[Frontiers in Bioscience 7, e263-275, May 1, 2002]

ALTERED COMMUNICATION BETWEEN L-TYPE CALCIUM CHANNELS AND RYANODINE RECEPTORS IN HEART FAILURE

Jean-Pierre Bénitah, Benoît Gilles Kerfant, Guy Vassort, Sylvain Richard, Ana María Gómez

INSERM U-390, Montpellier, France

TABLE OF CONTENTS

1. ABSTRACT

Heart failure (HF) is a progressive syndrome that appears as the final phase of most cardiac diseases and is manifested as a decreased contractile function. Contraction in cardiomyocytes arises by the Ca^{2+} induced Ca^{2+} release mechanism, where Ca^{2+} entry (I_{Ca}) through Ca^{2+} channels (DHPRs) activates Ca^{2+} release channels (RyRs) in the junctional sarcoplasmic reticulum (SR). This is the base of cardiac excitation-contraction (EC) coupling. To elucidate the mechanisms underlying depressed function of the failing heart, analysis of EC coupling main elements have been undertaken. I_{Ca} density is usually maintained in HF. However, failing myocytes show a reduced SR Ca^{2+} release. Then, if the trigger of SR Ca^{2+} release is maintained, why is SR Ca^{2+} release depressed in HF? Analyses of the DHPR-RyR coupling efficiency have revealed a decrease in the I_{Ca} efficacy to trigger Ca^{2+} release in failing myocytes. In terminal heart failure without hypertrophy, a decrease in SR Ca^{2+} load can account for the decreased SR Ca^{2+} release. Fewer Ca^{2+} sparks (elementary units of SR Ca^{2+} release) are triggered by an equivalent I_{Ca} in hypertrophied failing myocytes, suggesting a functional or spatial reorganization of the space T-tubule junctionnal SR. This theory is supported by new data showing that the T-tubule density is reduced in failing cells.

2. INTRODUCTION

The heart adapts to work in different situations to ensure adequate blood distribution. This adaptation can be fast, in response to an acute need, i.e. in a stress situation, adrenergic stimulation will have a fast inotropic effect. In response to a sustained pathological stimulus, the heart adapts to adjust cardiac output. In this case, the heart experiments biochemical, electrophysiological and structural changes, globally known as "remodeling" (1), that usually involve hypertrophy. Although hypertrophy is initially beneficial, sustained cardiac hypertrophy is a

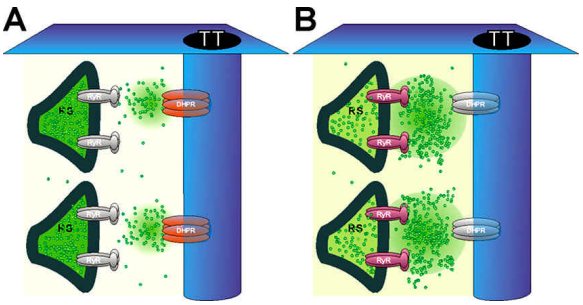

Figure 1. A: Cartoon showing the basis of cardiac excitation contraction coupling. Membrane depolarization during an action potential activates L-type Ca^{2+} channels, DHPRs, B: Ca^{2+} influx increases the local $[Ca^{2+}]_i$ activating neighboring ryanodine receptors, RyRs.

leading predictor for the development of sudden death and heart failure (HF) (2, 3).

For a long time, the process of heart remodeling has been divided in three phases (4). After the first phase ("development", hours to days after the initiating stimuli) the heart hypertrophies to meet the body needs. This period is called "compensated" hypertrophy. The compensation provided by hypertrophy is often limited and the cardiac pump function is compromised. During this new situation of "decompensated" hypertrophy, HF develops. The failing heart function is depressed and has less capacity to respond to acute needs. In systolic HF, the failing muscle pumps out blood with less strength than normal. In diastolic HF, the pump function may be normal, but it cannot relax normally and refilling of the ventricles is impaired. This last situation is characteristic of the HF found in the elderly.

Because Ca^{2+} activates contraction in the cardiac muscle, the major mechanism underlying contractile alterations during cardiac remodeling and its final outcome, heart failure, could be alterations in the Ca^{2+} signaling, or in the contractile proteins itself. We will focus here in collected data regarding Ca^{2+} signaling in heart remodeling.

2.1. Etiology and stage of disease do matter

HF is a progressive syndrome that appears as the final phase of most cardiac diseases. The initiating insult can be acute as in myocardial infarction, or gradual as in hemodynamic load (pressure or volume overload) or as in the case of many hereditary cardiomyopathies. Other acquired diseases, such as viral myocarditis can also induce heart remodeling. One must take into consideration the above discussion when integrating data regarding Ca^{2+} handling collected in different experimental models of hypertrophy and HF and define precisely the stage of the disease. Many apparent contradictory data may just reflect the different stages in the disease progression. Ideally the best tissues to analyze would be human tissues. However, one major difficulty resides in the access to normal hearts, which is highly limited for obvious reasons (5, 6). Only terminally failing hearts obtained from transplants are available. Regardless of the variability in etiology, age and gender, all failing tissues are obtained from patients with long-term medications, which clearly hampers analysis and proper understanding of the disease progression. Consequently, many animal models have been used in an attempt to overcome these problems and better standardize the experimental conditions. The etiology and stage of the pathology can be determined more accurately, and the effect of medication is avoided although one cannot always extrapolate from the many animal models to human heart. Moreover, no animal model reproduces the wide variety of causes and manifestations of clinical syndromes in human. In addition to these limitations, several variables reviewed recently (7) underlie discrepancies found in different animal models: (i) pathophysiology of myocardial failure which varies among models; (ii) the studied molecular level, since discrepancies can potentially exist between mRNA level, protein level and function, both in normal and diseased tissues (differences in terms of protein isoforms, sub-unit assembly, mRNA or protein degradation, regulation); (iii) the stage of the disease and time of measurement; and (iv) the experimental conditions themselves which are a major variable among groups.

3. Ca^{2+} HANDLING IN THE NORMAL HEART

Contraction arises when intracellular free Ca^{2+} level increases following activation by an action potential. Figure 1 shows the bases of cardiac excitation-contraction (EC) coupling. Membrane depolarization during an action potential activates voltage-dependent L-type Ca^{2+} channels (or dihydropyrydine receptors, DHPRs) concentrated in the transverse tubules (TT). Charge carried by Ca^{2+} entry through these channels generates a current referred to as I_{Ca}. The resultant increase in $[Ca^{2+}]_i$ activates Ca^{2+} release channels (or ryanodine receptors, RyRs) in the junctional sarcoplasmic reticulum (SR). This Ca^{2+}-induced Ca^{2+} release (CICR) mechanism is the basis of cardiac excitation-contraction (EC) coupling. By this mechanism, the initial Ca^{2+} signal is greatly amplified, then providing enough Ca^{2+} for contraction. CICR was first proposed by Fabiato (8) in skinned cardiac myocytes. Theoretical calculations on the CICR mechanisms suggested that Ca^{2+} release from the SR is not activated by elevation in the global $[Ca^{2+}]_i$ (common pool theory) but by the *local* increase in $[Ca^{2+}]_i$, taking place in the restricted space between L-type Ca^{2+} channels and RyRs (9). The common pool theory would result in an all-or-none response, while a graded response is expected to result from the local control of EC coupling theory; the latter being physiologically more relevant. This theory was supported by the finding of non-propagating localized elevations of $[Ca^{2+}]_i$ that correspond to the opening of one or few RyRs, named Ca^{2+} sparks (10) (Figure 1C). It was then demonstrated that a single L-type Ca^{2+} channel evokes one Ca^{2+} spark (11-13). Under the local control of EC coupling, the RyRs are relatively insensitive to Ca^{2+}, and only high $[Ca^{2+}]_i$, produced in the vicinity of a L-type Ca^{2+} channel, will be able to activate release through RyRs. Because Ca^{2+} diffuses away rapidly, the $[Ca^{2+}]_i$ sensed by the RyR is dependent on the physical distance from the DHPR (12). Thus, the efficient coupling of EC relies on a tight functional and spatial coupling of DHPRs and RyRs.

Relaxation occurs when cytoplasmic Ca^{2+} is both rapidly extruded from the cell through the Na^{+}-Ca^{2+}

exchanger and pumped back into the SR by the SR Ca^{2+} ATPase (SERCA). The sarcolemmal Ca^{2+} ATPase, different from SERCA, can also extrude some Ca^{2+}. However, its contribution appears to be minor (about 3% of total Ca^{2+} removal) and its physiological significance has yet to be determined. For equilibrium to occur, the amount of Ca^{2+} extruded through the Na^+ -Ca^{2+} exchanger should be equivalent to the amount of Ca^{2+} entering the cell through DHPRs, and the amount of Ca^{2+} transported by SERCA should be equivalent to Ca^{2+} released by the SR. These Ca^{2+}-extruding mechanisms not only allow relaxation but may also be indirectly involved in contraction, since they can modulate the amount of Ca^{2+} stored in the SR available for the next beat.

4. Ca^{2+} HANDLING IN HYPERTROPHIED AND FAILING MYOCYTES

Ca^{2+} release and uptake originate contraction and relaxation. Consequently, alteration of Ca^{2+} handling has been suspected to drive the progression of functional abnormalities in hypertrophy and HF. Numerous analyses over the years have now provided a great body of evidence that Ca^{2+} handling is indeed altered. However, while some alterations are consistent among various models of hypertrophy and HF, some others are found only in failure and not in hypertrophy and they differ with the etiology.

