Do Alterations in Intercellular Coupling Play a Role in Cardiac Contractile Dysfunction?
Jeffrey E. Saffitz and Kathryn A. Yamada

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Myocardial stunning\(^1\) and hibernation\(^2\) have been subjects of intense laboratory and clinical investigations to elucidate mechanisms responsible for contractile dysfunction after transient ischemia or chronic hypoperfusion. Considerable evidence has implicated the generation of oxygen-derived free radicals and derangements in myocardial energy metabolism and excitation-contraction coupling as major contributors to the pathogenesis of myocardial stunning and hibernation.\(^3\)\(^4\) Despite enormous progress in this area, however, our understanding of the cellular pathophysiology of contractile dysfunction in ischemic heart disease remains incomplete.

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In this issue of *Circulation*, Kaprielian et al\(^5\) provide evidence for a novel mechanism involving alterations in intercellular coupling that might contribute to the pathogenesis of contractile dysfunction in hibernating myocardium. Using digital image processing techniques and confocal immunofluorescence microscopy, they measured the amount of the major cardiac gap junction protein connexin43 (Cx43) in left ventricular samples obtained from patients at the time of coronary artery bypass graft surgery. Biopsies were taken from well perfused, normally contracting wall segments (identified with preoperative thallium scans and magnetic resonance imaging studies) and from “reversibly ischemic” segments (showing improved thallium uptake on stress/redistribution images but no improvement in contractile function after revascularization) or “hibernating” segments (showing improved contractile performance in postoperative MRI studies). Mean gap junction area, determined by measuring the amount of Cx43 immunoreactive signal at intercellular junctions and expressed per unit of intercalated disk area, was reduced by \(\approx23\%\) in reversibly ischemic segments and by \(33\%\) in hibernating tissue compared with normally perfused regions. The average size of an individual gap junction was also reduced by \(13\%\) and \(30\%\) in reversibly ischemic and hibernating regions, respectively, due mainly to loss of the largest gap junctions in myocytes of dysfunctional regions. The authors suggest, on the basis of these observations, that reduction of Cx43 content in gap junctions may contribute not only to arrhythmogenesis by creating anatomic substrates of abnormal conduction but also to wall motion impairment in hibernating myocardium.

Kaprielian et al\(^5\) developed and extensively validated sophisticated analytical techniques to measure the amount and spatial distribution of Cx43 in the heart. Despite the quantitative rigor of the analytical methods used to measure Cx43 immunoreactive signal, however, the data do not establish a causal role for diminished intercellular coupling in arrhythmogenesis or contractile dysfunction. No relation was shown between the extent of Cx43 downregulation and the development of arrhythmias. In addition, the amount of Cx43 signal in intercalated discs was not significantly different in reversibly ischemic versus hibernating tissues. Thus, claims that pathophysiologically important differences in these two types of contractile dysfunction may be attributable to different degrees of Cx43 downregulation are not supported strongly by the data. Nevertheless, a clear difference was observed between levels of Cx43 expression in normal versus abnormal segments. This observation focuses attention on the possibility that alterations in intercellular coupling at gap junctions could directly or indirectly contribute to the pathogenesis of hibernating myocardium. This idea is worthy of further consideration.

**Intercellular Coupling at Gap Junctions**

Nearly all eukaryotic cells communicate with their nearest neighbors by means of gap junctions, specialized regions of the cell surface containing densely packed arrays of membrane-spanning channels that directly link the cytoplasmic compartments of adjacent cells.\(^6\) Ubiquitous among metazoans, gap junctions play fundamental roles in mediating intercellular communication from the early stages of embryogenesis to the establishment and maintenance of coordinated multicellular functions in diverse tissues. Gap junction channels are composed of subunit proteins called connexins.\(^7\) More than a dozen individual connexins (each identified numerically on the basis of its predicted molecular mass in kilodaltons) have been cloned and sequenced. In most differentiated tissues, individual parenchymal cells have been found to express multiple connexins. Three different connexins have been identified in cardiac myocytes: Cx43, connexin45 (Cx45), and connexin40 (Cx40).\(^8\) These cardiac connexins are expressed in different amounts and combinations in different regions of the heart. For example, ventricular myocytes express Cx43 and Cx45, whereas atrial myocytes express Cx43, Cx45, and Cx40.\(^9\) When expressed individually in “communication-deficient” cells, each of the cardiac connexins forms channels with distinct unitary conductances, voltage sensitivities, and different capacities to pass anions, cations, or fluorescent dyes of...
varying sizes and charge densities. These observations suggest that the number, spatial distribution, and connexin composition of gap junction channels are important determinants of the conduction properties of a given cardiac tissue. Regional alterations in connexin expression phenotypes or gap junction distribution induced under a variety of pathophysiological conditions could produce considerable functional heterogeneity within the ventricle.

