even enhanced [28], supporting the importance of the regulation of activating Ca²⁺. It is to be noted, however, that in ischemic cardiomyopathy, alterations in myofilament density or phosphorylation may result in a decreased maximal force response or reduced sensitivity for the available Ca²⁺ [29]. This will then further enhance the effect of reduced Ca²⁺ availability.

One of the most striking properties of the failing heart is that the contractile deficit is strongly dependent on heart rate. Early reports did not describe a reduction in the amplitude of the [Ca²⁺], transient in vitro, as they were
recorded at very low frequencies of stimulation. However, with increasing rate within the physiological stimulation frequencies, the contraction amplitude and the underlying \([\text{Ca}^{2+}]_{i}\) transient are severely reduced \([30,31]\). This negative force frequency relationship (FFR) seems to be a hallmark of the failing heart, and is well described in studies on human tissues \([32]\). It is also present in animal models \([26,33]\).

Two major mechanisms could account for the reduced release: a failure of activation of the \(\text{Ca}^{2+}\) release channel, which could be described as defective coupling, and/or insufficient availability of \(\text{Ca}^{2+}\) within the SR (Fig. 2).

4. Regulation of sarcoplasmic reticulum \(\text{Ca}^{2+}\) release in HF

4.1. Triggers for activation of the RyR

The \(\text{Ca}^{2+}\) release channel of the SR is activated by \(\text{Ca}^{2+}\) influx, with the major pathway in the normal heart being the L-type \(\text{Ca}^{2+}\) channel. The \(\text{Ca}^{2+}\) current density in human myocytes was found to be unchanged although there were data from binding studies that suggested a decrease in channel number (reviewed in \([34]\)). A study on the properties of single channels, however, suggested an increased activity \([35]\), which was reminiscent of the effect of increased adrenergic drive. Together with an unchanged whole-cell current, these findings would suggest that the number of channels was decreased but with higher activity. More recent data also indicated that the baseline current density was similar in control and failing myocytes \([36]\). In this study, the response to adrenergic stimulation was decreased, and this persisted even when the cAMP concentration was directly increased, thus bypassing defects in the \(\beta\)-adrenergic receptor function. These findings are compatible with a higher baseline degree of phosphorylation of the \(\text{Ca}^{2+}\) channel.

Animal studies have suggested that the actual interaction of the \(\text{Ca}^{2+}\) channel with the RyR could be defective, leading to a reduced coupling ‘gain’ of \(\text{Ca}^{2+}\) influx and \(\text{Ca}^{2+}\) release, as reported for the SHR with HF \([37]\) and the rat with MI \([38]\). This has not been reported in other animal models of heart failure, but the mechanism may be difficult to verify as it needs a rigorous evaluation of the gain or efficiency of coupling between the \(\text{Ca}^{2+}\) influx and the

![Fig. 2. Changes with heart failure. The decrease in \(\text{Ca}^{2+}\) release from the sarcoplasmic reticulum is the result of changes in the triggering process (defective coupling) and of a decrease in available \(\text{Ca}^{2+}\). Several individual transporters are involved, and their function is regulated through phosphorylation, involving kinases and phosphatases as indicated. See text for details and references.](image-url)
Ca\textsuperscript{2+} release flux. The concept of gain has been (mis)used to describe any change in Ca\textsuperscript{2+} release for a similar trigger, i.e. Ca\textsuperscript{2+} current, but in its strictest sense should describe the change in Ca\textsuperscript{2+} release for a similar trigger and available SR Ca\textsuperscript{2+}, i.e. reflecting the coupling between Ca\textsuperscript{2+} channels and RyR. In the presence of altered filling capacity and Ca\textsuperscript{2+} content of the SR, this is difficult to establish, although not impossible (see, e.g., [39] for gain under \beta-adrenergic stimulation). Reduced coupling efficiency could be due to altered properties of the RyR (see below), but could also be due to a physical barrier, as suggested in the original report. More recently, He et al. described a reduction in T-tubule density in the dog with pacing-induced heart failure [40,41]. This was accompanied by a loss of Ca\textsuperscript{2+} channels and could also contribute to reduced coupling efficiency. We recently showed that loss of T-tubules results in a loss of synchronization at the subcellular level, which would translate into an apparent reduced Ca\textsuperscript{2+} release flux at the whole-cell level [42]. Though it remains to be determined whether this is important in human end-stage heart failure, we have preliminary data on such changes in chronic ischemic heart disease in a pig model [43].

The frequency-dependence of the Ca\textsuperscript{2+} current can also contribute to the negative FFR [44].

