

The role of myocardial gap junctions in electrical conduction and arrhythmogenesis

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Abstract

Electrical activation of the heart requires cell–cell transfer of current via gap junctions, arrays of densely packed protein channels that permit intercellular passage of ions and small molecules. Because current transfer occurs only at gap junctions, the spatial distribution and biophysical properties of gap junction channels are important determinants of the conduction properties of cardiac muscle. Gap junction channels are composed of members of a multigene family of proteins called connexins. As a general rule, individual cells express multiple connexins, which creates the potential for considerable functional diversity in gap junction channels. Although gap junction channels are relatively nonselective in their permeability to ions and small molecules, cardiac myocytes actively adjust their level of coupling by multiple mechanisms including changes in connexin expression, regulation of connexin trafficking and turnover, and modulation of channel properties. In advanced stages of heart disease, connexin expression and intercellular coupling are diminished, and gap junction channels become redistributed. These changes have been strongly implicated in the pathogenesis of lethal ventricular arrhythmias. Ongoing studies in genetically engineered mice are revealing insights into the role of individual gap junction channel proteins in normal cardiac function and arrhythmogenesis. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

Cardiac muscle is composed of individual cells each invested with an insulating lipid bilayer. As a result, electrical activation of the myocardium requires intercellular transfer of current. This process occurs at gap junctions, specialized regions of the membranes of adjacent cells containing arrays of densely packed intercellular channels that directly connect the cytoplasmic compartments of neighboring cells and permit intercellular passage of ions and small molecules. Because transfer of depolarizing current can occur only at gap junctions, it follows that the spatial distribution and biophysical properties of gap junction channels are important determinants of the

velocity and three-dimensional pattern of electrical activation of the heart. The cloning and sequencing of genes encoding gap junction channel proteins has led to great advances in knowledge of the molecular structure, distribution, and functional specializations of gap junction channels in the heart. This review summarizes selected aspects of the structure and function of gap junction channels as determinants of electrical conduction in the normal heart. It also considers mechanisms responsible for derangements in the expression, distribution, and function of gap junction channel proteins in the diseased heart and their role in arrhythmogenesis.

2. Expression of multiple connexins by cardiac myocytes

Gap junction channels are composed of members of a multigene family of proteins referred to as connexins. More than a dozen unique connexins, each encoded by a separate gene, have been identified and characterized [1,2]. These

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proteins are named by the abbreviation Cx followed by the molecular weight of the specific protein. A connexin molecule has four transmembrane domains and two extracellular loops, which are highly conserved among members of the connexin family (Fig. 1). In contrast, the intracellular loop that connects the second and third transmembrane domains and the carboxy terminus have unique amino acid sequences that are responsible for the different molecular weights and specific channel properties of the connexins (Fig. 1).