Relatively little is known about the composition and molecular regulation of the “flow of information” across gap junctions and how this information coordinates the activities of groups of cells that act together as a functional unit. Many low-molecular-weight (<1000 D) regulatory and signaling molecules probably traverse gap junctions to coordinate multicellular events involved in fundamental biological processes, such as cell metabolism, signal transduction, secretion, cell division, cell migration, and morphogenesis. In electrically excitable tissues such as smooth muscle and cardiac muscle, gap junction channels are critical determinants of passive conduction properties. It is important to remember, however, that in addition to subserving an obvious role in intercellular ionic conductance, gap junctions in the heart probably facilitate the exchange of molecular information that regulates metabolic activity and, possibly, contractile activity among groups of coupled cells.

**How Might Reduced Coupling Affect Contractile Function?**

Because gap junctions play a central role in conduction, alterations in connexin expression and gap junction distribution have been implicated in the pathogenesis of reentrant arrhythmias in patients with anatomic substrates characterized by slow, heterogeneous conduction or conduction block. Kaprielian et al speculated that by unmasking local inhomogeneities in individual cell action potential durations, reduced gap junction coupling in areas of hibernating myocardium could disrupt wavefront propagation, and this might interfere with local coordination of myocyte contraction. Although localized uncoupling clearly contributes to the pathogenesis of conduction abnormalities and unidirectional conduction block, it is not so apparent how uncoupling might contribute to contractile dysfunction. Before considering potential mechanisms, it should be stressed that immunohistochemical data reflect the amount but not necessarily the functional state of Cx43 located in gap junctions. The amount of a connexin is certainly an important determinant of total junctional conductance, but other factors play important roles. For example, immunostaining may not distinguish phosphorylated and non-phosphorylated isoforms of Cx43, although the extent of phosphorylation can have a major impact on channel conductance properties and turnover dynamics. Most of the Cx43 in the normal heart appears to be phosphorylated, but under pathophysiological conditions, the relative proportions of phosphorylated and nonphosphorylated Cx43 could change with resultant changes in channel function. Gap junction channel function also is modulated by changes in intracellular Ca\(^2+\) and H\(^+\) concentrations and by the lipid composition of the membrane in which the channels reside. It is possible, therefore, that changes in the intracellular milieu of dysfunctional myocytes could affect junctional coupling independent of changes in the amount of Cx43. Intracellular acidosis and Ca\(^2+\) overload may also affect contractility directly, which further complicates elucidation of potential causal relationships between changes in coupling and contractility. Other mechanisms (both direct and indirect) could link development of derangements in passive electrical properties and the development of alterations in active electrical properties, excitation-contraction coupling, and contractile performance. For example, recent evidence indicates that some voltage-sensitive potassium channels, voltage-gated sodium channels, and inositol trisphosphate receptors are concentrated at sites of intercellular junctions. Alterations in the number or distribution of gap junctions could affect the distribution and possibly even the function of sarcoplasmic reticulum that would affect excitation-contraction coupling and mechanical function.

Although Cx45 is clearly expressed in ventricular myocytes, Kaprielian et al did not detect an immunoreactive signal for Cx45, the other ventricular connexin, in formalin-fixed tissues from patients undergoing bypass surgery. They suggest that because no detectable changes in the levels of other connexin isoforms occur in myocytes of hibernating tissues, potential pathophysiological effects are likely related to alterations in expression levels of Cx43 per se. However, when a tissue expresses multiple connexins, the functional consequences of a change in the expression level of a single connexin may be more complex than simply reducing one type of channel. There is convincing evidence from experiments with transfected cell lines that Cx43 and Cx45 can combine to form hybrid gap junction channels composed of both proteins. Channels composed exclusively of Cx45 have a lower unitary conductance than Cx43 channels and fail to pass detectable amounts of the fluorescent dye Lucifer yellow (which passes readily through Cx43 channels). Hybrid channels composed of Cx45 from one member of a cell pair and Cx43 from the other member have low unitary conductance and do not pass Lucifer yellow, biophysical properties that resemble those of pure Cx45 channels more closely than Cx43 channels.