Studies of $[Ca^{2+}]_i$ transient have revealed several abnormalities. In a study comparing $[Ca^{2+}]_i$ transient characteristics in human failing and hypertrophied non-failing hearts, an alteration of Ca^{2+} handling was evident in both, but it was more pronounced in failing hearts (14). This suggests that altered $[Ca^{2+}]_i$ mobilization may develop early in the course of hypertrophy, before the onset of clinical signs of cardiac dysfunction. Abnormalities include decreased systolic Ca^{2+} and slowed decay phase. The slowing of the $[Ca^{2+}]_i$ transient decay phase is perhaps the most consistent alteration (15-18). It has been correlated with a decrease in SERCA activity or expression, which is observed in some cases. However, it seems that contraction kinetics and $[Ca^{2+}]_i$ transient characteristics are not constant in failing human ventricular cells, depending on dilated or ischemic cardiomyopathy etiology (19). These differences may account for the divergence in findings regarding SERCA in failing human myocytes that has been shown to be decreased or unaltered (for review see (20)). These alterations probably become critical at high beating rates because SR Ca^{2+} accumulation during shorter diastolic period may become dramatically inadequate to provide sufficient Ca^{2+} during systole. This process contributes largely to the negative force-frequency relationship reported in the failing human heart (21-23). Interestingly, after enhancing SERCA activity by either knocking out phospholamban or overexpressing SERCA, HF is prevented or rescued, respectively (24-27). Besides alterations in relaxation, the lengthening of the $[Ca^{2+}]_i$ transient decay could be responsible for lowering SR Ca^{2+} contents at higher frequencies and for frequency-dependent facilitation of I_{Ca} (see later). At the end of the action potential (AP), the Na^+-Ca^{2+} exchanger (NCX) can work in reverse mode favoring Ca^{2+} entry, maintaining the $[Ca^{2+}]_i$ transient and slowing relaxation. Thus, it has been proposed that the increased activity of the NCX can contribute to the decreased $[Ca^{2+}]_i$ transient decay (28).

Besides slowed kinetics, $[Ca^{2+}]_i$ transient amplitude seems modified in the remodeled heart, although different models have provided conflicting data. An increase of the SR Ca^{2+} release has been reported in models of early cardiac hypertrophy, together with hyperactivity, in spontaneous hypertensive rats (29-31). The increase in SR Ca^{2+} release during early stages of hypertrophy development has been correlated with longer AP duration (32) while SR Ca^{2+} load is maintained (31). Some other models of hypertrophy have shown normal $[Ca^{2+}]_i$ transient amplitude (33, 34), that can be reduced under some challenges, such as increase (35) or decrease (36) of extracellular $[Ca^{2+}]$. However, most analyses of the $[Ca^{2+}]_i$ transient in tissue or isolated myocytes from failing hearts have evidenced a reduced capacity of the SR to release Ca^{2+} (37-46). Taken together, these data strongly suggest that SR Ca^{2+} release changes with the state of the heart adaptive response. In agreement with this notion, cell shortening and $[Ca^{2+}]_i$ transient at two time points during autoimmune myocarditis in mice were normal at day 18^{th} but reduced at day 35^{th} (47). These observations suggest that the $[Ca^{2+}]_i$ transient is progressively reduced with disease and that the weak contraction in failing myocytes may be due, at least in part, to a smaller SR Ca^{2+} release. Now, the challenge is to determine the molecular mechanisms that weaken the $[Ca^{2+}]_i$ transient in failing hearts.

Theoretically, a decrease either in the triggering Ca^{2+} or in the EC coupling efficiency could account for reduced cellular contraction in HF. Defects may result from altered expression or function of proteins involved in Ca^{2+} homeostasis. There is indeed general agreement that disturbed SR function plays a significant role in the altered systolic and diastolic performance in human as well as in various animal models (48). To determine the defect(s) underlying the failure, investigators have long analyzed each of the elements involved in EC coupling. Here, we will overview these elements both in human and animal models of HF, focusing particularly on I_{Ca} and SR Ca^{2+} release. Simultaneous measurements of both I_{Ca} and SR Ca^{2+} release provide valuable information on the effectiveness of EC coupling.

4.1. Triggers of SR Ca^{2+} release

Calcium influx in cardiac myocytes is the initiating event in the EC coupling process. The sarcolemmal Ca^{2+} entry system includes two major proteins, namely the Na^+-Ca^{2+} exchanger and Ca^{2+} channels. Under certain circumstances, Ca^{2+} might also permeate through Na^+ channels and activate SR Ca^{2+} release (49). Activation of this slip-mode conductance in failing myocytes can improve Ca^{2+} signaling (40, 46).

4.1.1. Na^+-Ca^{2+} exchanger

The Na^+-Ca^{2+} exchanger (NCX) is a cation transporting protein. It exchanges 3 Na^+ for 1 Ca^{2+} (50, 51) in either way. Although the normal "forward" mode is Ca^{2+}

extrusion, which favors relaxation after each twitch, it can also work in the "reverse" mode inducing Ca^{2+} entry and thus probably triggering SR Ca^{2+} release (52), although with less efficiency than Ca^{2+} channels (53). In cardiac hypertrophy and HF, expression of the NCX seems altered. Increases in its mRNA and protein levels have been reported in human (54) and experimental (55) HF, although the functional meaning is controversial. For instance, Wang et al. (56) have confirmed the increase in NCX exchanger expression in a mice hypertrophy model but paradoxically the NCX current, I_{NCX}, was decreased. However, most investigators have found an increase in I_{NCX} both in hypertrophied and failing hearts (57-60). From an electrophysiological point of view, the increase in I_{NCX} may be arrhythmogenic (61); however, it is not clear whether the increase in NCX function is adaptive or deleterious for the Ca^{2+} handling of the hypertrophied and failing cell. In fact, because the NCX can work in both directions, it may be involved both in contraction and in relaxation (28). It seems that the increase in NCX function would be compensatory in cell relaxation. In human failing hearts with normal diastolic function, the NCX was upregulated while in hearts with impaired diastolic function the NCX expression was normal (62). Regarding the systolic function, a compensatory role has been proposed in compensatory hypertrophy, but the issue is more complicated. The underlying mechanism of the increased contraction would be an increase in SR Ca^{2+} load, favored by the *reverse* mode of the up regulated NCX (59). However in HF, up-regulation of the NCX could be deleterious because, working in *forward* mode would tend to decrease SR Ca^{2+} content (63). In summary, there is strong evidence that the NCX is up regulated both in hypertrophy and HF, but the physiopathologic consequences are unclear (64).

4.1.2. Ca^{2+} channels

Ca^{2+} channels open transiently in response to depolarization and serve as the major pathway for Ca^{2+} entry into the myocytes. They permeate Ca^{2+} driven by its electrochemical gradient in a time- and voltage-dependent manner. In the myocardium, the coexistence of at least two populations of voltage-dependent Ca^{2+} channels (L-type and T-type) is well established. The T-type Ca^{2+} current ($I_{Ca,T}$) has not been found in all mammalian species investigated, including human, but in the species where it is present, the efficacy of $I_{Ca,T}$ to trigger SR Ca^{2+} release is much weaker than that of the L-type I_{Ca} (65).

The L-type Ca^{2+} channels (DHPRs) have been linked to the plateau phase of the action potential and play a crucial role in EC coupling. Initially termed 'slow inward current' (66, 67), I_{Ca} activates at depolarizations positive to –40 mV, peaks near 0 mV and declines gradually at more positive voltages. Decay kinetics of the current include a fast Ca^{2+} -dependent component and a slow voltage-sensitive component. At the single channel level, this channel occupies three different basic modes: no opening or mode 0, short opening or mode 1 and long opening or mode 2 (68). The DHPR is constituted by a pore-forming subunit (α1c subunit), associated with auxiliary transmembrane subunits (α2-δ subunit) and cytoplasmic regulatory subunits (β subunit) (69).

Many studies of I_{Ca} in hypertrophy and HF have been conducted but the results remain ambiguous (reviewed in (6, 70-73)). When investigating Ca^{2+} entry via I_{Ca} at least two parameters should be examined: (i) peak amplitude because it grades Ca^{2+} release from the SR; and, (ii) the decay kinetics because inactivation, which terminates Ca^{2+} entry, is likely to influence the AP repolarization. Regarding I_{Ca} density, the general trend is that cardiac hypertrophy ranges from no change to significant increase, whereas HF ranges from no change to significant decrease. It was thus tentatively proposed that I_{Ca} density would increase at early stages, be normalized during compensated hypertrophy, to finally decrease in late stage failure (70). This hypothesis was first postulated by Scamps et al. (74) to reconcile the unmodified I_{Ca} density observed in hypertrophied myocytes with the increase shown in hypertensive non-hypertrophied myocytes (75). This hypothesis predicts a decrease of I_{Ca} in severe HF such as human samples (always at the end-stage). However, in most studies of human failing myocytes and in animal models, the density of I_{Ca} is unchanged (6).

Both amplitude and kinetics of I_{Ca} may in part contribute to AP shape (76). Even if I_{Ca} peak density is maintained, slowing of its inactivation has been described in HF (reviewed in (73)) that might contribute to AP prolongation. Increased net Ca^{2+} entry would consequentially tend to enhance SR Ca^{2+} load and increase contraction.

Whole-cell current depends on both, number and individual properties of functional channels. Therefore, even if the macroscopic current seems unchanged, any modification of individual channel activity may be important in cardiac hypertrophy and HF. In human HF, Ca^{2+} channels were shown to switch activity from mode 1 to mode 2, showing higher availability and open probability (77). Since only one study analyzed single channel properties during human HF, one cannot definitively conclude whether this reflects a general feature. Moreover, extrapolation to whole-cell currents is difficult. In particular, one would expect larger I_{Ca} in HF cells. Surprisingly, the same study showed unchanged whole-cell I_{Ca}. One possibility to reconcile this disparity would be that HF cells have fewer but more active Ca^{2+} channels (78) as a result of an increase in channel phosphorylation. The latter could underlie the mode switching, too (79).

