

# CardiologyRounds™

AS PRESENTED IN THE ROUNDS OF THE CARDIOVASCULAR DIVISION  
OF BRIGHAM AND WOMEN'S HOSPITAL, BOSTON, MASSACHUSETTS

## Contractile failure during ischemia and congestive heart failure: role of defective excitation-contraction coupling

JOSHUA I. GOLDHABER M.D.

Contractile dysfunction is a hallmark of major cardiac disorders, including acute ischemia, reperfusion, hypertrophy, and failure. Abnormal intracellular calcium ( $\text{Ca}^{2+}$ ) movements are thought to underlie these contractile abnormalities, but detailed pathophysiology has not been established. Contraction of heart muscle is mediated by a  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release mechanism, wherein extracellular  $\text{Ca}^{2+}$  entering subsarcolemmal microdomains via L-type  $\text{Ca}^{2+}$  channels controls the amount of  $\text{Ca}^{2+}$  released into the microdomain by ryanodine receptors on the sarcoplasmic reticulum. This hypothesis was confirmed recently when local  $\text{Ca}^{2+}$  release events were imaged in cardiac cells loaded with  $\text{Ca}^{2+}$ -sensitive indicators using laser scanning confocal microscopy (LSCM). These so-called " $\text{Ca}^{2+}$  sparks" represent the elementary events of excitation-contraction (E-C) coupling in myocytes. Currently, little is known about the behavior of these local  $\text{Ca}^{2+}$  release events in the context of cardiac disease. This issue of *Cardiology Rounds* will:

- review the current understanding of E-C coupling
- introduce the concept of defective E-C coupling in diseased myocardium
- describe work on defective E-C coupling during metabolic stress
- review other models of defective E-C coupling
- discuss the effects of common clinical maneuvers on E-C coupling.

### Normal E-C coupling

Excitation-contraction (E-C) coupling is a highly organized process of signal transduction that governs contractile force in muscle. In the heart, E-C coupling is characterized by a  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release (CICR) mechanism (Figure 1). CICR begins when L-type  $\text{Ca}^{2+}$  channels open during depolarization by the action potential. A small amount of  $\text{Ca}^{2+}$  enters the cell via L-type  $\text{Ca}^{2+}$  channels and triggers ryanodine receptors (RyRs) on the sarcoplasmic reticulum (SR) to open, allowing a much larger release of  $\text{Ca}^{2+}$  from intracellular stores. The released  $\text{Ca}^{2+}$  binds to myofilaments in the cytoplasm resulting in the generation of contractile force. About 20% of the released  $\text{Ca}^{2+}$  is removed from the cell by the sodium-calcium exchanger (NCX) in diastole. The other 80% is pumped back into the SR by its ATP-dependent  $\text{Ca}^{2+}$  pump (SERCA), which is regulated by the inhibitory protein phospholamban (PL). The *local control model* (Figure 2) of E-C coupling is a refinement of the CICR model. The local control model predicts that  $\text{Ca}^{2+}$  trigger and release events occur within *local* and *independent* intracellular microdomains.<sup>1</sup> These local  $\text{Ca}^{2+}$  release events, known as  $\text{Ca}^{2+}$  "sparks," can now be imaged using real-time LSCM,<sup>2</sup> and have been correlated with electrical events<sup>3</sup> using standard patch clamp techniques (Figure 3).



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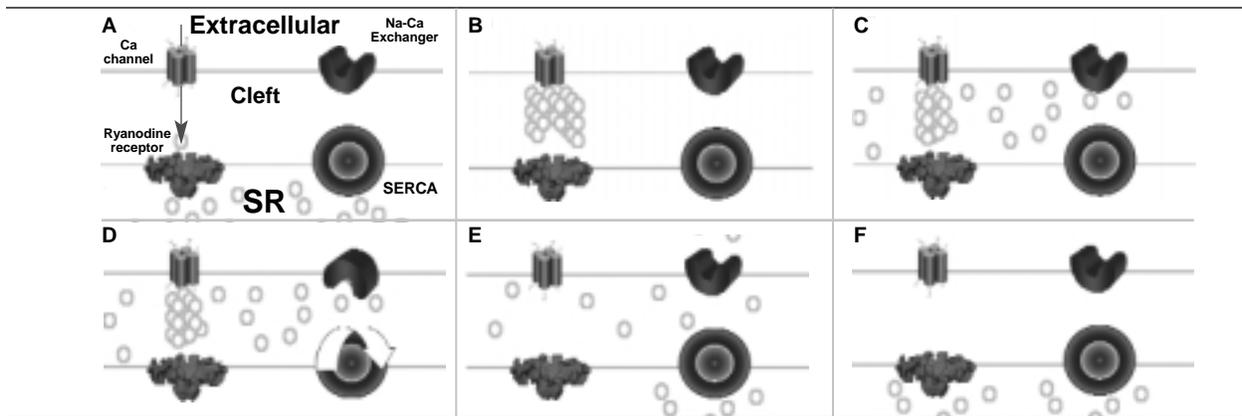
#### Brigham and Women's Hospital

