Myocardial Stunning and Hibernation
The Physiology Behind the Colloquialisms

Eduardo Marban, MD, PhD

The recent spectacular progress in the treatment of coronary artery disease has led to a growing awareness of the contractile abnormalities that accompany ischemia and reperfusion. Myocardium subjected to low coronary flow exhibits a reversible decrease in the force of contraction, a phenomenon known as “hibernation.” A different sort of contractile dysfunction, “stunning,” prevails during reperfusion after brief periods of ischemia: force development remains impaired for days despite the maintenance of histological integrity. Unfortunately, our understanding of perfusion-related contractile dysfunction remains quite limited, particularly in comparison with the richness of our therapeutic repertoire. In this paper, I wish to apply a new, more physiological approach to cardiac contractile dysfunction based on investigation of the various steps in excitation–contraction coupling. To accomplish this, I focus rather narrowly on research from my own laboratory. Several excellent reviews summarize other aspects of the biology of ischemia and reperfusion. The reader is also referred elsewhere for discussion of the potentially important clinical manifestations of stunning and hibernation.

Background

The new sophistication in revascularization therapy for coronary artery disease has heightened our awareness of the diverse effects of perfusion on cardiac function. Total ischemia leads to a prompt cessation of contraction. Reperfusion after periods of ischemia brief enough to prevent necrosis results in impaired contractile force generation, stunning, which persists for days to weeks after the ischemic event. The histological and metabolic abnormalities in stunned myocardium are relatively minor and provide few clues as to the origin of the dysfunction. Yet another manifestation of the effects of perfusion on contraction is myocardial hibernation, the reversible contractile depression that occurs during a mild-to-moderate decrease in coronary perfusion. Considerable evidence favors the notion that force can respond to changes in flow even in the absence of ischemia (“Gregg’s phenomenon”), but the mechanism remains unclear. The distinctive features of myocardial stunning and hibernation are summarized in Table 1.

Despite the rich variety of colloquialisms and eponyms that have evolved to describe the contractile abnormalities during ischemia and reperfusion, little is known about their pathophysiology at the cellular level. Do any of these conditions result from a failure of excitation? Is the availability of activator Ca\(^{2+}\) decreased by other means, such as by impaired Ca\(^{2+}\) release from the sarcoplasmic reticulum? Alternatively, is the myofilament responsiveness to Ca\(^{2+}\) reduced?

The various types of contractile dysfunction must involve changes in one or more steps of the final common pathway for contractile activation, depicted schematically in Figure 1. The left-hand column shows hypothetical Ca\(^{2+}\) transients (the phasic increase in cytoplasmic Ca\(^{2+}\) concentration during each heartbeat), whereas the right-hand column depicts the resulting twitch contractions. Myofilament Ca\(^{2+}\) responsiveness is given by the relation between intracellular free Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) and force, depicted graphically above the arrows in the central column. Panel A diagrams a normal Ca\(^{2+}\) transient and its corresponding twitch contraction. Panels B, C, and D show three distinct (but not mutually exclusive) mechanisms whereby twitch force can be depressed. If Ca\(^{2+}\) transients are made smaller (B), the force produced will be lower. Alternatively, force can fall by a decrease in myofilament Ca\(^{2+}\) responsiveness, even if Ca\(^{2+}\) transients remain unchanged. This can take either of two forms: a shift in Ca\(^{2+}\) sensitivity such that the range of contractile activation moves to higher [Ca\(^{2+}\)], (C), or a decrease in the maximal Ca\(^{2+}\)-activated force, which scales the overall response (D).

Hypoxia and Metabolic Inhibition

The experimental strategies required to dissect the various elements of excitation–contraction coupling in appropriate models have lagged behind the clinical progress. Ischemia, hibernation, and stunning are
vascularly induced phenomena by definition; realistic models must therefore rely on arterially perfused myocardium, not on isolated cells or superfused muscle preparations. On the other hand, hypoxia and metabolic inhibition are simple interventions that can be applied to isolated, superfused preparations and that mimic at least some of the features of ischemia. These interventions are also interesting in their own right because they enable the study of graded reduction in substrate and of various intermediates of metabolism. It is thus worth considering first what is known about excitation–contraction coupling in hypoxia.