In this regard, myocytes from failing hearts are less responsive to β-adrenergic stimulation, which may be due to β-adrenergic receptor down-regulation or de-sensitization (80). Another possibility is that the level of Ca^{2+} channel phosphorylation is increased in failing myocytes. Several facts support this interpretation: (1) the adrenergic tone is increased in HF patients (81) (2) single-channel records show increased activity (77), (3) some other proteins show increased phosphorylation state (82), and (4) β-adrenergic receptor blockers help in HF therapy (83). Furthermore, strengthening the β-adrenergic system has been proved beneficial to prevent the development of HF in transgenic mice, even in the presence of the instigating cause (45) (84). However, there is so far not

enough evidence that Ca^{2+} channels are phosphorylated in human HF. In fact, forskolin, which also activates PKA phosphorylation of Ca^{2+} channels but bypasses β-adrenergic receptors, can increase I_{Ca} similarly in failing and control cells (85).

At the molecular level, a large discrepancy has been, once again, reported. In human HF (86) was revealed that mRNA expression for the α1c-subunit and the abundance of dihydropyridine binding sites were reduced. More recently, unchanged expression of α1c-subunits was reported in end-stage or diastolic HF (77, 87-89). Ancillary subunits play important functional roles in the formation and stabilization of L-type Ca^{2+} channels (69, 90). Although mRNA levels of the α2/δ-subunit remained unchanged in diastolic human HF (87), either reduced or unchanged β-subunit mRNA levels were reported in human HF (77, 87). Nevertheless, the ratio of β-subunit mRNA over α1c-subunit mRNA was significantly reduced in both studies. Moreover, cardiac myocytes contain several isoforms of the α1c-subunit. Hypertrophy after myocardial infarction in rats is associated with re-emergence of the fetal isoform of the α1c gene (91). Similarly, the relative abundance of isoforms is changed in human ischemic HF (88), although the functional significance of isoform switching has not been established yet.

Regardless of the potential changes of I_{Ca} during cardiac hypertrophy and failure, two points that are usually underestimated have to be considered: (1) the electrophysiological landmark of hypertrophy and HF is the prolongation of AP duration induced, at least in part, by the down-regulation of the transient outward K^+ current (73, 92-94) that regulates the height of the AP plateau during which I_{Ca} is active. Prolonged depolarization during the extended AP will thus enhance I_{Ca} contribution (95-97); (2) assuming unaltered I_{Ca} density in hypertrophy implies that the magnitude of I_{Ca} (and by extension, the total number of functional channels) is significantly increased in parallel with enlargement of cardiac myocytes. Therefore, a decrease in I_{Ca} density or DHPRs means a down- or no-regulation, whereas unchanged or increased I_{Ca} density means up-regulation. Interestingly, recent studies on a transgenic mouse model indicate that cardiac-specific overexpression of the L-type Ca^{2+} channel induces the development of hypertrophy and sets the stage for late-onset HF (98).

4.2. Activity of RyRs

Biochemical analysis of human failing hearts has revealed that the RyRs are hyperphosphorylated (82) (detailed in other chapter of this issue), although no difference has been reported in RyR phosphorylation in the fast-paced dog HF model (99). Bilayer experiments have shown that RyRs phosphorylation would increase its open probability and accelerate adaptation (100). *In vivo*, RyRs are located in clusters that open synchronously (coupled gating) to produce one Ca^{2+} spark. Phosphorylation of RyRs unbinds FK 506 binding protein from the RyRs and induces uncoupled gating (101). So far the pathological implication is not clear. The RyR hyperphosphorylation in terminal human HF would be associated to changes in Ca^{2+} sparks characteristics. However, this kind of analysis has not been undertaken and the Ca^{2+} sparks characteristics in animal models of HF do not recapitulate the changes after PKA phosphorylation (38, 42, 45, 46).

4.3. Effectiveness of coupling

Even if I_{Ca} density is maintained, the AP duration is increased in cardiac hypertrophy and HF (38, 73, 102). The consequence should be an increased Ca^{2+} entry during the longer depolarization period in failing cells. Since HF cells contract less, despite AP prolongation, one must conclude that the prolongation of Ca^{2+} influx still does not provide enough Ca^{2+} for normal activation of contractile proteins. In fact, even AP-evoked $[Ca^{2+}]_i$ transients are reduced in HF (46, 103). The question remains, if Ca^{2+} entry seems not to be decreased in HF, then why the heart fails?

Simultaneous measurements of I_{Ca} and SR Ca^{2+} release in different models have suggested that the mechanism of EC coupling, more precisely the Ca^{2+} -induced Ca^{2+} -release (CICR), is altered in HF (see **Table** 1). It was shown that I_{Ca}, which triggers SR Ca^{2+} release, was normal in failing human myocytes, but the $[Ca^{2+}]_i$ transient was depressed (18, 37). This means that for the same triggering Ca^{2+}, less Ca^{2+} is released. Similar results have also been observed in animal models of HF (38, 40, 42, 45, 46, 78). Now the underlying defect of this *uncoupling* must be identified. A detailed analysis of EC coupling entails determination of the efficacy of I_{Ca} to trigger Ca^{2+} release by simultaneously measuring I_{Ca} and SR Ca^{2+} release in its elementary events. In this way, one can determine how many Ca^{2+} sparks are triggered by a given Ca^{2+} entry. Likewise, the analysis of Ca^{2+} sparks characteristics provides an insight of RyR activity. We have undertaken this kind of analysis in two different models of pressure-overload decompensated hypertrophy (38). The first was the Dahl rat model. These animals are a strain of Sprague-Dawley that was selected by its sensitivity to sodium intake. The salt-sensitive rats develop hypertension when fed with a high salt diet. With time, their hearts hypertrophy and ultimately develop HF. The salt-resistant rats do not develop any alteration at the same high salt diet (104). At the time of experiments, the salt-sensitive animals experienced cardiac hypertrophy although no signs yet of congestive HF. However, isolated myocytes were failing. They showed a normal I_{Ca} (although slowed inactivation kinetics, see later) but the associated $[Ca^{2+}]_i$ transient and contraction (shortening) was weak (38). To analyze the reason why the SR Ca^{2+} release was reduced, one approach was to estimate the SR Ca^{2+} load that was shown to be unchanged. Next was to count and analyze the Ca^{2+} sparks evoked by I_{Ca} at several potentials. Such analysis showed that the number of Ca^{2+} sparks triggered by equivalent I_{Ca} was greatly reduced in failing myocytes compared to match controls (38). That is, the coupling between L-type Ca^{2+} channels and RyRs was defective. Nevertheless, the cells maintained the ability to compensate by β-adrenergic stimulation. These results were compared with another model of pressure-overload disease in overt HF. Similar data was obtained, although in this case the β-adrenergic stimulation was ineffective to overcome the

Table 1. Several analysis of EC coupling in cardiac hypertrophy and heart failure

References	Model	I_{Ca}	$[Ca^{2+}]_i$ transient	Ca^{2+} sparks	SR load
	Compensated hypertrophy:				
31	SHR (rat)	=	↑	Bigger	=
59	AVB dog	=	↑		↑
36	Abdominal aortic constriction (rat)	=	= ($1\ mM\ Ca^{2+}]_o$) ↓ ($0.5\ mMCa^{2+}]_o$)		=
153	Thoracic aortic constriction rabbit	= (DHPR density)	↓ (RyR density)		=
	Heart failure:				
38	Dahl & SHHF rat	=	↓	Fewer, normal characteristics	=
44	Rat PMI	=	↓		
46	Rat PMI	=	↓	Fewer, normal characteristics	=
103	Rabbit PMI	↓	↓	Asynchronous	
43	Paced rabbit	↓	↓		
61	Aortic insufficiency + aortic constriction in Rabbit	=	↓		↓
41	Paced dog	=	↓		↓
	Transgenic mice:				
45	MLP K.O.	=	↓	Normal characteristics	=
40	Viral myocarditis	=	↓		
42	Calsequestrin overexpression	=	↓	Fewer, diffused	↑
	Human:				
18, 39, 107	Terminal Heart Failure	=	↓		↓

defect (38). This suggests that the defective EC coupling may be underlying HF and was unmasked when systemic compensation was not effective anymore. The decreased SR Ca^{2+} release was underlying the decreased cellular contraction. Because the global $[Ca^{2+}]_i$ transient is constituted by the temporal and spatial summation of Ca^{2+} sparks (105) the depressed $[Ca^{2+}]_i$ transient is due to fewer Ca^{2+} sparks. Today, it is generally accepted that a Ca^{2+} spark is produced by a group of RyRs acting in concert. Analysis of RyRs showed that the density of RyRs was unchanged in these failing myocytes, as was their Ca^{2+} sensitivity (38). This is further supported by the unchanged characteristics of Ca^{2+} sparks. The later finding suggests that there is no change in the activity of RyRs and confirms that the SR Ca^{2+} load is unchanged in these models of HF. In fact, once the RyR opens, Ca^{2+} is released down its concentration gradient. If the SR load were reduced, the Ca^{2+} spark amplitude would be reduced because it depends on SR Ca^{2+} load (106). Because RyRs were found normal in number and Ca^{2+} sensitivity, it seems that there are fewer Ca^{2+} sparks in failing myocytes because the release units failed to be triggered. Since the trigger (I_{Ca}) is normal, these data suggest that the relationship DHPRs – RyRs is altered in HF. Few other studies have analyzed the elementary EC coupling in other models of HF. Recently we have shown that the same defect is also present in a model of HF after myocardial infarct in the rat (46). In the calsequestrin overexpression transgenic mice, the number of Ca^{2+} sparks was also shown to be reduced even though the SR Ca^{2+} load was highly increased (42). But in this case, the fewer Ca^{2+} sparks could be due to the reduced RyR density of this transgenic model. During compensated hypertrophy, in the spontaneously hypertensive rats, bigger Ca^{2+} sparks underlie bigger $[Ca^{2+}]_i$ transients and enhanced contractility, while the triggering I_{Ca} was also unchanged (31). Taken together, these data provide evidence that the relationship between DHPR and RyRs is modulated during the development of cardiac hypertrophy with: (i) an increase in the EC coupling gain underlying the increased contractility of this adaptive process; and (ii) a decrease as HF develops, underlying the decreased contractility.

