Mechanisms of remodeling of gap junction distributions and the development of anatomic substrates of arrhythmias

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1. Introduction

Alteration of structure (remodeling) is a fundamental response of the heart to injury or disease. It originates in changes in gene expression which, in turn, alter the types and amounts of regulatory and structural proteins in myocytes, the form and distribution of subcellular organelles (including the contractile apparatus and other specialized subcellular structures responsible for cardiac function), and changes in the structure of whole cells, the extracellular matrix and, ultimately, the tissue and whole organ. A change in cardiac structure inevitably leads to a change in cardiac function, but the complex relationships between altered structure and function are only beginning to be revealed. Although structural remodeling serves important adaptive purposes, maladaptive consequences of remodeling are likely to contribute to morbidity and mortality in patients with heart disease.

An important clinical setting in which altered structure begets altered function and in which responses to injury may serve adaptive purposes, but also lead to maladaptive changes, is the development of anatomic substrates of ventricular arrhythmias. These ‘substrates’ arise as a consequence of structural alterations in response to common forms of heart disease such as myocardial infarction or systemic hypertension. Clearly, replacement of a transmural myocardial infarct by fibrous scar tissue limits the likelihood of fatal ventricular rupture and helps preserve optimal ventricular geometry. Similarly, development of myocyte hypertrophy allows the heart to function better in the face of chronic pressure overload. However, although these adaptations are beneficial, the structural alterations that result from these myocardial responses to injury can also change patterns of electrical activation of the heart that may enhance the risk of developing a serious ventricular arrhythmia. For example, sudden death in patients who have survived myocardial infarction often occurs by a reentrant mechanism in which derangements in conduction play a critical pathogenic role [1,2]. Conduction slowing and unidirectional conduction block, necessary for initiation and maintenance of a reentrant circuit, typically arise in viable, but structurally altered, myocardium bordering subacute or healed infarcts [3–7]. Although distinctly abnormal propagation of wavefronts persists in healed infarct border zone regions, intracellular recordings of resting membrane potentials and action potential upstroke velocities in myocytes adjacent to infarct scars return to normal or become nearly normal once infarct healing is complete [4,8]. Thus, abnormal conduction leading to reentry in regions bordering fully healed infarcts is a property of diseased myocardial tissue rather than a property that resides within each individual cell. The pathophysiologically relevant abnormality appears to be alterations in current transfer between myocytes at gap junctions.

In this review, we briefly consider recent advances in understanding mechanisms of remodeling of intercellular electrical junctions in diseased myocardium. We speculate on how these alterations in cardiac structure/function may be advantageous for the individual cell but also maladaptive by contributing to the development of regions of abnormal conduction that may function as anatomic substrates of arrhythmias. We focus primarily on remodeling of gap junctions in ventricular myocardium and its role in...
the pathogenesis of ventricular arrhythmias, but the general principles and mechanisms may apply to atrial myocardium and atrial fibrillation as well, and to other types of cardiac tissues and arrhythmias, such as those involving the cardiac conduction system. Space limitations preclude a detailed consideration of potential differences in the regulation and function of multiple cardiac gap junction channel proteins (connexins) in the development of anatomic substrates of arrhythmias. Recent evidence in mice with deficient connexin expression indicates that, of the two connexins expressed in ventricular myocytes, connexin43 (Cx43) plays a dominant role in electrical coupling [9,10], whereas connexin45 (Cx45), which is apparently present in considerably smaller amounts than Cx43 [11], may not be as important as Cx43 in impulse propagation. Accordingly, this review will focus primarily on alterations in the expression and distribution of the principal ventricular coupling protein, Cx43, in the development of anatomic substrates of ventricular arrhythmias.