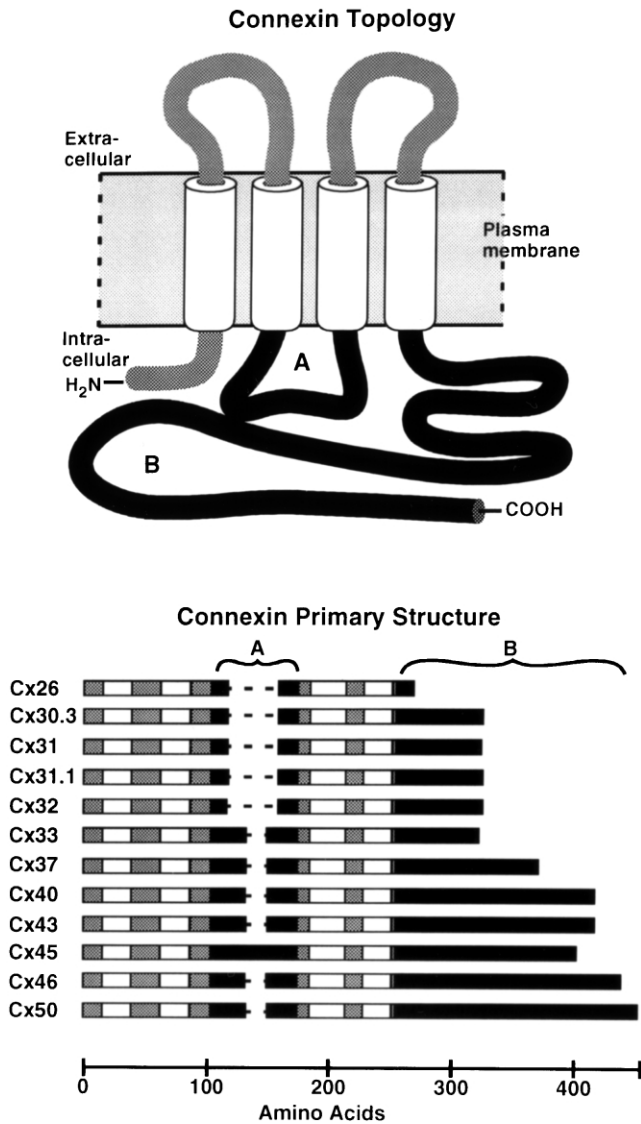


Fig. 1. Upper panel: A model of the topology of a connexin molecule. The transmembrane domains are shown in white, the N-terminus and extracellular domains are shown in gray (hatched), and the intracellular hydrophilic loop connecting the second and third transmembrane domains (A) and the C-terminal tail (B) are shown in black. Lower panel: Comparison of the sequences of multiple members of the connexin family. The amino acid number begins at the N-terminus. The gray (hatched) and white segments correspond to the regions of the molecules shown in the upper panel. Divergent sequences, shown in black, are located mainly in the intracellular loop (A) and the C-terminal tail (B) regions.

As a general rule, individual connexins are expressed in multiple tissues, and individual cells tend to express multiple connexins. For example, messenger RNAs encoding Cx37, Cx40, Cx43, Cx45, Cx46, and Cx50 have been detected in homogenates of mammalian heart muscle [3–8]. Not all of these transcripts are necessarily translated nor are all of the proteins necessarily expressed by cardiac myocytes. Expression of Cx37, for example, is confined to the endothelium of the coronary vasculature [6]. Cx43 and Cx40 are expressed by both cardiac myocytes and vessel wall cells.

It has been established unequivocally that mammalian cardiac myocytes express Cx43, Cx45, and Cx40, but different tissues of the heart express different amounts and combinations of these connexins. The major cardiac gap junction protein, Cx43, is expressed in atrial and ventricular muscle and in the distal His–Purkinje system [9–11]. Cx43 has also been identified in the rabbit and canine sinus nodes [12,13] but not in bovine sinus or atrioventricular nodes [14,15]. Expression of Cx40 in the heart is more restricted than Cx43. Cx40 is expressed by atrial myocytes and by the His–Purkinje fibers of the atrioventricular conduction system but not by adult ventricular myocytes [10,11,16–18].

Studies in genetically engineered mice have revealed tissue-specific functions for the cardiac connexins. Cx43-null mice die shortly after birth [19] but heterozygotes (Cx43^{+/-} mice) survive and breed. Although a study by Morley et al. [20] using optical mapping of transmembrane voltage showed no differences in conduction velocity in Cx43^{+/-} and Cx43^{+/+} hearts, earlier studies using an electrode array revealed modest slowing of ventricular conduction in heterozygotes [21,22]. More recent optical mapping studies have also revealed ventricular conduction slowing in both Cx43^{+/-} [23] and Cx43^{-/-} [24] mice. Atrial conduction abnormalities have been observed in Cx40-null mice [25,26] but no slowing of atrial conduction occurs in Cx43^{+/-} mice [22], even though both atrial and ventricular myocardia express Cx43 abundantly in roughly equal amounts. These observations indicate that Cx43 is the principal intercellular coupling protein in ventricular myocardium, whereas Cx40 fulfills this function in atrial muscle.