4.3.1. Defects underlying the EC uncoupling

Data from human HF cells have been collected from terminally failing hearts obtained during transplantation. Very often, different etiologies are mixed together and the patients have a long history of medication, which render conclusions difficult. Nevertheless, it is always useful and necessary to know what happens in the human heart; even if limited to the end-stage of HF. Ventricular myocytes isolated from terminally failing human hearts had a decreased EC coupling gain (37). In fact, while the triggering I_{Ca} was maintained in density, the triggered $[Ca^{2+}]_i$ transient was depressed. Moreover, the elevation in $[Ca^{2+}]_i$ due to the L-type Ca^{2+} channel (with

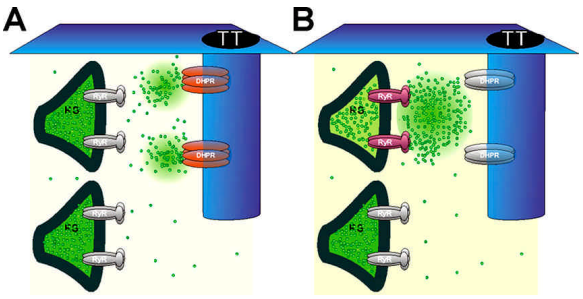

Figure 2. Proposed cartoon showing a spatial remodeling that can underlie the decrease in the EC coupling gain observed in heart failure (46). Excitation contraction coupling elements as in Figure 1.

empty SR) was similar in both failing and normal hearts. The depressed $[Ca^{2+}]_i$ transient was thus due to a smaller SR Ca^{2+} release (39) although in these non-hypertrophied failing cells, a decrease in SR Ca^{2+} content may, at least in part, explain the decrease in the efficacy of the I_{Ca} to trigger SR Ca^{2+} release (107). Similar explanation has been suggested in a model of paced-induced HF (41). Interestingly, this model also induces HF without cellular hypertrophy (60).

In cardiac hypertrophy and HF with cellular hypertrophy, the reorganization of the cellular structure as cell grows may be important determinants of defective EC coupling. In fact, there is a growing body of evidence suggesting that alterations in cardiac cytoskeletal proteins may induce dilated cardiomyopathy (108). Moreover, several transgenic mice with alterations in the cytoskeleton develop HF, such us the muscle LIM protein (MLP) knock out mice. They develop dilated cardiomyopathy and HF with hypertrophy (109) and decrease in the EC coupling efficiency (45). MLP is a protein involved in cytoarchitecture organization. Likewise, desmin (constituent of the intermediate filaments in muscle) deficiency induces cardiomyopathy (110) with cell hypertrophy and alterations in Ca^{2+} handling (111). Interestingly, acquired cardiac hypertrophy induces changes in the cytoskeleton. More precisely, it has been reported that HF following chronic hypertension is associated with an increase in the density of another cytoskeleton component, the microtubules (112). Very interestingly, microtubule proliferation in the failing cell may be involved in the contractile abnormalities since microtubule depolymerization normalizes contraction in failing cells (112). Moreover, it has been shown that microtubule proliferation can decrease $[Ca^{2+}]_i$ transient (113), whereas microtubule depolymerization increases $[Ca^{2+}]_i$ transient (114) and modifies Ca^{2+} sparks characteristics (115). Because CICR occurs mainly at the region of junctional SR (where RyRs are located) close to the TT (where the DHPRs are located), a rearrangement in the spatial organization of DHPRs and RyRs in the dyadic cleft or a disruption in the TT system during remodeling would have severe consequences in EC coupling in the failing myocytes (Figure 2). In this line, alterations in the TT system have been reported in hypertrophied cells from human failing myocardium (116). In a HF model of paced dog with significant cellular hypertrophy, a decrease in TT density has been shown (78), and it seems that a similar pattern could be present in human HF (117).

4.3.2. Feed-back RyR-DHPR

While the increase in the $[Ca^{2+}]_i$ produced by one L-type Ca^{2+} channel opening activates the RyR and induces one Ca^{2+} spark, the increase in $[Ca^{2+}]_i$ in the dyadic cleft induced by the RyRs opening inactivates the L-type Ca^{2+} channel (118). In fact, analysis of the I_{Ca} inactivation time constant provides an insight of the magnitude of release. In this regard, most studies have shown a lengthening of the fast component of I_{Ca} inactivation (38, 73, 119) that could reflect decreased $[Ca^{2+}]_i$ transient in HF. This hypothesis is supported by a theoretical model predicting that, under conditions of reduced SR Ca^{2+} release, there is less Ca^{2+} -dependent inactivation of $I_{Ca,}$ which helps to prolong the plateau phase of the AP (120). Indeed, SR Ca^{2+} release inactivates up to 50% of net Ca^{2+} entry through Ca^{2+} channels (121). Besides slowing of I_{Ca} inactivation at a given frequency, increasing stimulation frequency in cardiac myocytes slows I_{Ca} inactivation with a subsequent increase in net Ca^{2+} entry. This phenomenon is termed I_{Ca} facilitation and is dependent on SR Ca^{2+} release (119, 122). Facilitation of I_{Ca} occurs over a range of frequencies corresponding to rates encountered in pathophysiological states. Higher rates produce a marked lengthening of I_{Ca} inactivation and, as a direct consequence, a slight increase in peak current, which is unrelated to recruitment of new channels. Simultaneous measurements of I_{Ca} facilitation and SR $[Ca^{2+}]_i$ release have revealed that this phenomenon results from a decrease of the $[Ca^{2+}]_i$ transient at high frequencies in rat ventricular myocytes (122). Moreover, thapsigargin and ryanodine induced similar "pharmacological" I_{Ca} facilitation (119, 122). In this regard, I_{Ca} facilitation in HF can be used as an index of the coupling DHPR-RyR. Interestingly, I_{Ca} facilitation is rarely observed in human HF when comparing either with ventricular cells from other mammals or atrial cells from HF and non-HF patients (72, 119, 123, 124). Furthermore, even at low stimulation rates, inactivation of I_{Ca} is slow in HF cells which is consistent with weaker SR Ca^{2+}-release and, thereby, weaker Ca^{2+}-dependent inactivation (119).

5. PERSPECTIVES: IMPORTANCE OF Ca^{2+} CYCLING IN THE PROGRESSION OF HF

Remodeling during cardiac hypertrophy and failure is usually accompanied by complex changes in gene expression reprogramming (1). In recent years, it has become apparent that Ca^{2+} also plays a role in this longer-term regulation. This represents a second mechanism, via long-term adaptation, by which Ca^{2+} might alter the function of cardiac muscle. It has been suggested that alterations in Ca^{2+} handling proteins progressively exacerbate a hypertrophic or cardiomyopathic phenotype, in part, through sustained activation of Ca^{2+}-sensitive signaling pathways. The intricate signaling network that causes cardiac hypertrophy (125) involves Ca^{2+}-dependent gene regulation as a necessary component (126, 127). In the first phase of hypertrophy response (min to hours), early response genes, e.g. c-fos, c-myc, c-jun, are activated (128). In cardiac myocytes, transcriptional induction via c-

jun has been related to intracellular Ca^{2+} (129, 130). Moreover, Ca^{2+} influx through L-type Ca^{2+} channels is critical for early response gene stimulation by a variety of stimuli, including neurotransmitters and growth factors (131, 132). Intracellular [Ca^{2+}] may also affect expression of late genes. Subject of intense research over the past decade, the signaling cascades underlying alteration in gene expression in cardiac hypertrophy and HF converge on the MAPK cascade (133, 134). Activation of this pathway by hypertrophy stimulus depends on transsarcolemmal influx of Ca^{2+} (135). A Ca^{2+} sensitive mechanism, which involves Ca^{2+}/Calmodulin pathway, seems to be important for activation of MAPK cascade (136). Over-expression of calmodulin (CaM) in transgenic mice causes myocytes hypertrophy (137). Calmodulin, when activated by Ca^{2+}, modulates the activity of a number of enzymes, including CaM-dependent protein kinases involved in transcriptional regulation (47, 138, 139). Recently, it has been shown that the CaM kinase pathway is sufficient to activate many features of cardiac hypertrophy and failure in vivo (140, 141). Recent studies have shown that the Ca^{2+}/calcineurin pathway is a necessary component for the expression of hypertrophy markers (126) although this has been subject of controversy (142). As matter of fact, overexpressing calcineurin in a transgenic mouse produced a rapid development of hypertrophy and HF (143). Calcineurin is a specific Ca^{2+}-dependent phosphatase that dephosphorylates transcription factors, which turn on a panel of hypertrophy genes.

Thus, when considering all the published studies on HF, a central and perhaps essential role for intracellular Ca^{2+} is inescapable, not only on altered cardiac contraction, but also on altered gene expression. In cardiomyocytes, EC coupling thus regulates the transcription activity by process termed excitation-transcription coupling. This process represents an elementary pathway whereby, through Ca^{2+} signaling, the electrical activity of a cell feeds back upon and shapes the cellular genetic program (144, 145). This has been nicely evidenced in smooth muscle (146, 147). For instance, activation of Ca^{2+} influx through the L-type Ca^{2+} channel by membrane depolarization increased Ca^{2+} sparks frequency, which in turn activated the cAMP-responsive element-binding protein and subsequent c-fos expression.

Recently, we provided new data on the implication of this pathway in an aldosterone-induced down-regulation of the transient outward K^+ current ($I_{to,1}$) functional expression, which might be involved in hypertrophy and HF (148). This mineralocorticoid hormone might be one of the primary factors in the remodeling of hypertrophy and HF (149-151). Incubation of adult rat isolated cardiomyocytes in the presence of aldosterone induces a specific and genomic up-regulation of functional expression of L-type Ca^{2+} channels (152). When this stimulation is sufficient, then it induces an increase in Ca^{2+} sparks occurrence, which is able to down-regulate the functional expression of I_{to1} (148).