2. Myocyte interconnections at gap junctions

Cardiac muscle is not an electrical syncytium. Rather, current must be transferred from one discrete cell to another at gap junctions [12], which are specialized membrane regions of densely packed channels that directly connect the cytoplasmic compartments of two adjacent cells (Fig. 1). Many observations suggest that the number, size and spatial distribution of gap junctions play an important role in determining the conduction properties of different cardiac tissues. For example, patterns of intercellular connections are distinctly different in ventricular and atrial tissues [13,14], which also exhibit different anisotropic conduction properties [15,16]. A typical myocyte in the canine left ventricular subepicardium is physically connected by intercalated disks and gap junctions to an average of 11 or 12 other myocytes, of which, roughly half are oriented primarily in side-to-side apposition and the remaining half are connected in a predominant end-to-end orientation [14,17] (Fig. 2). In contrast, atrial myocytes of the canine crista terminalis of the right atrium are interconnected mainly end-to-end [14], consistent with the much greater degree of anisotropy of conduction in the crista compared with the ventricle [15,16] (Fig. 2). Reductions in the total amount of gap junction profile length and gap junction protein expression in diseased ventricular myocardium [7,17–20], described in more detail below, have been implicated in the pathogenesis of slow conduction and unidirectional conduction block leading to reentrant arrhythmogenesis. Conversely, an increase in the number of gap junctions and the content of gap junction channel proteins in cultured myocytes exposed to cAMP has been correlated with increased conduction velocity [21]. All of these observations suggest that the three-dimensional distribution of gap junctions is a critical determinant of tissue-specific conduction properties in different parts of the heart under both normal and pathophysiological settings. Little is known, however, about how specific patterns of intercellular connections are established or regulated in different tissues of the heart. Mechanisms responsible for remodeling of patterns of intercellular connections in diseased myocardium are also
poorly understood, but recent advances in basic aspects of gap junction biology have begun to provide insights into these important questions.

3. Enhanced connexin expression and intercellular coupling as a component of the acute hypertrophic response

When faced with a moderate increase in load, cardiac myocytes respond with compensatory hypertrophic growth characterized by increased protein synthesis, changes in cell structure and increased cardiac performance. Fundamental features of the hypertrophic response include increased synthesis of contractile proteins, assembly of new sarcomeres and improved contractile function. Although a detailed understanding of potential changes in gap junction protein expression and function in hypotrophied myocardium is lacking, the results of in vitro studies suggest that compensatory hypertrophic growth may also be associated with increased connexin levels, increased numbers of gap junctions and enhanced intercellular coupling. For example, long-term (24 h) exposure of neonatal rat ventricular myocyte cultures to dibutyryl cAMP, a membrane-permeant analogue of cAMP, increases the tissue content of Cx43 by approximately two-fold and increases the number of gap junctions interconnecting the cells [21]. Cultured neonatal rat ventricular myocytes exposed for 24 h to angiotensin II also exhibit a two-fold increase in Cx43 content and an increase in the number of gap junction profiles [22]. Interestingly, the Cx43 synthesis rate increases by two- to three-fold after exposure to angiotensin II for 24 h, although under similar conditions, exposure to dibutyryl cAMP does not increase Cx43 synthesis [21,22] (Fig. 3). Thus, increases in the total tissue content of Cx43 caused by cAMP or angiotensin II appear to be mediated by disparate molecular mechanisms. In response to angiotensin II, Cx43 synthesis increases whereas in response to cAMP, accumulation of Cx43 may depend primarily on diminished degradation.

Taken together, the results of these in vitro studies suggest that activation of disparate signal transduction pathways involving angiotensin II and cAMP may regulate connexin expression during the acute hypertrophic response. Remodeling of conduction pathways during early, compensatory responses to increased load in vivo may also be an active process involving enhanced connexin expression and rearrangements of gap junction distribution. For example, connexin expression is enhanced during the early stages of hypertrophy induced by renovascular hypertension in guinea pigs [23]. Bastide et al. [24] have shown that expression of Cx40 is enhanced in Purkinje fibers of the rat when hypertrophy is induced by hypertension. Although Cx40 is not apparently expressed in working ventricular myocytes, it is expressed abundantly in the cardiac conduction system (and in atrial myocardium as well) and its upregulation in hypertensive hypertrophy may be another example of enhanced connexin expression during early, adaptive hypertrophic growth in the heart. A more detailed understanding of signal transduction pathways regulating connexin expression during compensated hypertrophic growth may provide insights into the molecular mechanisms responsible for subsequent development of arrhythmia substrates in patients with chronic forms of heart disease.

4. Redistribution of gap junctions in chronic heart disease

The hypertrophic response is a dynamic continuum in which progressive changes in gene expression and the
structure of cells and extracellular matrix may mediate the transition from a phase of compensated structural and functional adaptation to an increasingly maladaptive state culminating in heart failure. Ventricular conduction delay, often reflected as prolongation of the QRS interval in the surface electrocardiogram, is a general feature of chronic left ventricular hypertrophy in man. Toyoshima et al. [25] suggested more than 16 years ago that conduction velocity increased in hypertrophied ventricles but then decreased in the failing ventricle, based on echocardiography and body surface potential mapping in patients. Indeed, conduction velocity appears to decrease with increasing severity of hypertrophy [26–28] and is likely to be affected by increases in extracellular resistance related to interstitial fibrosis [27,28] as well as increases in intercellular resistance due to decreased connexin expression [7,17–20,29].