75 Francis Street  
Boston, Massachusetts 02115  
Fax: (617) 732-5291  
Cardiovascular Division Website: [www.heartdoc.org](http://www.heartdoc.org)

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**Figure 1: Schematic of excitation-contraction coupling.**

- A) The four major proteins of E-C coupling are arranged on the sarcolemmal membrane (L-type  $\text{Ca}^{2+}$  channel, Sodium-Calcium exchanger) and on the sarcoplasmic reticulum membrane (Ryanodine Receptor, SERCA). The two membranes surround a restricted space known as the diadic cleft. Extracellular  $\text{Ca}^{2+}$  enters the diadic cleft via the L-type  $\text{Ca}^{2+}$  channel, binds to ryanodine receptors, and triggers  $\text{Ca}^{2+}$  release from the SR.
- B) The  $\text{Ca}^{2+}$  accumulates in the cleft space, giving rise to high localized concentrations of  $\text{Ca}^{2+}$  that can be imaged as  $\text{Ca}^{2+}$  “sparks”, before diffusing into the cytoplasm (C).
- D and E) Some of the  $\text{Ca}^{2+}$  is removed from the cell by the sodium-calcium exchanger, but most is pumped back into the SR by the SERCA.
- F) Finally,  $\text{Ca}^{2+}$  is resequestered by the SR and diastolic  $\text{Ca}^{2+}$  levels return to normal.



The local control model of E-C coupling helps explain a fundamental paradox of CICR; how the amount of  $\text{Ca}^{2+}$  entering via the L-type  $\text{Ca}^{2+}$  current ( $I_{\text{Ca}}$ ) can regulate the amount of  $\text{Ca}^{2+}$  released from the SR, and thus contractile force. True to the predictions of the model, it has been found that the size of  $I_{\text{Ca}}$  is a key determinate of local  $\text{Ca}^{2+}$  release events. A large current through  $\text{Ca}^{2+}$  channels (for example during administration of inotropes such as dobutamine) activates relatively many  $\text{Ca}^{2+}$  sparks and a strong contraction, whereas a smaller current (such as occurs during administration of  $\text{Ca}^{2+}$  channel blockers) activates fewer  $\text{Ca}^{2+}$  sparks and a weaker contraction (Figure 4). Thus the amplitude of  $I_{\text{Ca}}$  is an important regulator of E-C coupling and contractile force.

A second control point for  $\text{Ca}^{2+}$  release is the “gain” of the system, which refers to the effectiveness of  $I_{\text{Ca}}$  at triggering  $\text{Ca}^{2+}$  release from the SR. A decrease in gain implies a reduction in the efficiency of  $I_{\text{Ca}}$  at triggering  $\text{Ca}^{2+}$  release, and consequently a reduction in the force of contraction. An increase in gain may result in spontaneous  $\text{Ca}^{2+}$  release and triggered arrhythmias. Gain can be measured experimentally in patch clamped single ventricular myocytes by measuring how quickly  $\text{Ca}^{2+}$  is released by  $\text{Ca}^{2+}$  currents of varying amplitude. In a “high gain” situation, even small  $\text{Ca}^{2+}$  currents will rapidly release lots of  $\text{Ca}^{2+}$ . In a “low gain” situation, large  $\text{Ca}^{2+}$  currents will release relatively smaller amounts of  $\text{Ca}^{2+}$  at lower rates. Using real-time confocal microscopy, it has become clear that the extent of  $\text{Ca}^{2+}$  spark recruitment by  $I_{\text{Ca}}$  is a key determinate of gain. A high gain situation is one where a given  $I_{\text{Ca}}$  has a higher probability of activating  $\text{Ca}^{2+}$  sparks, whereas a low gain situation is one where the same  $I_{\text{Ca}}$