The role of Cx40 in the cardiac conduction system has also been revealed in studies of Cx40-null mice. These animals exhibit altered atrioventricular conduction and a right bundle branch block pattern on the surface ECG, indicating that Cx40 plays important roles in critical regions of the conduction system [26–28]. Expression of Cx40 is regulated developmentally. In mice, for example, Cx40 is expressed abundantly in the embryonic left ventricle but then undergoes down-regulation during the early postnatal period [29]. There is no current evidence to suggest that Cx40 is reexpressed by ventricular myocytes in diseased myocardium.

In the adult heart, Cx45 expression appears to be concentrated within the atrioventricular conduction system [30]. Like Cx40, Cx45 is more widely expressed during cardiac morphogenesis but then becomes down-regulated [31].

Whether it is expressed to an appreciable extent in adult mammalian ventricular myocytes is not clear at the present time. Targeted deletion of Cx45 in mice is embryonic lethal [32,33].

3. Structural and functional diversity of gap junction channels

An individual intercellular channel is created by stable, noncovalent interactions of two hemichannels, referred to as connexons, located in the plasma membranes of adjacent cells. Each connexon is formed by six connexin subunits [34]. Channels formed by individual connexins exhibit distinct biophysical properties including unitary conductances, pH dependence, voltage dependence, and selective permeability to ions and small molecules such as fluorescent dyes. Cx43 channels have main conductance states of 90–115 pS and are relatively insensitive to changes in transjunctional voltage compared with channels composed of Cx40 or Cx45 [35–38]. Cx40 forms channels with larger conductances (150–160 pS) than Cx43 channels [36, 39–41]. In contrast, Cx45 channels exhibit a much lower main state conductance of ~ 25 pS and are highly sensitive to transjunctional voltage [37,42].

Because individual differentiated cells generally express more than a single species of connexin, the possibility exists for the formation of individual channels composed of more than one isoform. Theoretical combinations of connexins in hybrid channels and a currently used system of nomenclature [43] are illustrated in Fig. 2. Individual connexons can be homomeric or heteromeric, and individual channels can be homotypic or heterotypic. The potential number of unique combinations of heteromeric connexons and heterotypic channels is large. Currently, little is known about the natural occurrence or biological significance of hybrid channels in the heart or other differentiated tissues. It has been clearly shown, however, using *Xenopus* oocytes or other “communication-deficient cells” transfected with known connexin sequences, that some but not all homomeric connexons can form heterotypic channels. For example, Cx43, Cx46, and Cx50, the multiple connexins expressed in the vertebrate lens, form hybrid channels selectively [44]. Connexons composed of Cx50 cannot form

heterotypic channels with connexons composed of Cx43, but functional Cx46/Cx43 and Cx46/Cx50 homomeric, heterotypic channels can occur and their biophysical properties are distinct from those of the corresponding homotypic channels.

The potential for structurally and functionally diverse forms of hybrid gap junction channels in the heart is great. Although earlier studies in *Xenopus* oocytes suggested that Cx40 cannot form functional heterotypic channels with Cx43 [45,46], more recent experiments involving transfected HeLa cells indicate that heteromeric Cx40/Cx43 channels do form and exhibit asymmetrical conductance properties [47]. Recent evidence also suggests that Cx43 and Cx45 can form both heteromeric connexons and homomeric, heterotypic channels [48,49]. These findings have potential implications for intercellular coupling in specific regions of the heart, such as the interface between the sinus node and atrial myocardium or Purkinje fibers and ventricular myocardium, both of which are characterized by a high degree of current-to-load (source–sink) mismatch. It is still not clear how a small amount of depolarizing current (source) provided by the limited number of cells in the sinus node or a thin bundle of Purkinje fibers can activate the much larger mass of atrial or ventricular muscle to which they are apparently well coupled and which should act as large current sinks. One attractive hypothesis is that disparate connexin phenotypes at junctions between sinus node and atrial myocytes or Purkinje fibers and ventricular myocytes may create specific types of hybrid channels with unique properties that ensure safe conduction. For example, Valiunas et al. [47] have provided evidence that in heterotypic Cx40/Cx43 channels, Cx40 gates with positive polarity and Cx43 gates with negative polarity. Thus, at the interface between Purkinje fibers (which express abundant Cx40) and ventricular myocytes (which express mainly Cx43), rectification of Cx40/Cx43 channels would facilitate intercellular current flow when the Cx40 cell is depolarized and impair current flow when the Cx43 cell is depolarized. The presence of Cx40/Cx43 channels at the Purkinje–ventricular interface could, therefore, promote preferential conduction in the antegrade direction and decrease the likelihood of developing reentrant arrhythmias dependent on retrograde conduction through the Purkinje system.