During the early stages of hypoxia, Allen and Orchard found that contractile failure is not accompanied by a decrease in Ca²⁺ transients reported by the Ca²⁺-activated photoprotein aequorin microinjected into ferret papillary muscles. The authors implicated a decrease in myofilament Ca²⁺ responsiveness, due either to acidosis or to inorganic phosphate (P_i) accumulation, as the basis of hypoxic contractile failure. The observation that Ca²⁺ transients are not reduced when aequorin is microinjected into cells contrasts with data of McKinnon et al. who reported that hypoxia decreases (but does not abolish) Ca²⁺ transients in muscles chemically loaded with aequorin. Unfortunately, the metabolic integrity of preparations thus loaded has been questioned. With severe hypoxia in single rat ventricular cells, the first harbingers of contractile dysfunction is failure of the action potential and a consequent cessation of Ca²⁺ transients. The variability of the results makes it important to reexamine the effects of hypoxia in a preparation that permits ready comparison to true ischemia, particularly if hypoxia is to be used rationally as an analogue of ischemia.

In the isovolumically contracting perfused heart, we first investigated the effects of hypoxia using a new strategy for measuring maximal Ca²⁺-activated pressure, which is homologous to maximal Ca²⁺-activated force in isolated muscle; this strategy called for tetanizing ferret hearts perfused with high-calcium solutions. Maximal Ca²⁺-activated pressure depends only on myofilament properties; during a tetanus, [Ca²⁺]_i reaches levels that are sufficiently high to saturate the myofilaments. We found that maximal Ca²⁺-activated pressure decreases during hypoxia, confirming that myofilament Ca²⁺ responsiveness is decreased and implicating changes in maximal Ca²⁺-activated pressure as at least part of the explanation (as in Figure 1D). By combining the measurements of maximal Ca²⁺-activated pressure with phosphorus-31 nuclear magnetic resonance (³¹P NMR) spectroscopy during hypoxia, we found that P_i accumulation, but not acidosis, correlated well with the observed contractile failure. Taken together, our results and those of Allen and Orchard imply that an accumulation of P_i, via its well-known inhibitory effect on crossbridge cycling, figures prominently in the pathogenesis of hypoxic contractile failure. In the same perfused heart model used for our measurements of maximal Ca²⁺-activated pressure, we recently confirmed the primacy of changes in P_i by verifying that Ca²⁺ transients are not decreased during relatively mild hypoxia.

**Methodological Challenges in Intact Hearts**

In contrast to hypoxia, which can be induced in superfused muscles and cells, realistic models of myocardial stunning and hibernation require the use of perfused myocardial preparations. Both phenomena are readily demonstrable in situ regional models, but isolated, perfused hearts allow considerably greater experimental flexibility. For stunned myocardium, whole-heart models have begun to clarify the nature of the impairment in excitation–contraction coupling. We found that the pressure developed by ferret hearts is decreased by 35%
during reperfusion after 15 minutes of total global ischemia, at a time when $^{31}$P NMR spectroscopy reveals the P, and intracellular pH (pH$_i$) have returned to normal levels.$^{27}$ (Modest ATP depletion is observed, but various lines of evidence indicate it has no causative role in the contractile impairment.$^{27-29}$)

We also found that maximal Ca$^{2+}$-activated pressure is decreased (Figure 1D), but only by 20%; the decrease in maximal Ca$^{2+}$-activated pressure thus contributes to, but cannot fully account for, the decrease in twitch force.$^{27}$ A superimposed decrease in Ca$^{2+}$ transients (Figure 1B) and/or a shift in Ca$^{2+}$ sensitivity (Figure 1C) must be sought to complete the characterization of excitation–contraction coupling in stunned myocardium.

Although the ability to measure maximal Ca$^{2+}$-activated pressure has made it possible to probe myofilament Ca$^{2+}$ responsiveness directly, measurements of [Ca$^{2+}$]$_i$ are also required for a comprehensive evaluation of excitation–contraction coupling. A decrease in the amplitude of Ca$^{2+}$ transients (Figure 1B) is an obvious possibility in either hibernating or stunned myocardium. In fact, the most frequently cited causal agents for stunning (free radicals$^5$ and ATP depletion$^9$) are implicitly or explicitly assumed to decrease force by reducing cytoplasmic activator Ca$^{2+}$.$^1$ Direct verification or exclusion of this hypothesis would help focus the effort to identify the specific mediators of injury that feed into the final common pathway of contractile activation sketched in Figure 1.