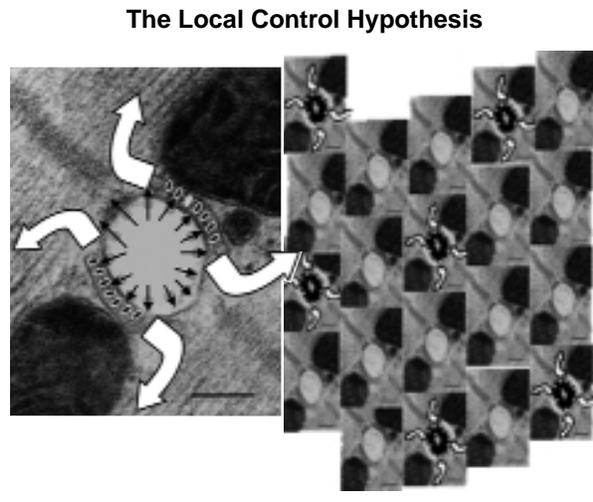
has a lower probability of activating  $\text{Ca}^{2+}$  sparks. Thus an understanding of the regulation of  $\text{Ca}^{2+}$  sparks is essential to understanding how gain, and hence contractility, are controlled in cardiac muscle.

The regulation of  $\text{Ca}^{2+}$  sparks is extremely complex and incompletely understood. The available evidence suggests that spark behavior can be affected by changes in the function of every single component of E-C coupling. These include SERCA-regulated SR  $\text{Ca}^{2+}$  content<sup>4</sup> (although this is an inconsistent finding),<sup>5</sup> RyR activity,<sup>4</sup> and activity of the L-type  $\text{Ca}^{2+}$  channel.<sup>2</sup> Using rapid (240 Hz) 2D LSCM, we have shown that NCX also regulates spontaneous spark frequency,<sup>6</sup> though direct triggering of Ca sparks by NCX has not been demonstrated conclusively.<sup>3</sup>

### Defective E-C coupling in diseased myocardium: metabolic stress

Abnormal  $\text{Ca}^{2+}$  regulation and effects of ischemia and heart failure on major proteins involved in E-C coupling have been studied in great detail. Nevertheless, the underlying pathogenesis of contractile abnormalities during ischemia and heart failure is not completely understood. Considerable attention has been applied to the nature and extent of expression and function of E-C coupling proteins in hypertrophy and heart failure. Confusion and controversy have resulted, in large part due to the extraordinary variety of experimental models of hypertrophy and failure. The lack of correlation between mRNA levels and protein expression, as well as a paucity of functional data in many heart failure models, further confounds our understanding of this heterogeneous disease. An excellent review on this subject was published recently by Hasenfuss.<sup>7</sup>

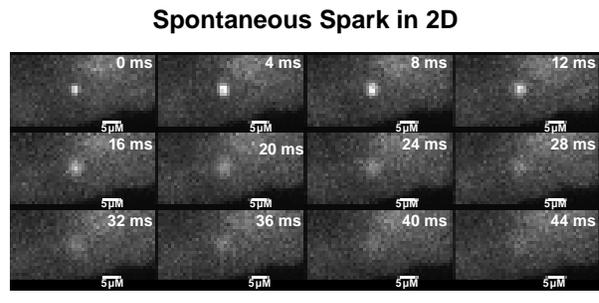
**Figure 2: The local control hypothesis of E-C coupling predicts that  $\text{Ca}^{2+}$ -induced  $\text{Ca}^{2+}$ -release occurs in local independent microdomains within the subcellular architecture of the cell. Under physiological conditions, it is hypothesized that the activation of one local site does not activate distant sites. Sites are located at transverse tubule spacing, shown here schematically in cross-section.**



Depressed metabolism, a potential cause of abnormal intracellular  $\text{Ca}^{2+}$  regulation and hence defective E-C coupling, is a common feature of both ischemia<sup>8</sup> and congestive heart failure.<sup>9</sup> In the myocardium, as well as other tissues, ATP synthesis and utilization are subcellularly localized.<sup>10</sup> Several studies suggest that glycolysis may play a special role in maintaining  $\text{Ca}^{2+}$  homeostasis during ischemia/reperfusion,<sup>11</sup> particularly when cardiac workload is elevated.<sup>12</sup>