4. Tissue-specific spatial distributions of gap junctions as determinants of anisotropic conduction

Reconstructions of the three-dimensional distribution of cell–cell junctions have revealed tissue-specific patterns of intercellular connections, which appear to confer distinct conduction properties. A good example of how different gap junction distributions may contribute to tissue-specific conduction properties involves a comparison of structure–function relations in ventricular muscle and the crista terminalis of the right atrium [50]. The velocity of conduc-

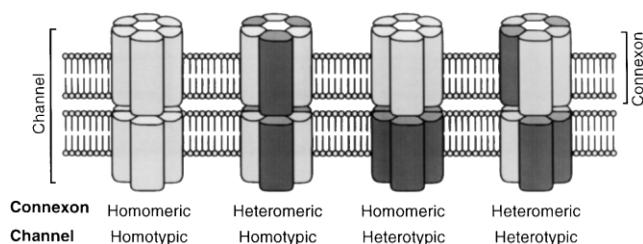


Fig. 2. Nomenclature for connexons and gap junction channels formed by one or more connexins. Modified from Kumar and Gilula [43].

tion in ventricular myocardium is ~ 0.6 m/s in the longitudinal direction (parallel to the long cell axis) and ~ 0.2 m/s in the transverse direction, yielding an anisotropy ratio of $\sim 3:1$. Reconstructions of intercellular connections in the canine left ventricular have revealed that individual ventricular myocytes are connected to an average of 11.3 neighbors (Fig. 3), approximately half of which are connected in a purely or predominantly side-to-side orientation, and the others are connected in an entirely or mainly end-to-end fashion [50]. This “blueprint” of intercellular connections is consistent with the moderate anisotropy of ventricular conduction and suggests that the principal determinant of anisotropic conduction in ventricular muscle is the elongated shape of the cells rather than an anisotropic distribution of junctions (i.e., numerous sites for intercellular current transfer exist in both directions but because of the elongated shape of the cells, a wavefront traveling in the transverse direction must cross more intercellular junctions and thus would encounter greater resistance and propagate more slowly than wavefronts traveling an equal distance in the longitudinal direction). The opposite seems to be true for the crista terminalis, a discrete bundle of atrial myocardium that conducts impulses from the sinus node to the atrioventricular junction. Myocytes in the crista terminalis have the same elongated shape as those in ventricular muscle, but conduction in the crista is more rapid (~ 1 m/s) and far more anisotropic (longitudinal-to-transverse conduction velocity $\sim 10:1$) than in the ventricle. Although myocytes of the crista terminalis are interconnected to fewer neighbors than ventricular myocytes, the great majority of these interconnections occur between cells oriented end-to-end [50] (Fig. 3), a pattern that undoubtedly contributes to the high degree of anisotropy in this tissue.