My coworkers and I recently developed an approach to measure Ca$^{2+}$ transients in perfused hearts that has enabled us to address these questions. NMR spectroscopy is used to detect signals from the fluorinated Ca$^{2+}$ indicator 5,5′-F$_2$-BAPTA (5F-BAPTA),$^{30,31}$ which can be readily loaded into perfused hearts by exposure to the acetoxymethyl ester derivative.$^{32,33}$ With gated data acquisition, [Ca$^{2+}$]$_i$ can be estimated at various times throughout the cardiac cycle.$^{34,35}$ Figure 2A shows gated $^{19}$F NMR spectra acquired from a ferret heart at the two times in the cardiac cycle indicated above the pressure record. Each spectrum consists of three peaks: the first at 8 parts per million (ppm), reporting 5F-BAPTA that is bound to calcium (B); the second at 2 ppm, arising from 5F-BAPTA free in the cytoplasm (F); and the third peak at 0 ppm, produced by a standard of 6F-tryptophan in the left ventricular cavity. [Ca$^{2+}$]$_i$ can be calculated by multiplying the ratio of the areas under the two cytoplasmic peaks, [B] and [F], by the dissociation constant for Ca-5F-BAPTA ($K_d$, $\sim$300 nM at 30°C$^{36}$):

**FIGURE 2.** Panel A: Gated fluorine-19 nuclear magnetic resonance spectra acquired at two times in cardiac cycle (a: 10 msec before stimulus; b: 75 msec after stimulus) from a heart loaded with 5,5′-F$_2$-BAPTA ([Ca]$_o$=8 mM). Panel B: Ca$^{2+}$ transients and contractile pressure in another heart; [Ca$^{2+}$]$_i$ was calculated at each point from gated spectra like those in A during perfusion with 2 mM [Ca]$_o$ (left) or 8 mM [Ca]$_o$ (right). Reprinted with permission.$^{34}$
was obtained under control perfusion conditions. The heart was then subjected to 15 minutes of total global ischemia at 37°C. After 20 minutes of reperfusion, the new developed pressure was approximately 40% lower than it had been in control; our previous research has shown that the contractile dysfunction produced by this intervention in the ferret heart fulfills the standard criteria for stunned myocardium. What is remarkable here is that the Ca$^{2+}$ transients underlying this contractile dysfunction (B) are, if anything, greater than in control (A); the availability of cytoplasmic activator Ca$^{2+}$ is certainly not decreased in this postischemic heart.

Pooled data from seven hearts are summarized in Figure 3C. Paradoxically, systolic [Ca$^{2+}$]i, was increased in the postischemic hearts; the changes in diastolic [Ca$^{2+}$]i, were relatively minor and not consistent. As a corollary to the increase in peak-systolic [Ca$^{2+}$]i, the mean amplitude of Ca$^{2+}$ transients was significantly higher in the postischemic hearts than in control (0.78 versus 0.43 μM, p < 0.01; see Kusuoka et al37 for details). The changes are in the wrong direction to explain the postischemic decrease in contractility; the force generated by the reperfused myocardium would have been even lower if Ca$^{2+}$ transients had remained unchanged. Thus, these results point to a decrease in myocardial Ca$^{2+}$ responsiveness, not in activator Ca$^{2+}$ availability, as the primary abnormality of excitation-contraction coupling in stunned myocardium.

NMR is particularly powerful in that [Ca$^{2+}$]i, and energy metabolism can be sampled in the same hearts. We thus verified that the impairment of contraction was not due to the well-known effects of either pH or Pi, on the myofilaments: we also determined the extent of ATP depletion. Unlike hypoxia, in which Pi is markedly elevated and modest acidosis occurs, neither Pi nor pH, was significantly different from control values in the reperfused hearts. Consistent with previous findings, only mild ATP depletion was observed. In summary, the observable changes in steady-state high-energy phosphate concentrations do not help explain postischemic dysfunction.

These observations point to a problem at the level of the myofilaments, but provide no indication as to why their responsiveness to Ca$^{2+}$ is reduced in the stunned heart. Several lines of evidence, reviewed elsewhere, suggest that transient cellular calcium overload may be the culprit. It is intriguing to wonder whether ischemia and/or reperfusion might facilitate calcium-mediated proteolysis of one or more of the contractile protein elements; the slow but complete recovery of contractile capacity could reflect the time course of replacement of the damaged contractile proteins by newly translated polypeptides.