Remodeling of tissue structure in chronic forms of heart disease leads to changes in patterns of gap junctions that are likely to alter the conduction properties of myocardium and contribute to arrhythmogenesis, independent of changes in the active membrane properties of individual cells. The best-studied disease setting in which alterations in gap junction distribution have been closely linked to reentrant arrhythmias is myocardial infarct healing. During the inflammatory and reparative phases of infarct healing, viable myocytes at the edges of the infarct scar develop complex structural alterations involving both cardiac myocytes and the extracellular matrix. A common pattern of structural alteration in peri-infarct tissue is accumulation of interstitial bundles of collagen oriented parallel to the long axis of groups of cardiac myocytes [3–6,17]. This ‘substrate’ has been observed in regions identified by activation mapping to be sites of slow conduction, conduction block and complex fractionated electrograms [1,3–5]. Ultrastructural measurements in a healed canine left ventricular infarct model have shown that epicardial border zone myocytes in bundles separated by interspersed collagen are connected by smaller gap junctions than normal myocytes [17]. Approximately 10% of total gap junctions observed by electron microscopy in normal myocardium have a profile length >3 μm [17]. Although they comprise only a small proportion of the total number of profiles, these long junction profiles constitute approximately 40% of total gap junctional area and, thus, probably play an important role in intercellular current transfer [17]. In contrast, myocytes in the epicardial border zone lack these large gap junction profiles, suggesting that the largest arrays of gap junctional channels are selectively lost during infarct healing [17] (Fig. 4). Furthermore, the number of cells connected to a single canine ventricular myocyte is reduced by nearly half in epicardial border zone regions, but the loss of intercellular connections is not uniformly distributed [17]. Compared with the number and spatial distribution of interconnected myocytes in normal tissue, the mean number of border zone myocytes connected to one another in side-to-side configuration is reduced by 75%, whereas connections between epicardial border zone cells in end-to-end orientation are reduced by only 22% [17]. The predicted pathophysiological consequences of these structural alterations are consistent with observations made in both experimental animals and human arrhythmia mapping studies. Longitudinal propagation through remodeled regions is relatively rapid because end-to-end connections are preserved. However, ventricular tachycardia is typically induced and maintained when wavefronts activate these critical regions in a direction transverse to the long myocyte axis [6,7]. Macroscopic propagation through the remodeled tissue in the transverse direction is greatly impaired because side-to-side connections are selectively disrupted and wavefronts are forced to zig-zag through the tissue [6] until they reenter post-refractory tissue and initiate the next beat of the tachycardia. The
complex pathways followed by such wavefronts probably account for the slow, heterogeneous conduction properties and the presence of fractionated electrograms and late (‘diastolic’) potentials in border zone regions [3–7]. Remodeling of gap junctions in peri-infarct myocytes and loss of side-to-side connections have been observed in canine infarcts only four days after infarction [7] before significant fibrosis has occurred, and also in healed canine [17] and human [6] infarcts. The inducibility of ventricular tachycardia by premature stimuli at selected intervals after infarction varies in canine and human infarcts and the locations of reentrant circuit vary as well (typically in the epicardial border zone in dogs but subendocardially in patients). Nevertheless, basic mechanisms responsible for the development of arrhythmia substrates following infarction are probably similar in canine and human infarcts.