Yet another pattern of intercellular connections occurs in the sinus node, a tissue characterized by exceptionally slow (~ 0.03 m/s) conduction. Although differences in active depolarizing currents between sinus node and ventricular myocytes play a major role, structural differences also contribute to the highly disparate conduction properties of these two tissues. For example, a typical sinus node myocyte is connected to an average of only 4.8 neighbors [51]





	Left Ventricle	Crista Terminalis	Sinoatrial Node
I 	3.3 \pm 1.4 (29)	0.8 \pm 0.6 (12)	0.7 \pm 0.5 (14)
II 	2.0 \pm 0.7 (18)	0.7 \pm 0.6 (11)	1.4 \pm 1.0 (30)
III 	2.1 \pm 0.9 (19)	1.1 \pm 0.7 (17)	2.1 \pm 0.9 (44)
IV 	3.9 \pm 1.1 (34)	3.8 \pm 0.7 (60)	0.6 \pm 0.7 (12)
	11.3 \pm 2.2 cells connected to each myocyte	6.4 \pm 1.7 cells connected to each myocyte	4.8 \pm 0.7 cells connected to each myocyte

Fig. 3. Diagram of the number and spatial orientation of cellular connections in canine left ventricle, crista terminalis, and sinus node. Values show the mean \pm S.D. of the number of each type of interconnection. The numbers in parenthesis indicate the percentage of each type of interconnection. From Saffitz et al. [51].

(Fig. 3). Furthermore, aggregate gap junction profile length measured in transmission electron micrographs and expressed as a proportion of myocyte area is >25 -fold less in canine sinus node myocytes than in ventricular myocytes [51]. Gap junctions interconnecting sinus node myocytes are contained within small intercalated disks on cytoplasmic projections arising at various points along the node myocytes. This arrangement creates complex packing of node myocytes interconnected in varying degrees of both side-to-side and end-to-end orientation [51]. Taken together, these structural features likely contribute to the slow, nonuniform conduction typical of the sinus node region.

Little is known about how tissue-specific patterns of intercellular junctions are established or how the number and size of gap junctions are determined in different cardiac tissues. Interestingly, the number of gap junctions is reduced in Cx43^{+/-} mice, which express only $\sim 50\%$ of the normal level of Cx43, but mean gap junction size is unchanged [52]. Mechanical junctions in the intercalated disk, which are also unaltered in Cx43^{+/-} mice [52], may stabilize certain regions of the membranes of interconnected cells and thereby create a local environment of low shear stress that favors formation and maintenance of large channel arrays. Thus, a normal distribution of mechanical junctions in Cx43^{+/-} mice may result in assembly of normally large gap junctions even though the diminished pool of Cx43 will result in fewer gap junctions. Whether any biological advantage is achieved by maintaining normal gap junction size rather than number remains a matter of speculation.

5. Rapid turnover of connexins

The number, size, and distribution of myocardial gap junctions may be relatively stable under physiological conditions, but the flux of connexins into and out of gap junctions appears to be highly dynamic. Rapid turnover of Cx43 was first demonstrated in cultured neonatal rat ventricular myocytes [53,54]. It is possible that disaggregation of ventricular myocytes followed by active reestablishment of cell junctions in culture could increase connexin synthesis and degradation rates compared with those in the adult heart. However, metabolic labeling and pulse-chase studies in isolated, perfused adult rat hearts have demonstrated monoexponential disappearance of radiolabeled Cx43 with a half-life of ~ 1.3 h [55], a rate similar to that seen in cultured myocytes. Intracellular proteolysis of Cx43 involves both of the major protein degradation pathways, the proteasome and the lysosome [56,57]. Inhibition of endosomal proteolysis in isolated perfused rat hearts leads to marked accumulation of phosphorylated isoforms of Cx43, whereas inhibition of proteasomal degradation causes nonphosphorylated Cx43 to accumulate [55]. Thus, changes in connexin phosphorylation may play a role in targeting the protein for degradation via different proteolysis pathways.

Metabolic labeling and pulse-chase studies cannot formally rule out the possibility that dynamic turnover of connexins occurs in an intracellular pool, while the protein located in gap junctions is sufficiently long lived that it does not become labeled during relatively brief pulse intervals. However, recent studies in which Cx43 has been visualized in living cells previously transfected to express Cx43 tagged with green fluorescent protein (Cx43–GFP) have demonstrated continuous transport of apparently newly synthesized Cx43–GFP to the plasma membrane where discrete patches of fluorescent signal (presumed gap junctional plaques) were seen to oscillate and coalesce [58]. Cx43–GFP was removed from the plasma membrane by budding and internalization and often formed distinct endocytic vesicles that could travel back to the cell surface [58]. These observations lend additional credence to the concept that the flow of connexins into and out of gap junctions is highly dynamic. If so, then modulation of this process could produce rapid changes in intercellular coupling.