**Hibernation**

Given the major differences in the factors that trigger stunning on the one hand and hibernation on the other, a fundamental discrepancy in the excita-
The range of coronary pressures above 60 mm Hg, pressure development by the left ventricle varies more than twofold (panel C, p < 0.001), despite the absence of significant changes in lactate production (A), pH, (B), or [P] (B) over this range (p ≥ 0.1 by analysis of variance). The dashed vertical line demarcates coronary pressures of more than 60 mm Hg, at which energy metabolism is comparable to control, from those of 55 mm Hg or less, at which significant differences begin to occur. The range above 60 mm Hg is presumably where pure “hibernation” occurs, given the paucity of metabolic evidence of ischemia and the complete reversibility of the contractile changes at these pressures.

The central abnormality of excitation–contraction coupling in hibernating myocardium is revealed in Figure 5. Column A shows left ventricular developed pressure, the Ca²⁺ transient, and a 31P NMR spectrum obtained under control conditions (80 mm Hg perfusion pressure). During each cardiac cycle, [Ca²⁺] increases from approximately 150 nM in diastole to 1.7 μM at peak systole, consistent with estimates from aequorin in papillary muscles. The 31P spectrum is typical of well-oxygenated myocardium and shows a very small P₁ peak at 4–5 ppm, a prominent phosphocreatine resonance at 0 ppm, and the three ATP peaks. The shift between P₁ and phosphocreatine reports a pH of 7.11. Coronary pressure was then decreased to 60 mm Hg, and the corresponding records in column B were obtained when developed pressure had reached a new steady state at slightly less than 50% of the control value (compare A with B, top panels). Interestingly, another Ca²⁺ transient acquired at this time reveals a marked decrease in amplitude; diastolic [Ca²⁺], was little changed, but [Ca²⁺], now reaches only ~500 nM in systole. On the other hand, the 31P spectrum changes very little; at 60 mm Hg, prominent phosphocreatine and ATP signals persist, and the P₁ resonance increases only slightly. Thus, the striking decrease in [Ca²⁺], is not associated with clear-cut metabolic evidence of ischemia. The decrease in the amplitude of Ca²⁺ transients almost certainly suffices to explain the negative inotropic effect. The decrease in force was quickly reversible (C, upper panel), as was that in systolic [Ca²⁺], (C, lower panel); this intervention thus satisfies the criteria described above for hibernating myocardium. Such changes in Ca²⁺ transients occurred consistently and were statistically significant.

The results indicate that a decrease in Ca²⁺ transients underlies the contractile dysfunction of myocardial hibernation. Unlike stunned myocardium, no decrease in myofilament Ca²⁺ responsiveness need be postulated here.

With moderate decreases in perfusion pressure, as in Figure 5, the paucity of changes in the 31P NMR spectra contrast with the clear-cut effect of coronary pressure on Ca²⁺ transients. Such perfusion-induced changes in [Ca²⁺], are striking, but their mechanism is not yet clear. Whereas changes in P₁, pH, and ATP...
certainly contribute to contractile failure when ischemia is prolonged and severe,3 such factors cannot explain the modulation of contractile force by less extreme alterations of coronary perfusion. Notably, force can actually increase when perfusion pressure is increased above ordinary control levels,12,44 suggesting that factors other than ischemia must be operative. An alternative mechanism that has attracted considerable attention is the “garden hose effect”: distension of the intracardiac coronary arteries is proposed to stretch the myocardial cells surrounding the vessels, resulting in increased contractile force via a Frank-Starling mechanism.45 This hypothesis is supported by histological examination of arrested hearts, which exhibit a modest increase in sarcomere length at supranormal coronary pressures.46 In contrast, Kitakaze and I42 found that an increase in perfusion pressure leads to an increase in force, even in hearts in which the end-diastolic length has already been stretched to optimal levels. This observation argues against a change in sarcomere length as the sole cause of the changes in the force of contraction and in \([\text{Ca}^{2+}]\). A thorough reexamination of the biochemical basis of vascularity-induced alterations in cardiac contraction is clearly warranted.

Limitations

Although the definition of stunning used here is generally accepted, there is little consensus in the literature regarding the meaning of hibernation. The term has been used to encompass dysfunction that may be associated with moderate or severe ischemia and exhibits only delayed reversibility.10,11,47–49 Taken to its logical extreme, the intuitive notion that hibernating myocardium is dysfunctional muscle that can be awakened by reperfusion, without lasting consequences, might even include the process of stunning. To avoid such potential overlap, I have chosen a pure but much more restrictive definition of hibernation than is commonly used. The distinction between stunning and hibernation becomes particularly problematic in classifying individual patients with coronary artery disease.5 Nevertheless, the features that characterize the ideal models may eventually help to distinguish stunning from hibernation in humans, as in vivo metabolic assay techniques (including, but not limited to, NMR spectroscopy) become more refined.