4.2. Properties of the RyR

Early studies of RyR focused primarily on the levels of expression. Some reported a reduction, whereas others saw no change (reviewed in [45]). More recently, the attention has shifted towards the regulation of RyR function through phosphorylation [46]. In vitro, phosphorylation of RyR leads to dissociation of FK506BP, which enhances the open probability by increasing the Ca\textsuperscript{2+} sensitivity [47]. Enhanced open probability of RyR could translate as ‘leaky’ channels, with on the one hand loss of Ca\textsuperscript{2+} from the SR during diastole and decreased availability for release during the action potential, and on the other hand a more trigger-happy channel that could lead to potentially arrhythmogenic Ca\textsuperscript{2+} release. Several studies have reported increased phosphorylation at the Ser\textsuperscript{2809} site [48], the site that is postulated to be under control of PKA, but not all [49,50]. More importantly, there are some controversies about the impact of this finding, as functional evidence for a leaky channel in intact cells has been hard to obtain [51]. Experiments in non-failing mouse myocytes could not detect increased spontaneous RyR activity after phosphorylation of RyR [52]. Recently, Shannon et al. devised a specific protocol to measure the SR leak in intact cells and found an increase of SR Ca\textsuperscript{2+} leak in a rabbit model for heart failure [53]. They pointed out, however, that this is only one of several changes in this model that contributes to the reduced SR Ca\textsuperscript{2+} release and contractile defect in heart failure.

The Ser\textsuperscript{2809} is also phosphorylated by Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II (CaMKII), but there are additional CaMKII sites [54]. The RyR can also be phosphorylated at the Ser\textsuperscript{2815} site by CaMKII, and this site could be the more relevant one for regulation of function [55,56]. In addition to regulation by kinases, phosphatase activity is an important determinant of the phosphorylation status, and PP2a as well as PP1 appear to be associated with the RyR [47].

If the hyperphosphorylated RyR is more sensitive to activating Ca\textsuperscript{2+}, this cannot account for a potential reduction in coupling efficiency between Ca\textsuperscript{2+} influx and Ca\textsuperscript{2+} release, as it would on the contrary enhance the response. Only indirectly, through a reduction in SR Ca\textsuperscript{2+} content, could RyR hyperphosphorylation lead to reduced Ca\textsuperscript{2+} release. Other regulatory proteins associated with the RyR such as calsequestrin, sorcin, triadin and junctin [57] are also candidates to affect the gain of Ca\textsuperscript{2+} release, but their potential role in heart failure is currently not clear.

4.3. Ca\textsuperscript{2+} availability in the SR and the role of SERCA—not only the numbers count

A potential reduction in the expression of SERCA or number of these pumps was one of the first molecular changes studied in human heart failure [58]. The interest in SERCA stemmed from the observations that in heart failure, diastolic dysfunction and slowed relaxation were one of the consistent changes, even more so than systolic dysfunction. In several subsequent studies, SERCA expression was measured at the mRNA and protein levels, or activity was measured during Ca\textsuperscript{2+} uptake studies in SR vesicles. From a review of the available data, it is clear that in end-stage human heart failure a reduction in SERCA is a very common finding, even if not in all studies [45]. Overexpression of SERCA in myocytes also enhances both the rate of relaxation and the amount of Ca\textsuperscript{2+} release [59].

In animal studies, there is less consistency. Again, a reduction in SERCA expression is seen very often, but not always. In the rabbit with heart failure due to mixed pressure/volume overload, e.g., the reduction is very modest [60].

An important factor in SERCA function is its regulation by phospholamban (PLN). An increase in PLN with reduction of the ratio SERCA/PLN could potentially also reduce SR Ca\textsuperscript{2+} uptake [61]. The expression levels of PLN have not been studied as extensively as the SERCA levels, but the available results are mixed, without a clear trend to change [45,61]. On the other hand, when in human failing myocytes the SERCA/PLN ratio was increased by suppression of PLN, a functional improvement was observed [62]. The results from PLN ablation in mouse models of heart failure are more mixed, with rescue in some, but not in all models (reviewed in [61]). However, PLN expression levels and stoichiometry are insufficient to evaluate the regulation of SERCA, as the level of inhibition of SERCA by PLN depends on the degree of phosphorylation [61]. A decrease in the level of PLN phosphorylation at Ser\textsuperscript{16} was shown in human heart failure [63] and some experimental
animal models [64], prompting further study of the regulation of the SR-associated PLN phosphorylation. Increased activity of the SR-associated phosphatase PP1, which dephosphorylates PLN, had already been reported [64,65], and overexpression of PP1 in transgenic mice resulted in heart failure [66]. PP1 activity was further shown to be regulated by the inhibitor I-1 [67], and I-1 was found to be reduced in human heart failure [66]. Pathak et al. also found that the I-1 activity in human heart failure was decreased, and showed that in mice, expression of constitutively active I-1 prevented the development of heart failure following aortic constriction [68]. Interestingly, overexpression of I-1 did not affect the degree of phosphorylation of RyR or TnI. These findings suggest that in heart failure the lower degree of PLN phosphorylation is due to hyperactivity of PP1 because of reduced inhibition by I-1.

In contrast to these findings indicating a role for excessive inhibition of SERCA by PLN in heart failure, some studies have reported an increase in PLN phosphorylation in certain animal models of heart failure [69,70]. In this case, it could be argued that the high level of baseline phosphorylation limits the response to increased demand for phosphorylation such as during adrenergic stimulation; increased Thr17 phosphorylation (also reported in [68]) may limit adaptation to higher frequencies of stimulation. The observation of the association of dilated cardiomyopathy with a null mutation in the PLN gene can perhaps be explained by similar mechanisms [71], and cautions against generalizing beneficial effects of PLN ablation observed in mouse models.