6. Altered intercellular coupling in the pathogenesis of arrhythmias in acute myocardial ischemia

The risk of developing lethal ventricular arrhythmias is high in the setting of acute myocardial ischemia. The pathogenesis of these malignant arrhythmias is multifactorial, involving marked reductions in tissue pH, increases in interstitial K^+ and intracellular Ca^{2+} levels, and neuro-humoral changes, all of which interact in a complex, integrative, pathophysiological milieu to slow conduction, alter excitability and refractoriness, promote electrical uncoupling, and generate spontaneous electrical activity [59]. The great complexity of biochemical and electrophysiological alterations caused by acute ischemia has made it difficult to sort out the contributions of specific derangements in arrhythmogenesis. Studies in genetically engineered mice have been particularly informative in defining the role of specific gene products in complex pathophysiological processes. For example, because Cx43 expression may be down-regulated by 25–50% in patients with chronic ventricular dysfunction [60,61], Cx43^{+/-} mice can provide insights into the role of diminished coupling in arrhythmogenesis induced by acute ischemia.

Recent studies in which a region of acute ischemia was created in isolated, perfused hearts by occlusion of the left anterior descending coronary artery have demonstrated clear phenotypic differences between Cx43^{+/-} and wild-type mice [62]. Spontaneous ventricular arrhythmias occurred in more than twice as many Cx43-deficient hearts than wild-type hearts. Ventricular tachycardia could be induced in nearly three times as many Cx43-deficient hearts. Multiple runs and prolonged runs of spontaneous ventricular tachycardia were more frequent and the onset of the first run of ventricular tachycardia occurred significantly earlier in Cx43-deficient hearts. These results provide compelling

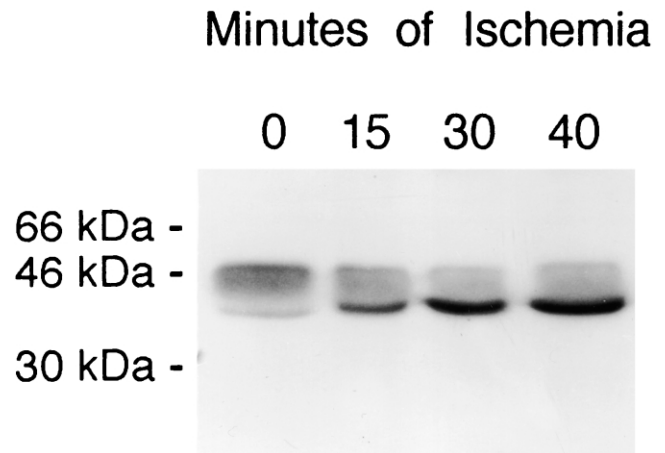


Fig. 4. Immunoblot of Cx43 in ventricular homogenates from isolated rat hearts subjected to selected intervals of ischemia. Phosphorylated isoforms of Cx43, migrating at 44–46 kDa, comprise ~85% of total Cx43 under basal conditions. During ischemia, there is progressive reduction in the amount of phosphorylated Cx43 and concomitant accumulation of non-phosphorylated Cx43 that migrates at 41 kDa. From Beardslee et al. [55].

evidence that a background level of reduced expression of Cx43 accelerates the onset and increases the incidence, frequency, and duration of ventricular tachyarrhythmias after coronary occlusion. In other studies, changes in electrical coupling induced by acute myocardial ischemia have been correlated with a marked reduction in the amount of phosphorylated Cx43 (which normally makes up ~85% of total Cx43) and accumulation of nonphosphorylated Cx43 [63] (Fig. 4). Although the total cellular content of Cx43 does not change during a 40-min interval of ischemia, immunohistochemistry with isoform-specific antibodies has shown progressive reduction in total Cx43 signal and concomitant accumulation of nonphosphorylated Cx43 signal at sites of intercellular junctions during acute ischemia [63]. These observations provide further evidence implicating changes in phosphorylation in the regulation of connexin function.