We should expect, in the near future, new insights into the mechanisms by which intracellular Ca^{2+} influences the progression of hypertrophy and HF.

6. ACKNOWLEDGMENTS

We wish to thank Dr. W.J. Lederer for numerous discussions about Ca^{2+} signaling in heart failure. We acknowledge FRM (Fondation pour la recherche médicale, S.R., INE20001117051) for financial support. AMG and SR are CNRS (Centre National de la Recherche Scientifique) scientists.

7. REFERENCES

1. Swynghedauw, B.: Molecular mechanisms of myocardial remodeling. *Physiol Rev* 79, 215-62 (1999)
2. Kannel, W. B.: Left ventricular hypertrophy as a risk factor: the Framingham experience. *J Hypertens Suppl* 9, S3-8; discussion S8-9. (1991)
3. Pye, M. P. and Cobbe, S. M.: Mechanisms of ventricular arrhythmias in cardiac failure and hypertrophy. *Cardiovasc Res* 26, 740-50. (1992)
4. Meerson, F. Z.: The myocardium in hyperfunction, hypertrophy and heart failure. *Circ Res* 25, Suppl 2:1-163. (1969)
5. Lorente, P., Delgado, C., Bénitah, J., Bailly, P. & Gómez, A. in *Cardiac electrophysiology. From cell to bedside.* (eds. Zipes, D. & Jalife, J.) 277-291 (WB. Saunders company, Philadelphia, 1995).
6. Bénitah, J. P., Gómez, A. M., Fauconnier, J., Kerfant, B. G., Perrier, E., Vassort, G. and Richard, S.: Voltage-gated Ca^{2+} currents in the human pathophysiologic heart: a review. *Basic Res Cardiol*, in press (2002)
7. Hasenfuss, G.: Animal models of human cardiovascular disease, heart failure and hypertrophy. *Cardiovasc Res* 39, 60-76. (1998)
8. Fabiato, A. and Fabiato, F.: Use of chlorotetracycline fluorescence to demonstrate Ca2+-induced release of Ca^{2+} from the sarcoplasmic reticulum of skinned cardiac cells. *Nature* 281, 146-8. (1979)
9. Stern, M. D.: Theory of excitation-contraction coupling in cardiac muscle. *Biophys J* 63, 497-517. (1992)
10. Cheng, H., Lederer, W. J. and Cannell, M. B.: Calcium sparks: elementary events underlying excitation-contraction coupling in heart muscle. *Science* 262, 740-4 (1993)
11. Lopez-Lopez, J. R., Shacklock, P. S., Balke, C. W. and Wier, W. G.: Local calcium transients triggered by single L-type calcium channel currents in cardiac cells. *Science* 268, 1042-5 (1995)
12. Santana, L. F., Cheng, H., Gómez, A. M., Cannell, M. B. and Lederer, W. J.: Relation between the sarcolemmal Ca^{2+} current and Ca^{2+} sparks and local control theories for cardiac excitation-contraction coupling. *Circ Res* 78, 166-71 (1996)
13. Wang, S. Q., Song, L. S., Lakatta, E. G. and Cheng, H.: Ca^{2+} signalling between single L-type Ca^{2+} channels and ryanodine receptors in heart cells. *Nature* 410, 592-6. (2001)
14. Gwathmey, J. K., Bentivegna, L. A., Ransil, B. J., Grossman, W. and Morgan, J. P.: Relationship of abnormal intracellular calcium mobilisation to myocyte hypertrophy in human ventricular myocardium. *Cardiovasc Res* 27, 199-203. (1993)
15. Gwathmey, J. K. and Morgan, J. P.: Altered calcium handling in experimental pressure-overload hypertrophy in the ferret. *Circ Res* 57, 836-43 (1985)

16. Gwathmey, J. K., Copelas, L., MacKinnon, R., Schoen, F. J., Feldman, M. D., Grossman, W. and Morgan, J. P.: Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. *Circ Res* 61, 70-6. (1987)
17. Bentivegna, L. A., Ablin, L. W., Kihara, Y. and Morgan, J. P.: Altered calcium handling in left ventricular pressure-overload hypertrophy as detected with aequorin in the isolated, perfused ferret heart. *Circ Res* 69, 1538-45 (1991)
18. Beuckelmann, D. J. and Erdmann, E.: Ca(2+)-currents and intracellular [Ca2+]i-transients in single ventricular myocytes isolated from terminally failing human myocardium. *Basic Res Cardiol* 87 Suppl 1, 235-43. (1992)
19. Sen, L., Cui, G., Fonarow, G. C. and Laks, H.: Differences in mechanisms of SR dysfunction in ischemic vs. idiopathic dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol* 279, H709-18. (2000)
20. Houser, S. R., Piacentino, V., 3rd and Weisser, J.: Abnormalities of calcium cycling in the hypertrophied and failing heart. *J Mol Cell Cardiol* 32, 1595-607. (2000)
21. Schillinger, W., Lehnart, S. E., Prestle, J., Preuss, M., Pieske, B., Maier, L. S., Meyer, M., Just, H. and Hasenfuss, G.: Influence of SR Ca(2+)-ATPase and Na(+)-Ca(2+)-exchanger on the force-frequency relation. *Basic Res Cardiol* 93, 38-45. (1998)
22. Mulieri, L. A., Hasenfuss, G., Leavitt, B., Allen, P. D. and Alpert, N. R.: Altered myocardial force-frequency relation in human heart failure. *Circulation* 85, 1743-50. (1992)
23. Pieske, B., Kretschmann, B., Meyer, M., Holubarsch, C., Weirich, J., Posival, H., Minami, K., Just, H. and Hasenfuss, G.: Alterations in intracellular calcium handling associated with the inverse force-frequency relation in human dilated cardiomyopathy. *Circulation* 92, 1169-78. (1995)
24. Minamisawa, S., Hoshijima, M., Chu, G., Ward, C. A., Frank, K., Gu, Y., Martone, M. E., Wang, Y., Ross, J., Jr., Kranias, E. G., Giles, W. R. and Chien, K. R.: Chronic phospholamban-sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. *Cell* 99, 313-22 (1999)
25. del Monte, F., Harding, S. E., Schmidt, U., Matsui, T., Kang, Z. B., Dec, G. W., Gwathmey, J. K., Rosenzweig, A. and Hajjar, R. J.: Restoration of Contractile Function in Isolated Cardiomyocytes From Failing Human Hearts by Gene Transfer of SERCA2a. *Circulation* 100, 2308-2311 (1999)
26. Reilly, A. M., Petrou, S., Panchal, R. G. and Williams, D. A.: Restoration of calcium handling properties of adult cardiac myocytes from hypertrophied hearts. *Cell Calcium* 30, 59-66. (2001)
27. Sato, Y., Kiriazis, H., Yatani, A., Schmidt, A. G., Hahn, H., Ferguson, D. G., Sako, H., Mitarai, S., Honda, R., Mesnard-Rouiller, L., Frank, K. F., Beyermann, B., Wu, G., Fujimori, K., Dorn, G. W., 2nd and Kranias, E. G.: Rescue of contractile parameters and myocyte hypertrophy in calsequestrin overexpressing myocardium by phospholamban ablation. *J Biol Chem* 276, 9392-9. (2001)
28. Gaughan, J. P., Furukawa, S., Jeevanandam, V., Hefner, C. A., Kubo, H., Margulies, K. B., McGowan, B. S., Mattiello, J. A., Dipla, K., Piacentino, V., Li, S. and Houser, S. R.: Sodium/calcium exchange contributes to contraction and relaxation in failed human ventricular myocytes. *Am J Physiol* 277, H714-24. (1999)
29. Gao, W. D., Perez, N. G., Seidman, C. E., Seidman, J. G. and Marbán, E.: Altered cardiac excitation-contraction coupling in mutant mice with familial hypertrophic cardiomyopathy. *J Clin Invest* 103, 661-6 (1999)
30. Nagata, K., Liao, R., Eberli, F. R., Satoh, N., Chevalier, B., Apstein, C. S. and Suter, T. M.: Early changes in excitation-contraction coupling: transition from compensated hypertrophy to failure in Dahl salt-sensitive rat myocytes. *Cardiovasc Res* 37, 467-77. (1998)
31. Shorofsky, S. R., Aggarwal, R., Corretti, M., Baffa, J. M., Strum, J. M., Al-Seikhan, B. A., Kobayashi, Y. M., Jones, L. R., Wier, W. G. and Balke, C. W.: Cellular mechanisms of altered contractility in the hypertrophied heart: big hearts, big sparks. *Circ Res* 84, 424-34 (1999)
32. Brooksby, P., Levi, A. J. and Jones, J. V.: Investigation of the mechanisms underlying the increased contraction of hypertrophied ventricular myocytes isolated from the spontaneously hypertensive rat. *Cardiovasc Res* 27, 1268-77 (1993)
33. Anand, I. S., Liu, D., Chugh, S. S., Prahash, A. J., Gupta, S., John, R., Popescu, F. and Chandrashekhar, Y.: Isolated myocyte contractile function is normal in postinfarct remodeled rat heart with systolic dysfunction. *Circulation* 96, 3974-84 (1997)
34. Perez, N. G., Hashimoto, K., McCune, S., Altschuld, R. A. and Marbán, E.: Origin of contractile dysfunction in heart failure: calcium cycling versus myofilaments. *Circulation* 99, 1077-83 (1999)
35. Tajima, M., Bartunek, J., Weinberg, E. O., Ito, N. and Lorell, B. H.: Atrial Natriuretic Peptide Has Different Effects on Contractility and Intracellular pH in Normal and Hypertrophied Myocytes From Pressure-Overloaded Hearts. *Circulation* 98, 2760-2764 (1998)
36. McCall, E., Ginsburg, K. S., Bassani, R. A., Shannon, T. R., Qi, M., Samarel, A. M. and Bers, D. M.: Ca flux, contractility, and excitation-contraction coupling in hypertrophic rat ventricular myocytes. *Am J Physiol* 274, H1348-60 (1998)
37. Beuckelmann, D. J., Nabauer, M. and Erdmann, E.: Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. *Circulation* 85, 1046-55. (1992)
38. Gómez, A. M., Valdivia, H. H., Cheng, H., Lederer, M. R., Santana, L. F., Cannell, M. B., McCune, S. A., Altschuld, R. A. and Lederer, W. J.: Defective excitation-contraction coupling in experimental cardiac hypertrophy and heart failure. *Science* 276, 800-6. (1997)
39. Beuckelmann, D. J.: Contributions of Ca(2+)-influx via the L-type Ca(2+)-current and Ca(2+)-release from the sarcoplasmic reticulum to [Ca2+]i-transients in human myocytes. *Basic Res Cardiol* 92 Suppl 1, 105-10. (1997)
40. Wessely, R., Klingel, K., Santana, L. F., Dalton, N., Hongo, M., Jonathan Lederer, W., Kandolf, R. and Knowlton, K. U.: Transgenic expression of replication-restricted enteroviral genomes in heart muscle induces defective excitation-contraction coupling and dilated cardiomyopathy. *J Clin Invest* 102, 1444-53 (1998)
41. Hobai, I. A. and O'Rourke, B.: Decreased sarcoplasmic reticulum calcium content is responsible for defective