### 4.4. Na/Ca exchange—interaction with SERCA

Na/Ca exchange (NCX) activity is usually thought to be increased in heart failure, although the changes are not always consistent. With reduced SERCA activity and or increased PLN inhibition, forward mode and Ca$^{2+}$ extrusion can shunt Ca$^{2+}$ away from the SR and contribute to the lower SR Ca$^{2+}$ load. Changes in NCX have been studied extensively (reviewed in [72–74]) but less is known about a role for phosphorylation in regulation of its activity. Phosphorylation of NCX by PKA or PKC in healthy cells probably increases activity (reviewed in [75]). In a pig model of heart failure following rapid ventricular pacing, increased NCX activity and decreased responses to PKA phosphorylation strongly indicated a higher level of baseline phosphorylation [76]. Recent data obtained in the rat heart underscore the potential for modulation by PKA-dependent phosphorylation and the association of PKA, PKC, PP1 and PP2a with the NCX [77].

### 4.5. The Ca$^{2+}$-arrhythmia connection

Changes in Ca$^{2+}$ regulation during hypertrophy and heart failure have been linked to the increased incidence of arrhythmias and sudden death (see, e.g., [78–80] and review [81]). A major mechanism could be increased NCX exchange current, which would act both on the action potential plateau and duration and on delayed afterpolarizations at the diastolic membrane potential [72,74,82]. Loss of I$_{K1}$ as a factor enhancing excitability in heart failure was proposed earlier [78,83], but until now not directly linked to changes in Ca$^{2+}$ handling. A novel hypothesis to explain reduction of I$_{K1}$ in a rat model of heart failure after MI is proposed by Fauconnier et al. in the current issue of *Cardiovascular Research* [84]. In this animal model, the observed reduction in I$_{K1}$ is linked to a diastolic increase in Ca$^{2+}$ that is putatively ascribed to ‘leaky’ RyR. Similar effects on I$_{K1}$ were obtained by application of FK506 to healthy myocytes, mimicking dissociation of FK506BP from the RyR with hyperphosphorylation in failing myocytes. This hypothesis is innovative and could also help to explain arrhythmogenesis in the congenital PVT syndrome [85]. This indirect effect on I$_{K1}$ would reinforce any effect of a diastolic leak of Ca$^{2+}$ on NCX current. This hypothesis deserves and needs further study. In particular, as pointed out also by the authors, the functional evidence and role for the diastolic leak of Ca$^{2+}$ due to RyR phosphorylation is as yet still limited.

### 5. Emphasis on modulated activity of Ca$^{2+}$ transporters, not expression

The view on the mechanisms underlying contractile dysfunction in heart failure thus still centers on decreased and altered Ca$^{2+}$ release from the sarcoplasmic reticulum. However, whereas some years ago emphasis was on the alterations in protein expression, it is now clear that altered function of Ca$^{2+}$ handling proteins to a large extent can result from changes in phosphorylation status. Increased kinase activity as well as increased phosphatase activity is involved, through excessive adrenergic stimulation and PKA activity, through CaMKII activation, and possibly by PKC; some proteins have increased phosphorylation (RyR, possibly NCX), while others show decreased phosphorylation (PLN). The importance of the phosphorylation mechanism is also underscored by the association between partial recovery of the abnormal phosphorylation under therapy in parallel with functional recovery [36,86].

An important concept that has emerged from these recent studies is the co-localization of kinases and phosphatases in large protein complexes. This concept has been developed extensively for the RyR [47] and is probably more generally valid, as reviewed recently [87]. It could explain discrepancies between phosphorylation in different proteins as, e.g., RyR opposed to PLN. Apparently, I-1 regulates PLN phosphorylation only and not phosphorylation of the RyR or TnI, and this could be explained by a unique association of I-1 and PLN (Fig. 2). For the PKA-dependent regulation of Ca$^{2+}$ channels, PKA forms a complex with the α-subunit through AKAP [88]. Furthermore, compartmentalization of
cAMP via specific distribution of PDE will also contribute to the localization of phosphorylation [89,90].

Kinases and phosphatases are involved in the hypertrophic response and the regulation of gene expression (reviewed in [91]), and include PKA and PKC which also play a role in the altered phosphorylation of Ca\(^{2+}\) transporters. Recent evidence put also CaMKII forward as a central player in development of heart failure [92,93], providing another link between different levels of regulation at the expression level as well as at the phosphorylation level. The activation of different pathways, e.g., CaMKII or CaN, appears to be specific for the stimulus.

6. Conclusions

These developments open the way for alternative and additional therapeutic approaches to heart failure centering on modulation of Ca\(^{2+}\) transport through phosphorylation. Specificity of the target could be achieved through specific, associated components as shown for I-1. On the other hand, the targeting of elements of the signal transduction to prevent and/or reverse cardiac remodeling may at the same time benefit directly the phosphorylation and function of Ca\(^{2+}\) transport. These concepts need to be studied further, in different animal models and conditions, to avoid generalizing findings.

References