The effects of metabolic stress can readily be studied in patch clamped ventricular myocytes by rendering bathing solutions hypoxic or by applying metabolic inhibitors. The patch clamp technique is used to record isolated membrane currents, such as the  $\text{Ca}^{2+}$  and NCX currents. This technique also allows investigators to calculate changes in total ionic movements across cell membranes during experimental interventions.<sup>13</sup> When  $\text{Ca}^{2+}$  sensitive indicators are included in the patch electrode solution, intracellular  $\text{Ca}^{2+}$  concentration can be monitored simultaneously. This approach was used in guinea pig ventricular myocytes loaded with the  $\text{Ca}^{2+}$  sensitive indicator fura-2 to study the effects of metabolic stress on E-C coupling.<sup>14</sup>  $I_{\text{Ca}}$ , intracellular  $\text{Ca}^{2+}$  concentration and cell contraction amplitude were simultaneously monitored. The study indicated that inhibition of metabolism with a mitochondrial uncoupler, FCCP (carbonyl cyanide-p-trifluoromethoxyphenylhydrazone), and a glycolytic inhibitor, 2-deoxyglucose (2-DG), simultaneously reduces  $I_{\text{Ca}}$ , SR  $\text{Ca}^{2+}$  release, and cell shortening amplitude (Figure 5). Since E-C coupling theory predicts that the amplitude of the  $\text{Ca}^{2+}$  current determines the extent of  $\text{Ca}^{2+}$  release from the SR, it is not surprising that reduced  $\text{Ca}^{2+}$

**Figure 3: Successive images at 4 ms intervals showing a spontaneous  $\text{Ca}^{2+}$  “spark” recorded with rapid 2-dimensional laser scanning confocal microscopy in a patch clamped rat ventricular myocyte loaded with the  $\text{Ca}^{2+}$  indicator fluo-3<sup>®</sup>. Scale = 5  $\mu\text{M}$ .**



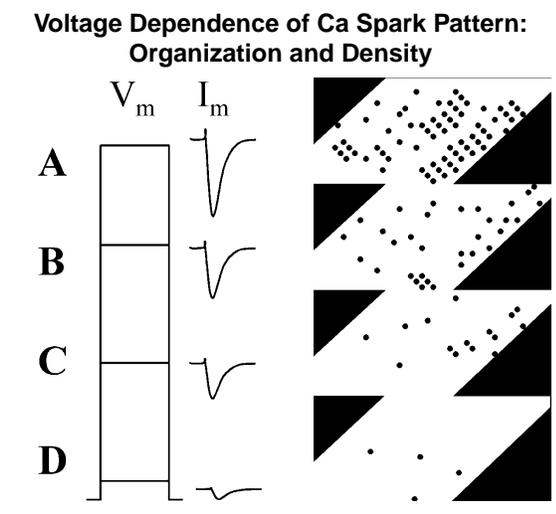
current leads to reduced  $\text{Ca}^{2+}$  release. However, surprisingly, metabolic inhibition also reduced the gain of E-C coupling. Such a result might have been easily explained by a depletion of SR  $\text{Ca}^{2+}$  resulting from the cell's profound metabolic challenge, but further experiments demonstrated that SR  $\text{Ca}^{2+}$  stores were completely unaffected. Since SR  $\text{Ca}^{2+}$  stores remained intact, it was hypothesized that metabolic inhibition interfered with the ability of  $\text{Ca}^{2+}$  entering via L-type  $\text{Ca}^{2+}$  channels to trigger the opening of RyRs.

The SERCA pump is ATP-dependent. Thus, at first glance it is surprising that SR  $\text{Ca}^{2+}$  stores remain intact during metabolic inhibition. It is speculated that even a metabolically compromised SERCA can maintain normal SR  $\text{Ca}^{2+}$  stores as long as SR  $\text{Ca}^{2+}$  release is limited by ineffective triggering of RyRs by  $I_{\text{Ca}}$ .