excitation-contraction coupling in canine heart failure. *Circulation* 103, 1577-84. (2001)
42. Jones, L. R., Suzuki, Y. J., Wang, W., Kobayashi, Y. M., Ramesh, V., Franzini-Armstrong, C., Cleemann, L. and Morad, M.: Regulation of Ca^{2+} signaling in transgenic mouse cardiac myocytes overexpressing calsequestrin. *J Clin Invest* 101, 1385-93 (1998)
43. Yao, A., Su, Z., Nonaka, A., Zubair, I., Spitzer, K. W., Bridge, J. H., Muelheims, G., Ross, J., Jr. and Barry, W. H.: Abnormal myocyte Ca^{2+} homeostasis in rabbits with pacing-induced heart failure. *Am J Physiol* 275, H1441-8 (1998)
44. Holt, E., Tonnessen, T., Lunde, P. K., Semb, S. O., Wasserstrom, J. A., Sejersted, O. M. and Christensen, G.: Mechanisms of cardiomyocyte dysfunction in heart failure following myocardial infarction in rats. *J Mol Cell Cardiol* 30, 1581-93. (1998)
45. Esposito, G., Santana, L. F., Dilly, K., Cruz, J. D., Mao, L., Lederer, W. J. and Rockman, H. A.: Cellular and functional defects in a mouse model of heart failure. *Am J Physiol Heart Circ Physiol* 279, H3101-12 (2000)
46. Gómez, A. M., Guatimosim, S., Dilly, K. W., Vassort, G. and Lederer, W. J.: Heart Failure After Myocardial Infarction: Altered Excitation-Contraction Coupling. *Circulation* 104, 688-693 (2001)
47. Stull, L. B., Matteo, R. G., Sweet, W. E., Damron, D. S. and Schomisch Moravec, C.: Changes in calcium cycling precede cardiac dysfunction during autoimmune myocarditis in mice. *J Mol Cell Cardiol* 33, 449-60. (2001)
48. Hasenfuss, G.: Alterations of calcium-regulatory proteins in heart failure. *Cardiovasc Res* 37, 279-89. (1998)
49. Santana, L. F., Gómez, A. M. and Lederer, W. J.: Ca^{2+} flux through promiscuous cardiac Na+ channels: slip-mode conductance [see comments]. *Science* 279, 1027-33 (1998)
50. Reeves, J. P. and Hale, C. C.: The stoichiometry of the cardiac sodium-calcium exchange system. *J Biol Chem* 259, 7733-9. (1984)
51. Egger, M. and Niggli, E.: Regulatory function of Na-Ca exchange in the heart: milestones and outlook. *J Membr Biol* 168, 107-30. (1999)
52. Leblanc, N. and Hume, J. R.: Sodium current-induced release of calcium from cardiac sarcoplasmic reticulum [see comments]. *Science* 248, 372-6 (1990)
53. Sipido, K. R., Maes, M. and Van de Werf, F.: Low efficiency of Ca^{2+} entry through the $Na(+)$-Ca^{2+} exchanger as trigger for Ca^{2+} release from the sarcoplasmic reticulum. A comparison between L-type Ca^{2+} current and reverse-mode $Na(+)$-Ca^{2+} exchange. *Circ Res* 81, 1034-44 (1997)
54. Studer, R., Reinecke, H., Bilger, J., Eschenhagen, T., Bohm, M., Hasenfuss, G., Just, H., Holtz, J. and Drexler, H.: Gene expression of the cardiac $Na(+)$-Ca^{2+} exchanger in end-stage human heart failure. *Circ Res* 75, 443-53. (1994)
55. Pogwizd, S. M., Qi, M., Yuan, W., Samarel, A. M. and Bers, D. M.: Upregulation of $Na+/Ca^{2+}$ Exchanger Expression and Function in an Arrhythmogenic Rabbit Model of Heart Failure. *Circ Res* 85, 1009-1019 (1999)
56. Wang, Z., Nolan, B., Kutschke, W. and Hill, J. A.: $Na+$-Ca^{2+} exchanger remodeling in pressure overload cardiac hypertrophy. *J Biol Chem* 276, 17706-11. (2001)
57. Hatem, S. N., Sham, J. S. and Morad, M.: Enhanced $Na(+)$-Ca^{2+} exchange activity in cardiomyopathic Syrian hamster. *Circ Res* 74, 253-61 (1994)
58. Litwin, S. E. and Bridge, J. H.: Enhanced $Na(+)$-Ca^{2+} exchange in the infarcted heart. Implications for excitation-contraction coupling. *Circ Res* 81, 1083-93 (1997)
59. Sipido, K. R., Volders, P. G. A., de Groot, S. H. M., Verdonck, F., Van de Werf, F., Wellens, H. J. J. and Vos, M. A.: Enhanced Ca^{2+} Release and Na/Ca Exchange Activity in Hypertrophied Canine Ventricular Myocytes : Potential Link Between Contractile Adaptation and Arrhythmogenesis. *Circulation* 102, 2137-2144 (2000)
60. Hobai, I. A. and O'Rourke, B.: Enhanced Ca(2+)-activated Na(+)-Ca(2+) exchange activity in canine pacing-induced heart failure. *Circ Res* 87, 690-8. (2000)
61. Pogwizd, S. M., Schlotthauer, K., Li, L., Yuan, W. and Bers, D. M.: Arrhythmogenesis and Contractile Dysfunction in Heart Failure : Roles of Sodium-Calcium Exchange, Inward Rectifier Potassium Current, and Residual {beta}-Adrenergic Responsiveness. *Circ Res* 88, 1159-1167 (2001)
62. Hasenfuss, G., Schillinger, W., Lehnart, S. E., Preuss, M., Pieske, B., Maier, L. S., Prestle, J., Minami, K. and Just, H.: Relationship Between Na+-Ca2+–Exchanger Protein Levels and Diastolic Function of Failing Human Myocardium. *Circulation* 99, 641-648 (1999)
63. Pogwizd, S. M.: Increased $Na+$-Ca^{2+} Exchanger in the Failing Heart. *Circ Res* 87, 641-643 (2000)
64. Pu, J., Robinson, R. B. and Boyden, P. A.: Abnormalities in Ca(i)handling in myocytes that survive in the infarcted heart are not just due to alterations in repolarization. *J Mol Cell Cardiol* 32, 1509-23. (2000)
65. Sipido, K. R., Carmeliet, E. and Van de Werf, F.: T-type Ca^{2+} current as a trigger for Ca^{2+} release from the sarcoplasmic reticulum in guinea-pig ventricular myocytes. *J Physiol* 508, 439-51. (1998)
66. Reuter, H.: The dependence of slow inward current in Purkinje fibres on the extracellular calcium-concentration. *J Physiol* 192, 479-92. (1967)
67. Rougier, O., Vassort, G., Garnier, D., Gargouil, Y. M. and Coraboeuf, E.: Existence and role of a slow inward current during the frog atrial action potential. *Pflugers Arch* 308, 91-110. (1969)
68. Hess, P., Lansman, J. B. and Tsien, R. W.: Different modes of Ca channel gating behaviour favoured by dihydropyridine Ca agonists and antagonists. *Nature* 311, 538-44. (1984)
69. Catterall, W. A.: Structure and regulation of voltage-gated Ca^{2+} channels. *Annu Rev Cell Dev Biol* 16, 521-55. (2000)
70. Hart, G.: Cellular electrophysiology in cardiac hypertrophy and failure. *Cardiovasc Res* 28, 933-46 (1994)
71. Mukherjee, R. and Spinale, F. G.: L-type calcium channel abundance and function with cardiac hypertrophy and failure: a review. *J Mol Cell Cardiol* 30, 1899-916. (1998)
72. Richard, S., Leclercq, F., Lemaire, S., Piot, C. and Nargeot, J.: Ca^{2+} currents in compensated hypertrophy and heart failure. *Cardiovasc Res* 37, 300-11. (1998)
73. Tomaselli, G. F. and Marbán, E.: Electrophysiological remodeling in hypertrophy and heart failure. *Cardiovasc Res* 42, 270-83. (1999)
74. Scamps, F., Mayoux, E., Charlemagne, D. and Vassort, G.: Calcium current in single cells isolated from