Given the important limitations of the 5F-BAPTA/NMR technique for measuring \([\text{Ca}^{2+}]\), (particularly calcium buffering and limited time resolution46), it would be highly desirable to compare our results with those obtained by complementary methods. One such method uses fluorescence rather than NMR to detect the \([\text{Ca}^{2+}]\) indicator indo-1 in perfused hearts.50,51 Although this technique can provide important qualitative information regarding \([\text{Ca}^{2+}]\) transients, \([\text{Ca}^{2+}]\), has defied quantitation: in rabbit
hearts loaded with indo-1-AM, only 5–7% of the signal is sensitive to changes in cytoplasmic [Ca\textsuperscript{2+}], presumably because of compartmentalization or partial deesterification.\textsuperscript{51} Aequorin can yield real-time measurements of Ca\textsuperscript{2+} transients with an excellent signal-to-noise ratio in perfused hearts\textsuperscript{52}; this important new method relies on a chemical loading technique that transiently disrupts cell membrane integrity. A recent report in which aequorin has been directly microinjected into the interventricular septum of perfused ferret hearts\textsuperscript{53} points to a promising alternative approach. It is encouraging to note that [Ca\textsuperscript{2+}]	extsubscript{i} measurements from directly microinjected aequorin reveal no decrease in Ca\textsuperscript{2+} transients in stunned hearts,\textsuperscript{53} consistent with our findings.

Finally, it should be noted that this article presents a highly personal perspective and is meant to be taken as an interim synopsis rather than as the final word on a complex topic. Future research with alternative approaches is necessary to test and refine these concepts.

**Summary**

The information presented enables us to begin to classify the various forms of cardiac dysfunction within a rational framework that individually considers each step in excitation–contraction coupling. Table 2 summarizes our observations with regard to hypoxic,\textsuperscript{21,22} stunned,\textsuperscript{27,37} and hibernating\textsuperscript{42} myocardium. The crucial deficit in hypoxia involves a decrease in myofilament responsiveness to Ca\textsuperscript{2+} that is straightforwardly attributable to changes in P\textsubscript{i} (and, to a lesser extent, pH\textsubscript{i}). The lesion of excitation–contraction coupling in stunning also occurs at the level of myofilament Ca\textsuperscript{2+} responsiveness, but neither P\textsubscript{i} nor pH\textsubscript{i} can be implicated because each quickly returns to normal upon reperfusion. Finally, the availability of activator Ca\textsuperscript{2+} appears to be the limiting factor in hibernation.

The findings are of immediate interest in producing a better understanding of the pathophysiology of cardiac contractile disorders, but the long-term payoff promises to be even greater: clear identification of the site of injury in any given condition will help focus the often haphazard biochemical approaches that have been brought to bear on these problems. For example, the finding that Ca\textsuperscript{2+} transients are not decreased in stunned myocardium sheds doubt on the relevance of explanations that focus on impairments in the pathways that control activator Ca\textsuperscript{2+}.\textsuperscript{1} Instead, attention can now be more profitably directed to determining the nature and origin of the decrease in myofilament Ca\textsuperscript{2+} responsiveness. Quite a different path will have to be followed to elucidate hibernation. The demonstration that perfusion pressure modulates the amplitude of Ca\textsuperscript{2+} transients should further motivate the ongoing search for specific mediators of excitation–contraction coupling elaborated by endothelial cells, smooth muscle cells, or myocardial cells in response to changes in coronary perfusion.\textsuperscript{24}

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**References**


**TABLE 2. Pathophysiology of Contractile Dysfunction in Myocardial Hypoxia, Stunning, and Hibernation**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Ca\textsuperscript{2+} transient amplitude</th>
<th>P\textsubscript{i}/pH\textsubscript{i}</th>
<th>Myofilament Ca\textsuperscript{2+} sensitivity</th>
<th>Maximal Ca\textsuperscript{2+}-activated pressure</th>
</tr>
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<td>Hypoxia</td>
<td>↑</td>
<td>↑ / ↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Stunning</td>
<td>↑</td>
<td>= / =</td>
<td>↓</td>
<td>↓</td>
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<tr>
<td>Hibernation</td>
<td>↓</td>
<td>= / =</td>
<td>=</td>
<td>=</td>
</tr>
</tbody>
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P\textsubscript{i}, inorganic phosphate; pH\textsubscript{i}, intracellular pH. ↑, increased relative to control; ↓, decreased; =, unchanged.


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E Marban

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