### The response of $\text{Ca}^{2+}$ sparks to metabolic stress

A new development in confocal imaging is the rapid (240–480 frames per second) 2D LSCM. Using this technique in conjunction with aggressive  $\text{Ca}^{2+}$  buffering, Cleemann et al<sup>15</sup> found that depolarization leads to the coordinated release of  $\text{Ca}^{2+}$  from distinct sites (assumed to be recruited sparks) along the transverse tubules of the myocyte, eliciting a pattern of stripes that run along sarcomeres. Recruitment of  $\text{Ca}^{2+}$  release sites appeared to follow the voltage dependence of E-C coupling (Figure 4).<sup>15</sup> Cleemann's study and others like it support the results of earlier experiments suggesting that recruitment of  $\text{Ca}^{2+}$  release sites and summation of independently functioning  $\text{Ca}^{2+}$  sparks determine the characteristics of the whole cell  $\text{Ca}^{2+}$  transient. If sparks are stereotypical unitary events controlled by single channel properties of the L-type  $\text{Ca}^{2+}$  channel, as predicted by the local control hypothesis, then we would anticipate that the integrity of the 2D pattern could be used to calculate E-C coupling gain. A decrease in gain (reduced efficacy of  $I_{\text{Ca}}$ ) would result in a loss of release sites without a corresponding drop in macroscopic  $I_{\text{Ca}}$  (A in Figure 6). If sparks do not conform to the local control hypothesis and are not stereotypical, then a decrease

**Figure 4: The density of Ca<sup>2+</sup> “sparks” is graded by the amplitude of the macroscopic Ca<sup>2+</sup> current, which was manipulated in this schematic example by increasing the voltage of a test potential. Note that the Ca<sup>2+</sup> “sparks” align themselves into “lines” or “strings” as the Ca<sup>2+</sup> current becomes larger during stronger depolarizations to higher potentials.**

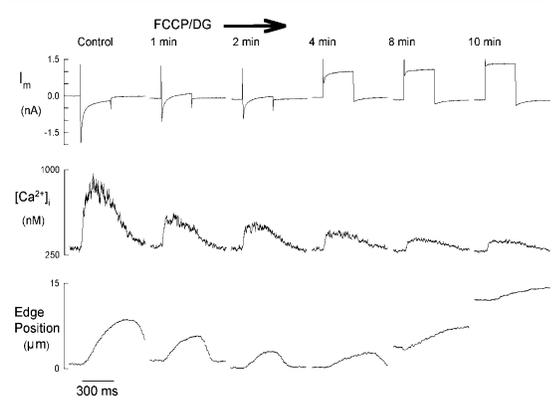


in gain could manifest itself as a decrease in Ca<sup>2+</sup> flux across intact release sites without any drop-out whatsoever (B in Figure 6). A combination of both effects must also be considered. Preliminary experiments suggest that metabolic inhibition leads to a disintegration of the Ca<sup>2+</sup> release pattern without affecting Ca<sup>2+</sup> flux across individual Ca<sup>2+</sup> release sites, well before there were significant reductions in I<sub>Ca</sub> or SR Ca<sup>2+</sup> stores.<sup>16</sup> These results are consistent with defective E-C coupling due a reduced ability of I<sub>Ca</sub> to activate local Ca<sup>2+</sup> release sites. The underlying mechanism of this decrease in gain remains to be determined, but may play a role in the impaired contractile state observed during metabolic inhibition.

#### Defective E-C coupling in a model of heart failure: Similarities to metabolic stress

Loss of E-C coupling gain has been described previously in hypertrophic and failing myocytes from the Dahl salt-sensitive rat.<sup>17</sup> Patch clamped myocytes isolated from rats with cardiac hypertrophy or heart failure had smaller Ca<sup>2+</sup> transients and fewer Ca<sup>2+</sup> sparks in response to depolarization (compared to controls), despite normal I<sub>Ca</sub> density, RyR function and SR Ca<sup>2+</sup> content. In other words, they had observed a decrease in gain at the subcellular level, which suggested a defect in the coupling of the Ca<sup>2+</sup> channel to the RyR. The authors proposed that a widening of the diadic cleft was responsible for this uncoupling, and speculated that such cleft widening might be a

**Figure 5: L-type Ca<sup>2+</sup> currents (top row, recorded during voltage clamps using the patch clamp technique), simultaneous intracellular Ca<sup>2+</sup> transients (middle row, recorded with the Ca<sup>2+</sup> indicator fura-2), and simultaneous cell edge motion (bottom row, recorded using a cell edge detector) from an enzymatically isolated guinea pig ventricular myocyte during exposure to metabolic inhibitors (FCCP and 2-DG). Metabolic inhibition reduced the amplitude of the inward Ca<sup>2+</sup> current, which resulted in a reduction in the Ca<sup>2+</sup> transient and cell edge motion. Further analysis revealed that the reduction in the Ca<sup>2+</sup> transient exceeded the amount predicted by the decrease in the Ca<sup>2+</sup> current, indicating a reduction in E-C coupling gain.<sup>14</sup>**