Recently, it has been demonstrated immunohistochemically that gap junction protein expression is reduced in segments of hibernating myocardium in patients with chronic ischemic heart disease [20]. Interestingly, the reduced gap junction protein expression appears to be attributable mainly to a loss of the larger gap junctions normally seen at the major intercalated disks at the ends of cells [20]. These results and others suggest that reduced gap junction channel protein levels occur as a general rule in chronic myocardial disease states, including healed myocardial infarction [7,17–19,29], chronic hibernation [20], end-stage disease in chronic aortic stenosis [19] and even with aging [30,31]. Reentrant arrhythmias, dependent on slow, heterogeneous conduction and unidirectional conduction block, occur frequently in these chronic forms of heart disease, thus implicating remodeling of gap junction distributions and reduced coupling in the development of anatomic substrates of ventricular arrhythmias. Recently, van der Velden et al. [32] demonstrated that the distribution of Cx40, a major coupling protein in the atria, is altered in goats with chronic atrial fibrillation, consistent with the hypothesis that altered coupling in the atria could also contribute to reentrant arrhythmogenesis. Increasing evidence indicates that changes in gap junction distribution in chronic forms of heart disease are only one feature of a more generalized, stereotypical response of cardiac myocytes to chronic injury [20,33–38]. This response has been referred to by various names including ‘dedifferentiation’ and ‘myocytolysis’. The term ‘dedifferentiation’ has been used perhaps to refer to the expression of fetal isoforms of some contractile proteins and cardiac enzymes in myocytes that have undergone this process, and the superficial resemblance of the altered myocytes to developmentally immature myocytes. However, because ‘dedifferentiation’ implies a step-wise reversal of the normal differentiation pathway, its application to the complex cellular changes seen in chronic heart disease would appear to be inappropriate. ‘Myocytolysis’, which aptly describes the most prominent feature of the myocytic response to chronic injury, may be a more appropriate term.

5. Alterations in connexin expression and distribution as a feature of myocytolysis

The results of in vitro studies with cAMP [21] and angiotensin II [22] suggest that the compensatory hypertrophic response creates a situation of enhanced intercellular communication to coordinate the activities of individual cells and optimize electrophysiological and mechanical functions of the tissue. However, when the load placed on a cardiac myocyte exceeds a certain threshold or a myocyte becomes severely injured, it undergoes a marked phenotypic transformation characterized most conspicuously by a loss of sarcomeres and diminished contractile performance. This phenotypic transformation is an adaptive response on the part of a seriously injured cardiac myocyte whose viability is threatened. Under these dire circumstances, it appears that the individual cell withdraws from the ‘community of cells’ that constitutes myocardial tissue and enters a ‘survival phase’, during which, its contribution to the function of the multicellular tissue becomes subordinated to the goal of cell survival.

Because cardiac myocytes expend most of their energy to support the pump function of the heart, the principal cell survival strategy involves a reduction in contractile activity and disassembly of the contractile apparatus within cells. The range of specific molecular and biochemical changes during this phenotypic transformation is undoubtedly vast. Multiple intracellular regulatory processes must be affected, including a significant shift in expression of enzymes responsible for energy metabolism from those using fatty acid substrates to those using glucose [39]. Among the most prominent of these changes, however, appears to be a reduction in the content of subcellular organelles that support contraction, especially the sarcomeres. However, as a component of this response, other organelles, including gap junctions, also appear to undergo marked alterations. The morphological picture of the myocyte survival response is non-specific. The same structural changes occur in response to diverse forms of sub-lethal injury and include partial or nearly complete loss of sarcomeres, accumulation of glycogen, and disorganization and loss of sarcoplasmic reticulum [33–35], as well as a reduction in gap junction protein expression and loss of large gap junctions, which has been described in many forms of chronic tissue remodeling [17,19,20]. These stereotypical morphological changes, referred to as myocytolysis, are universally observed, at least to some extent, in dysfunctional wall segments in patients with chronic ischemic heart disease (hibernating myocardium) [20] and in diverse forms of cardiomyopathy in patients with heart failure.
Chronic forms of heart disease implicates activation of cellular proteolytic systems as a fundamental mechanism in myocytolysis [41]. Significant advances have been achieved recently in elucidating processes by which cells regulate the balance of protein synthesis and degradation. Multiple cellular systems participate in proteolysis, including the lysosomal pathway and the cytoplasmic ubiquitin-proteasomal pathway [42,43]. The latter pathway has been implicated in cellular stress responses through targeting of proteins for proteolysis by ubiquitination and/or changes in phosphorylation. We and others have observed rapid turnover of Cx43 in cultured neonatal rat myocytes [44,45]. Recent studies have characterized mechanisms of Cx43 degradation in primary cultures of neonatal rat ventricular myocytes with specific inhibitors of both the lysosomal pathway and the proteasomal pathway [46]. To characterize connexin turnover dynamics in the adult heart and to elucidate its potential role in remodeling of gap junctions, we measured Cx43 turnover kinetics and characterized the proteolytic pathways involved in Cx43 degradation in intact, perfused adult rat hearts. We observed that Cx43 disappeared from adult rat hearts with a half-life of only 1.3 h [47] (Fig. 5). Hearts perfused with specific inhibitors of either lysosomal or proteasomal proteolysis demonstrated significant increases in Cx43 content (Fig. 6). In general, degradation of internalized extracellular proteins and some membrane proteins occurs via the lysosomal pathway [42]. In contrast, proteasomal proteolysis is thought to be the major pathway for degradation of cytosolic and nuclear proteins and for digestion of misfolded proteins trafficking through the endoplasmic reticulum and Golgi apparatus [42,43]. These observations indicate that even in the normal adult heart, in which ventricular myocytes appear to be stably interconnected under basal conditions, the proteins that form gap junction channels are turning over at a high rate and may be degraded by multiple proteolytic pathways. The high throughput of protein in gap junctions could mean that connexin synthesis and degradation are tightly regulated and that even modest changes in production or removal could result in rapid alterations in channel number and, presumably, the level of intercellular coupling. Alternatively, connexin synthesis and degradation could be more loosely regulated. Constitutively active synthetic pathways could be counterbalanced by regulated degradation pathways that determine the final channel number. In any event, the results of recent studies in Cx43-deficient mice [9,10] and in neonatal rat myocytes exposed to cAMP in vitro [21] suggest that even relatively modest changes in Cx43 expression levels (50 to 100% above or below the baseline level) are associated with significant gain or loss of function. Thus, most of the total cellular pool of connexin may be involved in coupling and changes in the total cellular content of Cx43 probably have functional consequences. The biological significance of connexin degradation by both lysosomal and proteasomal pathways is not clear, but this observation provides further evidence.