This raises the possibility that ventricular myocytes may be coupled by a heterogeneous population of gap junction channels, including homotypic Cx43 and Cx45 channels and hybrid channels composed of both proteins. If the relative proportions of homotypic and hybrid channels are related to the stoichiometries of Cx43 and Cx45, then downregulation of Cx43 expression could affect the numbers of both homotypic and hybrid channels even if the total level of Cx45 were unchanged. Thus far, there has been no direct proof of the existence of hybrid channels in the heart. Nevertheless, there is emerging consensus among gap junction biologists that hybrid channels can form and, if so, vastly extend the range of possible channel properties. The known differences in channel properties of individual connexins suggest that specific channel subtypes could subserve specific functions, including electrical coupling, metabolic coupling and coordination of contractile activity.
New Tools for Elucidating Pathophysiological Effects of Altered Connexin Expression

One promising approach to the study of the physiological and pathophysiological roles of different connexins in the heart is to investigate mice in which expression of a specific connexin has been manipulated genetically. Reaume et al20 created a Cx43 knockout mouse through homologous recombination techniques in embryonic stem cells. Mice that are homozygous for the null mutation develop to term but die shortly after being born. These animals consistently have a malformation of the right ventricular outflow tract, which has been implicated in their failure to survive once the maternal-fetal circulation has been disrupted and the neonate becomes reliant on its own cardiac function. Interestingly, a similar type of cardiac malformation has been observed in mice expressing a transgene encoding Cx43 under the control of a cytomegaloviral promoter that results in targeted overexpression of Cx43 in neural crest cells that ultimately participate in cardiac conotruncal development.21 Thus, both deletion and overexpression of Cx43 can be associated with similar types of cardiac malformations, suggesting that either gain or loss of intercellular coupling may have deleterious morphogenetic consequences.

Recent studies have shown that mice heterozygous for the Cx43 null mutation exhibit slow ventricular conduction.22 Although these animals express only 50% of the wild-type level of Cx43 (an even greater degree of downregulation than that seen by Kaprielian et al in hibernating myocardium), they show no outward signs of ventricular dysfunction. It should be emphasized, however, that detailed studies of contractile function have not been reported in Cx43 knockout mice. Furthermore, reduced intercellular coupling in Cx43 heterozygous mice is global in distribution. Contractile dysfunction in hibernating myocardium could be a consequence, in part, of regional loss of coordinated conduction.

Kaprielian et al23 report that myocardial biopsies of both normally contracting and hibernating regions exhibited ultrastructural abnormalities characterized by marked loss of myofibrils. Depletion of myofibrils may not, by itself, account for contractile dysfunction in hibernating myocardium, but the combination of this structural alteration and a defect in coupling could contribute to contractile dysfunction in hibernating myocardium. It is possible that downregulation of Cx43 expression in chronically hibernating myocardium is only one component of a generalized disassembly of subcellular organelles. It is noteworthy, however, that although the control and hibernating tissues demonstrated similar degrees of myofibrillar depletion, only hibernating tissues showed downregulation of Cx43. This suggests that diminished expression of Cx43 is not an obligatory feature of degenerative changes in myocytes in hearts of patients with complex ischemic heart disease. Further studies will be required to elucidate mechanisms of downregulation of Cx43 expression in selected regions of the heart and understand the potential pathophysiological roles that uncoupling might play in contractile dysfunction.

In conclusion, the time has come to broaden our thinking about myocardial gap junction channels beyond their electrical coupling role and to consider their possible roles in coordinating metabolic and contractile functions as well. Ventricular remodeling has both mechanical and electrophysiological consequences. These two major types of pathophysiological changes may interact in complex ways to contribute to both contractile dysfunction and enhanced arrhythmogenesis in patients with chronic ischemic heart disease.

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References