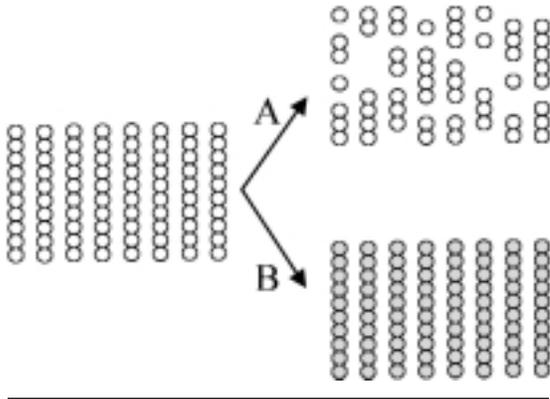
common cause of contractile dysfunction in heart failure. A recent study has confirmed that changes in transverse-tubule orientation can occur in another heart failure model.<sup>18</sup> However, it seems unlikely that changes in t-tubule structure explain our results with metabolic inhibition, which we have shown also gives rise to defective E-C coupling with reduced gain. Since metabolic stress is a common feature of heart failure, it is speculated that metabolic abnormalities could explain the findings in the Dahl salt-sensitive rat.

Previous observations suggest that sodium-calcium exchange (NCX) activity can influence the frequency of Ca<sup>2+</sup> spark activity in resting cells. The effect on frequency implies the potential to alter gain and E-C coupling during depolarization. In many models of hypertrophy and heart failure, NCX activity is significantly increased.<sup>7</sup> However, an effect of metabolic inhibition on NCX activity has not been observed.<sup>14</sup>

#### The hyperphosphorylation hypothesis of defective E-C coupling

Marx *et al.* have reported that RyRs become hyperphosphorylated in several different heart failure models, including human heart failure, presumably due to the chronic elevation in systemic catecholamines.<sup>19</sup> Hyperphosphorylation apparently disassociates FK binding protein (FKBP12.6) from RyRs,

**Figure 6: Possible responses of Ca<sup>2+</sup> release sites in a cell exposed to metabolic inhibitors.**

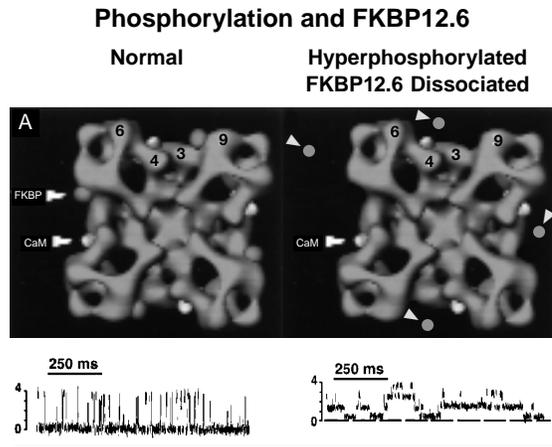


causing RyRs to increase their rate of Ca<sup>2+</sup> “leak” during diastole, resulting in SR Ca<sup>2+</sup> depletion and reduced E-C coupling efficiency (Figure 7). Reversal of hyperphosphorylation was observed in several patients placed on left ventricular assist device support.<sup>19</sup> Although these findings await confirmation from other laboratories, Marks has speculated that RyR hyperphosphorylation may underlie the defective E-C coupling common to all forms of CHF.<sup>20</sup> Since Ca<sup>2+</sup> stores generally remain intact during metabolic inhibition,<sup>14</sup> it seems unlikely that the hyperphosphorylation hypothesis can by itself explain all aspects of defective E-C coupling during ischemia and heart failure. Indeed, a profound depression of SERCA activity would be required to prevent compensation for the leak due to hyperphosphorylation. It turns out that SERCA activity is reduced in many, but not all heart failure models, and contractile amplitude has been significantly improved by transfecting failing myocytes with SERCA DNA.<sup>21</sup>

#### Effects of clinical maneuvers on E-C coupling gain

Pharmacologic treatments for heart failure necessarily affect E-C coupling. The prototypical treatment of heart failure patients with digoxin has no demonstrated survival benefit, but has been shown to improve clinical measures of well-being, and decrease frequency of hospitalization for heart failure exacerbation.<sup>22</sup> Digoxin increases contractile amplitude by inhibiting the Na<sup>+</sup> pump, which results in increased intracellular Na<sup>+</sup> concentration and consequently reduced efflux of Ca<sup>2+</sup> by the NCX. This favors increased sequestration of Ca<sup>2+</sup> by the SR. The increased SR Ca<sup>2+</sup> is expected to increase E-C coupling gain, and therefore the Ca<sup>2+</sup> current becomes more efficient at releasing Ca<sup>2+</sup>.