6. Connexin turnover and mechanisms of diminished coupling in chronic heart disease

The stereotypical appearance of myofibrillar loss in chronic forms of heart disease implicates activation of
Fig. 5. Cx43 turns over rapidly in intact, adult rat hearts. Isolated, Langendorff-perfused hearts were metabolically labeled with buffer containing [35S]methionine for 30 min and then chased with buffer containing unlabeled methionine for 0 to 240 min. As shown in the representative blots, the amount of radioactivity present in Cx43 or actin was determined at selected chase intervals with immunoprecipitation assays. The lower plot shows degradation kinetics in four hearts at each time point (means ± SD). Analysis of the Cx43 decay curve in the lower panel revealed a monoexponential loss of radioactivity with a half-life of approximately 1.3 h. Over the same interval, the long-lived protein actin showed no significant loss of radioactivity. (Reprinted from Ref. [47] with permission).

Fig. 6. Cx43 is degraded by both lysosomal and proteasomal proteolysis pathways. Isolated adult rat hearts were perfused for 4 h with buffer alone (A) or with buffer containing the proteasomal pathway inhibitor N-acetyl-leucyl-leucyl-norleucinal (B) or the lysosomal pathway inhibitor NH4Cl (C). The distribution of Cx43 in sections of left ventricle was analyzed by immunofluorescence microscopy. Enhanced Cx43 immunoreactive signal is readily apparent at sites of intercellular apposition in hearts perfused with either proteolysis pathway inhibitor. (Reprinted from Ref. [47] with permission).
that mechanisms regulating intercellular coupling are complex and that cells may have many ways to fine-tune intercellular communication.

7. Conclusions

In order for the heart to function optimally, the activities of individual cells must be organized and coordinated. Because gap junctions play such a fundamental role in regulating patterns of impulse propagation crucial to effective contractile performance, it is not surprising that cardiac myocytes have evolved multiple regulatory mechanisms to control the level of intercellular communication in the heart. In general, these regulatory mechanisms are poorly understood. The rapid turnover of cardiac connexins suggests that a principal mechanism controlling coupling is rapid adjustments in the number of channels between adjacent cells. Other mechanisms controlling channel function not even mentioned in this brief review, such as connexin phosphorylation, are also likely to play crucial roles, especially under pathophysiological conditions.

In response to extreme injury such as acute, lethal ischemia, cardiac myocytes uncouple rapidly and completely as part of the process of irreversible cell injury and death. In the face of sub-lethal injury, however, it appears that cardiac myocytes uncouple partially. This uncoupling response is only one component of a more general cellular response to injury (myocytolysis) involving disassembly of the contractile apparatus and other complex structural and functional adaptations. Partial uncoupling in viable but structurally altered myocardium inevitably changes the conduction properties of the tissue and, at least in some settings, increases the likelihood of developing arrhythmias dependent on derangements in conduction. Greater understanding of the molecular and biochemical mechanisms underlying cellular responses to injury will not only provide novel insights into fundamental disease processes that promote arrhythmogenesis, but will also lead to novel therapies to prevent lethal rhythm disturbances in patients with heart disease.

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References