normal and hypertrophied rat heart. Effects of beta-adrenergic stimulation. *Circ Res* 67, 199-208 (1990)
75. Keung, E. C.: Calcium current is increased in isolated adult myocytes from hypertrophied rat myocardium. *Circ Res* 64, 753-63. (1989)
76. Hume, J. R. and Uehara, A.: Ionic basis of the different action potential configurations of single guinea-pig atrial and ventricular myocytes. *J Physiol* 368, 525-44. (1985)
77. Schroder, F., Handrock, R., Beuckelmann, D. J., Hirt, S., Hullin, R., Priebe, L., Schwinger, R. H., Weil, J. and Herzig, S.: Increased availability and open probability of single L-type calcium channels from failing compared with nonfailing human ventricle. *Circulation* 98, 969-76. (1998)
78. He, J., Conklin, M. W., Foell, J. D., Wolff, M. R., Haworth, R. A., Coronado, R. and Kamp, T. J.: Reduction in density of transverse tubules and L-type Ca(2+) channels in canine tachycardia-induced heart failure. *Cardiovasc Res* 49, 298-307. (2001)
79. Yue, D. T., Herzig, S. and Marbán, E.: Beta-adrenergic stimulation of calcium channels occurs by potentiation of high-activity gating modes. *Proc Natl Acad Sci U S A* 87, 753-7. (1990)
80. Bristow, M. R.: Changes in myocardial and vascular receptors in heart failure. *J Am Coll Cardiol* 22, 61A-71A (1993)
81. Bristow, M. R., Hershberger, R. E., Port, J. D., Gilbert, E. M., Sandoval, A., Rasmussen, R., Cates, A. E. and Feldman, A. M.: Beta-adrenergic pathways in nonfailing and failing human ventricular myocardium. *Circulation* 82, I12-25 (1990)
82. Marx, S. O., Reiken, S., Hisamatsu, Y., Jayaraman, T., Burkhoff, D., Rosemblit, N. and Marks, A. R.: PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts. *Cell* 101, 365-76 (2000)
83. Bristow, M. R.: Mechanism of action of beta-blocking agents in heart failure. *Am J Cardiol* 80, 26L-40L (1997)
84. Dorn, G. W., 2nd, Tepe, N. M., Lorenz, J. N., Koch, W. J. and Liggett, S. B.: Low- and high-level transgenic expression of beta2-adrenergic receptors differentially affect cardiac hypertrophy and function in Galphaq-overexpressing mice. *Proc Natl Acad Sci U S A* 96, 6400-5. (1999)
85. Mewes, T. and Ravens, U.: L-type calcium currents of human myocytes from ventricle of non-failing and failing hearts and from atrium. *J Mol Cell Cardiol* 26, 1307-20. (1994)
86. Takahashi, T., Allen, P. D., Lacro, R. V., Marks, A. R., Dennis, A. R., Schoen, F. J., Grossman, W., Marsh, J. D. and Izumo, S.: Expression of dihydropyridine receptor (Ca^{2+} channel) and calsequestrin genes in the myocardium of patients with end-stage heart failure. *J Clin Invest* 90, 927-35. (1992)
87. Hullin, R., Asmus, F., Ludwig, A., Hersel, J. and Boekstegers, P.: Subunit expression of the cardiac L-type calcium channel is differentially regulated in diastolic heart failure of the cardiac allograft. *Circulation* 100, 155-63. (1999)
88. Yang, Y., Chen, X., Margulies, K., Jeevanandam, V., Pollack, P., Bailey, B. A. and Houser, S. R.: L-type Ca^{2+} channel alpha 1c subunit isoform switching in failing human ventricular myocardium. *J Mol Cell Cardiol* 32, 973-84. (2000)
89. Kaab, S., Dixon, J., Duc, J., Ashen, D., Nabauer, M., Beuckelmann, D. J., Steinbeck, G., McKinnon, D. and Tomaselli, G. F.: Molecular basis of transient outward potassium current downregulation in human heart failure: a decrease in Kv4.3 mRNA correlates with a reduction in current density. *Circulation* 98, 1383-93. (1998)
90. Singer, D., Biel, M., Lotan, I., Flockerzi, V., Hofmann, F. and Dascal, N.: The roles of the subunits in the function of the calcium channel. *Science* 253, 1553-7. (1991)
91. Gidh-Jain, M., Huang, B., Jain, P., Battula, V. and el-Sherif, N.: Reemergence of the fetal pattern of L-type calcium channel gene expression in non infarcted myocardium during left ventricular remodeling. *Biochem Biophys Res Commun* 216, 892-7. (1995)
92. Bénitah, J. P., Gómez, A. M., Bailly, P., Da Ponte, J. P., Berson, G., Delgado, C. and Lorente, P.: Heterogeneity of the early outward current in ventricular cells isolated from normal and hypertrophied rat hearts. *J Physiol (Lond)* 469, 111-38 (1993)
93. Gómez, A. M., Bénitah, J. P., Henzel, D., Vinet, A., Lorente, P. and Delgado, C.: Modulation of electrical heterogeneity by compensated hypertrophy in rat left ventricle. *Am J Physiol* 272, H1078-86 (1997)
94. Takimoto, K. & Levitan, E. S. in Potassium channels in cardiovascular biology (eds. Archer, S. L. & Rusch, N. J.) Kluver Academic/Plenum, New York, 773-83 (2001)
95. Wickenden, A. D., Kaprielian, R., Kassiri, Z., Tsoporis, J. N., Tsushima, R., Fishman, G. I. and Backx, P. H.: The role of action potential prolongation and altered intracellular calcium handling in the pathogenesis of heart failure. *Cardiovasc Res* 37, 312-23. (1998)
96. Kaprielian, R., Wickenden, A. D., Kassiri, Z., Parker, T. G., Liu, P. P. and Backx, P. H.: Relationship between K+ channel down-regulation and [Ca2+]i in rat ventricular myocytes following myocardial infarction. *J Physiol* 517, 229-45. (1999)
97. Volk, T., Nguyen, T. H.-D., Schultz, J.-H. and Ehmke, H.: Relationship between transient outward K+ current and Ca^{2+} influx in rat cardiac myocytes of endo- and epicardial origin. *J Physiol (Lond)* 519, 841-850 (1999)
98. Muth, J. N., Bodi, I., Lewis, W., Varadi, G. and Schwartz, A.: A Ca(2+)-dependent transgenic model of cardiac hypertrophy: A role for protein kinase Calpha. *Circulation* 103, 140-7. (2001)
99. Ming, T. J., Lokuta, A. J., Wolf, M. R., Haworth, R. A. and Valdivia, H. H.: Depression of ryanodine receptor density, but not function, may decrease calcium release in heart failure (HF). *Biophys J* 78, 376A (abstract) (2000)
100. Valdivia, H. H., Kaplan, J. H., Ellis-Davies, G. C. and Lederer, W. J.: Rapid adaptation of cardiac ryanodine receptors: modulation by Mg^{2+} and phosphorylation. *Science* 267, 1997-2000 (1995)
101. Marx, S. O., Gaburjakova, J., Gaburjakova, M., Henrikson, C., Ondrias, K. and Marks, A. R.: Coupled Gating Between Cardiac Calcium Release Channels (Ryanodine Receptors). *Circ Res* 88, 1151-1158 (2001)
102. Aronson, R. S.: Characteristics of action potentials of hypertrophied myocardium from rats with renal hypertension. *Circ Res* 47, 443-54 (1980)