**Figure 7: Hyperphosphorylation of the ryanodine receptor (RyR) is thought to occur in heart failure, resulting in dissociation of FK binding protein (FKBP12.6). This appears to prevent RyRs from closing completely (see single channel records below), and may result in chronic leak of Ca<sup>2+</sup> from the SR and depletion of SR Ca<sup>2+</sup> stores if SERCA is compromised.<sup>19,25</sup>**



Beta agonists (eg, dobutamine) and phosphodiesterase inhibitors (eg, milrinone) also increase SR Ca<sup>2+</sup> stores, but their mechanism of action is different than digoxin. These agents work by phosphorylating multiple E-C coupling proteins, including L-type Ca<sup>2+</sup> channels, and the SERCA-associated inhibitory protein phospholamban (PL). When PL is phosphorylated, its inhibitory effect on the SERCA pump is reduced. The net result is increased loading of SR Ca<sup>2+</sup> stores and an increase in the Ca<sup>2+</sup> transient amplitude (and thus contractile force) upon depolarization. However, chronic treatment with these agents is associated with increased sudden death,<sup>23</sup> likely caused by an increased rate of spontaneous diastolic release of Ca<sup>2+</sup> from the SR. Spontaneous Ca<sup>2+</sup> release in diastole produces arrhythmic transient inward currents through NCX, which result in delayed after-depolarizations and arrhythmia initiation. Although SR Ca<sup>2+</sup> overload can by itself provoke spontaneous Ca<sup>2+</sup> release, it seems likely that increased openings of L-type Ca<sup>2+</sup> channels at diastolic potentials (caused by beta agonist-induced phosphorylation of I<sub>Ca</sub>) contribute to the increased frequency of pathologic diastolic SR Ca<sup>2+</sup> release and sudden death observed with chronic beta agonist administration.

Beta-blockers acutely reduce contractile force by depressing I<sub>Ca</sub> and therefore Ca<sup>2+</sup> release from the SR. Although reduced contractility is observed upon initiation of beta-blocker therapy, long-term treatment of heart failure patients with beta-blockers has paradoxi-

cally proven to be an effective method of improving ejection fraction. The neurohormonal hypothesis has provided an excellent rationale for the beneficial effect of chronic beta-blockers on ventricular remodeling.<sup>24</sup> E-C coupling studies suggest that long-term beta-blockade may also improve contractility by reversing the maladaptive hyperphosphorylation of RyRs. This would help to reduce SR Ca<sup>2+</sup> leak, resulting in preserved SR Ca<sup>2+</sup> stores and improved E-C coupling gain.

It remains uncertain how the many complex changes in metabolism and E-C coupling protein expression and function interact to impair E-C coupling and cardiac function. Improved understanding of E-C coupling may lead to novel therapies for improving contractility in patients with heart failure and reducing susceptibility to acute contractile failure during ischemia syndromes.

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**Joshua I. Goldhaber, MD** is an Associate Professor of Medicine at the UCLA School of Medicine, Division of Cardiology. He chairs the Cardiovascular Pathophysiology course at UCLA, and has won numerous teaching awards. He is a Council member of the Western Society of Clinical Investigation, and a Principal Investigator in UCLA's Cardiovascular Research Laboratories, a collaborative research unit administered jointly by the Departments of Medicine and Physiology. His research interests include abnormal E-C coupling and arrhythmogenesis during ischemia, reperfusion and congestive heart failure. Dr. Goldhaber received his M.D. from Harvard Medical School in 1984, and subsequently completed his internship and residency in Internal Medicine at UCLA. His clinical fellowship in Cardiology was also at UCLA. He can be reached at [jgoldhaber@mednet.ucla.edu](mailto:jgoldhaber@mednet.ucla.edu).

Brigham and Women's Hospital,  
Cardiovascular Division website:  
[www.heartdoc.org](http://www.heartdoc.org)

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