7. Up-regulation of connexin expression during hypertrophic growth

Compensatory hypertrophic growth of cardiac myocytes in response to a moderate increase in load is characterized by increased synthesis of contractile proteins, assembly of new sarcomeres, and improved contractile function. It may also be associated with increased expression of connexins leading to an increased number of gap junctions and enhanced intercellular coupling. Changes in connexin expression have not been studied extensively in patients with physiologic hypertrophy. Studies in vitro, however, have provided support for the concept that connexin expression and coupling are enhanced during initial phases of hypertrophic growth. For example, long-term (24 h) expo-

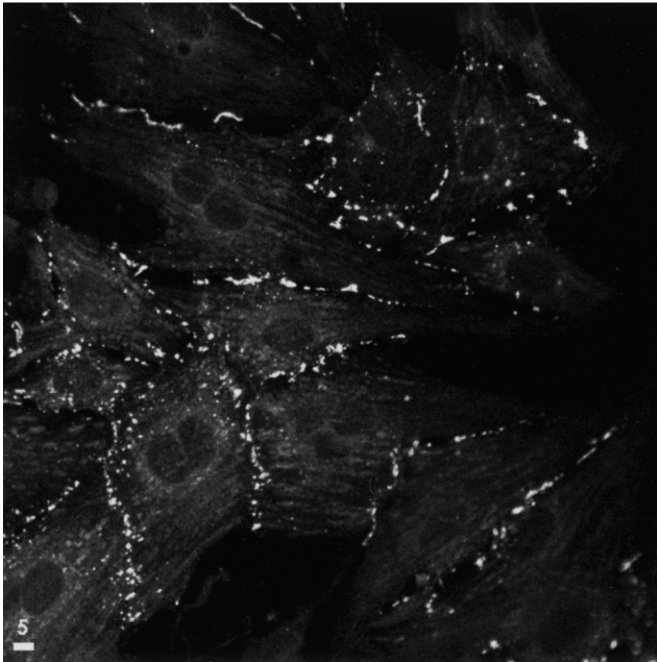
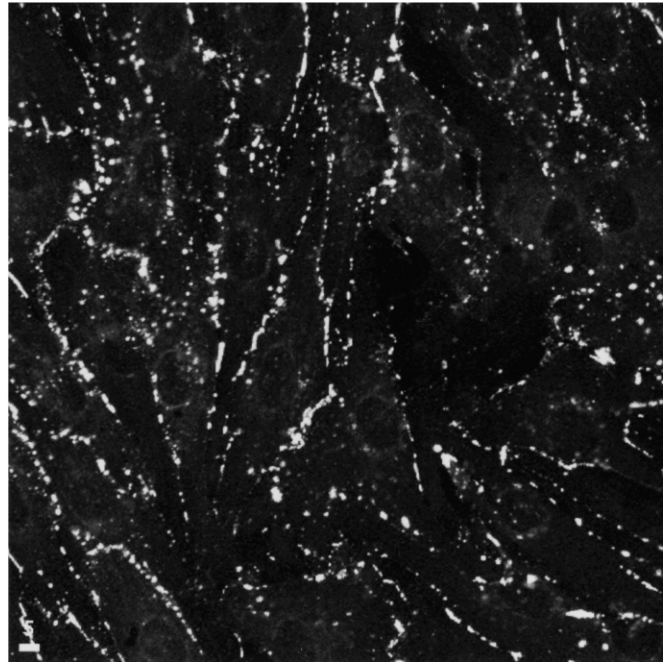
CONTROL**PULSATILE STRETCH**

Fig. 5. Confocal images of Cx43 immunofluorescence signal in control monolayers of neonatal rat ventricular myocytes or monolayers subjected to pulsatile stretch (110% of resting cell length at a frequency of 3 Hz for 6 h). From Zhuang et al. [66].