103. Litwin, S. E., Zhang, D. and Bridge, J. H. B.: Dyssynchronous Ca^{2+} Sparks in Myocytes From Infarcted Hearts. *Circ Res* 87, 1040-1047 (2000)
104. Inoko, M., Kihara, Y., Morii, I., Fujiwara, H. and Sasayama, S.: Transition from compensatory hypertrophy to dilated, failing left ventricles in Dahl salt-sensitive rats. *Am J Physiol* 267, H2471-82 (1994)
105. Cheng, H., Lederer, M. R., Xiao, R. P., Gómez, A. M., Zhou, Y. Y., Ziman, B., Spurgeon, H., Lakatta, E. G. and Lederer, W. J.: Excitation-contraction coupling in heart: new insights from Ca^{2+} sparks. *Cell Calcium* 20, 129-40 (1996)
106. Santana, L. F., Gómez, A. M., Kranias, E. G. and Lederer, W. J.: Amount of calcium in the sarcoplasmic reticulum: influence on excitation-contraction coupling in heart muscle. *Heart Vessels* Suppl 12, 44-9 (1997)
107. Lindner, M., Erdmann, E. and Beuckelmann, D. J.: Calcium content of the sarcoplasmic reticulum in isolated ventricular myocytes from patients with terminal heart failure. *J Mol Cell Cardiol* 30, 743-9. (1998)
108. Chen, J. and Chien, K. R.: Complexity in simplicity: monogenic disorders and complex cardiomyopathies. *J Clin Invest* 103, 1483-5. (1999)
109. Arber, S., Hunter, J. J., Ross, J., Jr., Hongo, M., Sansig, G., Borg, J., Perriard, J. C., Chien, K. R. and Caroni, P.: MLP-deficient mice exhibit a disruption of cardiac cytoarchitectural organization, dilated cardiomyopathy, and heart failure. *Cell* 88, 393-403 (1997)
110. Thornell, L., Carlsson, L., Li, Z., Mericskay, M. and Paulin, D.: Null mutation in the desmin gene gives rise to a cardiomyopathy. *J Mol Cell Cardiol* 29, 2107-24 (1997)
111. Gómez, A. M., Aimond, F., Alvarez, J., Artiles, A., Li, Z. L. and G., V.: Cardiac function in desmin-deficient transgenic mice. *Biophys J* 76, A462 (abstract) (1999)
112. Tsutsui, H., Ishihara, K. and Cooper, G. t.: Cytoskeletal role in the contractile dysfunction of hypertrophied myocardium. *Science* 260, 682-7 (1993)
113. Howarth, F. C., Calaghan, S. C., Boyett, M. R. and White, E.: Effect of the microtubule polymerizing agent taxol on contraction, Ca^{2+} transient and L-type Ca^{2+} current in rat ventricular myocytes. *J Physiol (Lond)* 516, 409-19 (1999)
114. Gómez, A. M., Kerfant, B. G. and Vassort, G.: Microtubule Disruption Modulates Ca(2+) Signaling in Rat Cardiac Myocytes. *Circ Res* 86, 30-36 (2000)
115. Kerfant, B. G., Vassort, G. and Gómez, A. M.: Microtubule disruption by colchicine reversibly enhances calcium signaling in intact rat cardiac myocytes. *Circ Res* 88, E59-65. (2001)
116. Kaprielian, R. R., Stevenson, S., Rothery, S. M., Cullen, M. J. and Severs, N. J.: Distinct patterns of dystrophin organization in myocyte sarcolemma and transverse tubules of normal and diseased human myocardium. *Circulation* 101, 2586-94 (2000)
117. Wong, C., Soeller, C., Burton, L. and Cannell, M. B.: Changes In Transverse-Tubular System Architecture In Myocytes From Diseased Human Ventricles. *Biophysical Journal* 80, 588a (abstract) (2001)
118. Adachi-Akahane, S., Cleemann, L. and Morad, M.: Cross-signaling between L-type Ca^{2+} channels and ryanodine receptors in rat ventricular myocytes. *J Gen Physiol* 108, 435-54 (1996)
119. Barrere-Lemaire, S., Piot, C., Leclercq, F., Nargeot, J. and Richard, S.: Facilitation of L-type calcium currents by diastolic depolarization in cardiac cells: impairment in heart failure. *Cardiovasc Res* 47, 336-49. (2000)
120. Ahmmed, G. U., Dong, P. H., Song, G., Ball, N. A., Xu, Y., Walsh, R. A. and Chiamvimonvat, N.: Changes in Ca(2+) cycling proteins underlie cardiac action potential prolongation in a pressure-overloaded guinea pig model with cardiac hypertrophy and failure. *Circ Res* 86, 558-70. (2000)
121. Bers, D. M. and Perez-Reyes, E.: Ca channels in cardiac myocytes: structure and function in Ca influx and intracellular Ca release. *Cardiovasc Res* 42, 339-60 (1999)
122. Delgado, C., Artiles, A., Gómez, A. M. and Vassort, G.: Frequency-dependent increase in cardiac Ca(2+)Current is due to reduced Ca(2+)Release by the sarcoplasmic reticulum [In Process Citation]. *J Mol Cell Cardiol* 31, 1783-93 (1999)
123. Piot, C., Lemaire, S., Albat, B., Seguin, J., Nargeot, J. and Richard, S.: High frequency-induced upregulation of human cardiac calcium currents. *Circulation* 93, 120-8. (1996)
124. Lemaire, S., Piot, C., Leclercq, F., Leuranguer, V., Nargeot, J. and Richard, S.: Heart rate as a determinant of L-type Ca^{2+} channel activity: mechanisms and implication in force-frequency relation. *Basic Res Cardiol* 93 Suppl 1, 51-9. (1998)
125. Molkentin, J. D. and Dorn, I. G., 2nd: Cytoplasmic signaling pathways that regulate cardiac hypertrophy. *Annu Rev Physiol* 63, 391-426. (2001)
126. Molkentin, J. D.: Calcineurin and beyond: cardiac hypertrophic signaling. *Circ Res* 87, 731-8. (2000)
127. Frey, N., McKinsey, T. A. and Olson, E. N.: Decoding calcium signals involved in cardiac growth and function. *Nat Med* 6, 1221-7. (2000)
128. Chien, K. R., Knowlton, K. U., Zhu, H. and Chien, S.: Regulation of cardiac gene expression during myocardial growth and hypertrophy: molecular studies of an adaptive physiologic response. *Faseb J* 5, 3037-46. (1991)
129. Marbán, E. and Koretsune, Y.: Cell calcium, oncogenes, and hypertrophy. *Hypertension* 15, 652-8. (1990)
130. McDonough, P. M., Hanford, D. S., Sprenkle, A. B., Mellon, N. R. and Glembotski, C. C.: Collaborative roles for c-Jun N-terminal kinase, c-Jun, serum response factor, and Sp1 in calcium-regulated myocardial gene expression. *J Biol Chem* 272, 24046-53. (1997)
131. Morgan, J. I. and Curran, T.: Calcium as a modulator of the immediate-early gene cascade in neurons. *Cell Calcium* 9, 303-11. (1988)
132. Ginty, D. D.: Calcium regulation of gene expression: isn't that spatial? *Neuron* 18, 183-6. (1997)
133. Sugden, P. H. and Clerk, A.: Cellular mechanisms of cardiac hypertrophy. *J Mol Med* 76, 725-46 (1998)
134. Hefti, M. A., Harder, B. A., Eppenberger, H. M. and Schaub, M. C.: Signaling pathways in cardiac myocyte hypertrophy. *J Mol Cell Cardiol* 29, 2873-92. (1997)
135. Yamazaki, T., Tobe, K., Hoh, E., Maemura, K., Kaida, T., Komuro, I., Tamemoto, H., Kadowaki, T., Nagai, R. and Yazaki, Y.: Mechanical loading activates mitogen-activated protein kinase and S6 peptide kinase in

cultured rat cardiac myocytes. *J Biol Chem* 268, 12069-76. (1993)
136. Soderling, T. R.: The Ca-calmodulin-dependent protein kinase cascade. *Trends Biochem Sci* 24, 232-6. (1999)
137. Gruver, C. L., DeMayo, F., Goldstein, M. A. and Means, A. R.: Targeted developmental overexpression of calmodulin induces proliferative and hypertrophic growth of cardiomyocytes in transgenic mice. *Endocrinology* 133, 376-88. (1993)
138. Soderling, T. R., Chang, B. and Brickey, D.: Cellular signaling through multifunctional Ca2+/calmodulin-dependent protein kinase II. *J Biol Chem* 276, 3719-22. (2001)
139. Corcoran, E. E. and Means, A. R.: Defining Ca2+/calmodulin-dependent protein kinase cascades in transcriptional regulation. *J Biol Chem* 276, 2975-8. (2001)
140. Passier, R., Zeng, H., Frey, N., Naya, F. J., Nicol, R. L., McKinsey, T. A., Overbeek, P., Richardson, J. A., Grant, S. R. and Olson, E. N.: CaM kinase signaling induces cardiac hypertrophy and activates the MEF2 transcription factor in vivo. *J Clin Invest* 105, 1395-406. (2000)
141. Chien, K. R.: Meeting Koch's postulates for calcium signaling in cardiac hypertrophy. *J Clin Invest* 105, 1339-42. (2000)
142. Leinwand, L. A.: Calcineurin inhibition and cardiac hypertrophy: A matter of balance. *Proc Natl Acad Sci U S A* 98, 2947-9. (2001)
143. Sussman, M. A., Lim, H. W., Gude, N., Taigen, T., Olson, E. N., Robbins, J., Colbert, M. C., Gualberto, A., Wieczorek, D. F. and Molkentin, J. D.: Prevention of cardiac hypertrophy in mice by calcineurin inhibition. *Science* 281, 1690-3. (1998)
144. Atar, D., Backx, P. H., Appel, M. M., Gao, W. D. and Marbán, E.: Excitation-transcription coupling mediated by zinc influx through voltage-dependent calcium channels. *J Biol Chem* 270, 2473-7. (1995)
145. Anderson, M. E.: Connections count : excitation-contraction meets excitation-transcription coupling. *Circ Res* 86, 717-9. (2000)
146. Cartin, L., Lounsbury, K. M. and Nelson, M. T.: Coupling of Ca(2+) to CREB activation and gene expression in intact cerebral arteries from mouse : roles of ryanodine receptors and voltage-dependent Ca(2+) channels. *Circ Res* 86, 760-7. (2000)
147. Stevenson, A. S., Cartin, L., Wellman, T. L., Dick, M. H., Nelson, M. T. and Lounsbury, K. M.: Membrane depolarization mediates phosphorylation and nuclear translocation of CREB in vascular smooth muscle cells. *Exp Cell Res* 263, 118-30. (2001)
148. Bénitah, Perrier, E., Gómez, A. and Vassort, G.: Effects of aldosterone on transient outward K+ current density in rat ventricular myocytes. *J Physiol* 537, 151-160 (2001)
149. Holmer, S. R. and Schunkert, H.: Adaptive and genetic alterations of the renin angiotensin system in cardiac hypertrophy and failure. *Basic Res Cardiol* 91, 65-71. (1996)
150. Pitt, B., Zannad, F., Remme, W. J., Cody, R., Castaigne, A., Perez, A., Palensky, J. and Wittes, J.: The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med* 341, 709-17. (1999)
151. Delcayre, C. and Silvestre, J. S.: Aldosterone and the heart: towards a physiological function? *Cardiovasc Res* 43, 7-12. (1999)
152. Bénitah, J. P. and Vassort, G.: Aldosterone upregulates Ca(2+) current in adult rat cardiomyocytes. *Circ Res* 85, 1139-45. (1999)
153. Milnes, J. T. and MacLeod, K. T.: Reduced ryanodine receptor to dihydropyridine receptor ratio may underlie slowed contraction in a rabbit model of left ventricular cardiac hypertrophy. J Mol Cell Cardiol 33, 473-85. (2001)

Key Words: Heart failure, excitation-contraction coupling, Ca^{2+} signaling, $[Ca^{2+}]_i$ transient, Ca^{2+} sparks, L-type Ca^{2+} current, Ryanodine receptors, Review

Send correspondence to: Dr Ana M. Gómez, Ph.D., Chargée de Recherche CNRS, INSERM-U.390, C.H.U. A. de Villeneuve, 34295 Montpellier, France, Tel: (33) 467 41 52 37, Fax: (33) 467 41 52 42, E-mail: agomez@montp.inserm.fr