sure of neonatal rat ventricular myocyte cultures to chemical mediators of hypertrophy, such as cAMP [64] or angiotensin II [65], increases the tissue content of Cx43, the number of gap junctions, and conduction velocity. More recent studies have shown that a mechanical load produced by linear pulsatile stretch (110% of resting cell length at 3 Hz) causes rapid (within 1 h) and marked (greater than twofold) up-regulation of Cx43 expression and a significant increase in conduction velocity in cultured neonatal ventricular myocytes [66] (Fig. 5). Nonpulsatile (static) stretch produced qualitatively similar but significantly smaller changes than pulsatile stretch. The application of linear pulsatile stretch *in vitro* may be a model for exercise-induced cardiac hypertrophy or early compensatory hypertrophic growth in response to pressure or volume overload seen in patients with heart disease. Mechanisms responsible for up-regulation of intercellular coupling proteins during hypertrophy have not been defined but changes in both connexin synthesis and degradation probably play a role.

8. Down-regulation of connexin expression in chronic heart disease

The hypertrophic response is a dynamic continuum in which progressive changes in gene expression and alterations in the structure of cells and the extracellular matrix occur during a transition from a phase of compensatory adaptation to an increasingly maladaptive state culminating in heart failure. Although conduction velocity is typically

increased in hypertrophied ventricles, it subsequently decreases as hypertrophy becomes more severe [67,68]. Consistent with these clinical observations, it has been clearly demonstrated that ventricular Cx43 expression is reduced in patients with ischemic cardiomyopathy and other chronic myocardial disease states such as end-stage aortic stenosis [60,61]. Gap junction distribution is also altered in cardiac myocytes infected with *Trypanosoma cruzi*, the cause of Chagas' disease, and could contribute to conduction derangements and arrhythmias [69,70].

Remodeling of gap junction distributions has been closely linked to development of reentrant arrhythmias in patients with healed myocardial infarcts [71]. Viable myocardium at the edges of healed infarcts typically exhibits interstitial fibrosis and rearrangements of gap junctions that likely contribute to slow conduction, conduction block, and complex fractionated electrograms characteristic of these regions [71–73]. Significant reductions in the number of cells connected to an individual myocyte, as well as selective loss of intercellular connections between cells oriented side to side, have been observed in infarct border zones [74,75]. This pattern of remodeling is consistent with observations in experimental animals and human arrhythmia mapping studies in which propagation through remodeled regions during sinus rhythm generally occurs in a direction parallel to the long axis of the myocytes. Under these circumstances, conduction would be expected to remain relatively rapid because end-to-end connections are preserved. In contrast, ventricular tachycardia is typically induced and maintained when wavefronts activate remodel-

eled regions in the transverse direction. Because side-to-side connections are selectively disrupted, transverse propagation is likely to be impaired and wavefronts must zigzag through the tissue until they reenter postrefractory tissue and initiate the next beat of the tachycardia [75]. These observations directly implicate diminished connexin expression and remodeling of gap junction distributions as principal components of anatomic substrates of ventricular arrhythmias.

9. Conclusions

Much remains to be learned about the role of gap junctions in normal cardiac function and arrhythmogenesis. In addition to fulfilling an obvious role in current transfer, intercellular communication via gap junctions may also play important roles in coordinating mechanical function and disseminating diverse chemical signals that help the heart respond to ever-changing demands of the periphery. The functional significance of multiple cardiac connexins remains to be elucidated. Similarly, the full implications of changes in connexin expression and gap junction channel function and distribution in various forms of heart disease must be defined. Although down-regulation of connexin expression in chronic heart disease may be regarded as maladaptive because it contributes to the development of arrhythmia substrates, it might also exert protective effects by limiting intercellular spread of biochemical mediators of injury. Additional studies will be required to determine whether new therapies designed to modulate intercellular coupling will be beneficial in lowering the risk of developing lethal arrhythmias in patients with acute and chronic myocardial diseases.

Acknowledgments

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