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Remodeling of Gap Junctions in Ischemic and Nonischemic Forms of Heart Disease

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Abstract Electrical activation of the myocardium to produce effective pumping of blood depends on the orderly coordinated spatial and temporal transfer of current from one cell to another via gap junctions. Normal ventricular myocytes are extensively coupled by gap junctions and have the capacity to rapidly increase the amount of connexin within gap junction plaques to meet physiological demands for enhanced cell-cell communication. However, myocytes can also rapidly uncouple in response to injury or disease. In general, both acute and chronic forms of heart disease caused by diverse etiologies are associated with changes in the expression of connexins and remodeling of gap junctions. Such remodeling may have both adaptive and maladaptive consequences and contribute to major clinical processes such as heart failure and sudden cardiac death. Our laboratory has investigated mechanisms regulating cell-cell electrical coupling in the heart under physiological and pathophysiological conditions. This review is focused on selected aspects of this work pertaining to changes in coupling in response to acute and chronic ischemic heart disease and in familial cardiomyopathies caused by mutations in genes encoding desmosomal proteins.

Keywords Gap junction · Heart disease · Ischemia

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Introduction

Cardiac muscle is not a true electrical syncytium. Rather, it is composed of individual cells, each invested with a continuous lipid bilayer which provides a considerable degree of electrical insulation. Electrical activation of the heart requires intercellular transfer of current, a process that can only occur at gap junctions (Saffitz, Lerner & Yamada, 2004). Thus, the number, size and distribution of gap junctions are important determinants of impulse propagation in cardiac muscle. Furthermore, alterations in the structure or function of gap junctions can give rise to conduction disturbances that may contribute to arrhythmogenesis. This review is focused on selected aspects of gap junction remodeling in ischemic and nonischemic forms of heart disease in which sudden cardiac death is likely related to abnormal electrical coupling.

Adaptive and Maladaptive Consequences of Electrical Uncoupling in Acute and Chronic Ischemic Heart Disease

Ventricular myocytes are connected by numerous gap junctions that are among the largest that occur in mammalian tissues (Saffitz et al., 2004). The extensive gap junctional coupling seen in the heart is likely an evolutionary adaptation that is critical to normal cardiac function. However, maintenance of a high level of intercellular coupling carries the risk that when the heart is injured, chemical mediators of injury will spread from severely affected areas to less diseased areas and thereby increase the damage to the heart.

Our current thinking about adaptive/maladaptive aspects of electrical uncoupling in response to injury has been

shaped in part by studies involving genetically engineered mice that are heterozygous for a null mutation in the gene encoding Cx43, the principal ventricular connexin ($Cx43^{+/-}$ mice). These mice express approximately 50% of the wild-type level of Cx43 and exhibit a modest degree of conduction slowing but are otherwise apparently normal and live a normal life span (Thomas et al., 1998; Eloff et al., 2001). However, they exhibit distinct phenotypes in response to provocative maneuvers, which shed light on the specific role of gap junctions in complex pathophysiological processes such as arrhythmogenesis in the setting of acute myocardial ischemia. This has been well demonstrated in studies of hearts from wild-type ($Cx43^{+/+}$) and $Cx43^{+/-}$ mice which have been subjected to acute occlusion of the left anterior descending coronary artery. Such studies have shown that the incidence, duration and frequency of ventricular arrhythmias are significantly greater in $Cx43^{+/-}$ hearts compared with $Cx43^{+/+}$ hearts in response to acute regional ischemia induced by coronary occlusion (Lerner et al., 2000). Arrhythmias also develop sooner after the onset of ischemia in $Cx43^{+/-}$ compared with $Cx43^{+/+}$ hearts (Lerner et al., 2000). These results unequivocally prove that a diminished level of Cx43 expression present at the onset of ischemia is a potent, independent risk factor in arrhythmogenesis.

While at first glance, these observations indicate that uncoupling during ischemia is maladaptive because it creates acute arrhythmia substrates, we know that, in general, responses to injury have evolved to fulfill protective and adaptive purposes. Another study took this pathophysiological scenario to its logical conclusion by characterizing healed infarcts in mice subjected to permanent coronary occlusion (as opposed to the immediate effects of acute coronary occlusion). Thus, myocardial infarction was induced by ligating the left anterior descending coronary artery, and animals were allowed to recover for several weeks, during which time infarcts underwent inflammatory and reparative events leading to replacement by a fibrous scar. We observed that $Cx43^{+/-}$ mice had significantly smaller infarcts than $Cx43^{+/+}$ mice despite the fact that in both groups the coronary arteries had been ligated at precisely the same location (Kanno et al., 2003). Taken together, these results illustrate an important principle of intercellular coupling in the heart. On the one hand, it is beneficial for cardiac myocytes to be extremely well coupled. Normal levels of coupling ensure rapid and safe conduction and facilitate the unfettered exchange of signaling molecules to coordinate the activities of individual cells so that they function as a tissue. On the other hand, maintenance of a high level of coupling carries the risk that when the heart is injured, chemical mediators of injury will spread from severely affected areas to less diseased areas and thereby increase damage to the heart. Thus, it is advantageous for cardiac myocytes to be as well coupled as possible to meet physiological demands, but it is also advantageous for viable cells to rapidly dissociate themselves from injured neighbors by uncoupling.

Remodeling of Gap Junctions in the Cell-Cell Junction Cardiomyopathies

Cardiac myocytes are connected by large intercellular junction complexes which play a critical role in both electrical and contractile functions in the heart. Because cardiac myocytes contract, they require more extensive and robust adhesion junctions than noncontractile cells in other solid organs. It is no surprise, therefore, that adherens junctions and desmosomes, organelles responsible for physically connecting one cardiac myocyte to another, are highly concentrated at the ends of individual cells, where they form elaborate complexes that can be readily identified at the light microscopic level of resolution and which have been given a specific name – the *intercalated disk*. Intercalated disks are composed of arrays of adherens junctions, each located at the end of a row of sarcomeres, and desmosomes which link cell-cell adhesion junctions to desmin filaments of the cytoskeleton. As highlighted above, cardiac myocytes also have a special requirement for extensive electrical coupling. However, membrane regions containing gap junctions are rigid and nonfluid because of the high concentration of protein within the lipid bilayer; as a result, these regions are vulnerable to shear stress. This presumably explains why gap junctions in cardiac myocytes are located within intercalated disks, where they are surrounded by mechanical junctions. These mechanical junctions apparently act as ‘‘spot welds’’ to create membrane domains that are protected from shear stress caused by contractile activities of neighboring cells and which thereby facilitate assembly and maintenance of large arrays of intercellular electrical channels.

We have studied a group of human cardiomyopathies caused by mutations in genes encoding proteins that function as linkers in cell-cell adhesion junctions. These heart diseases, which we have termed *cell-cell junction cardiomyopathies*, are caused by mutations in intracellular proteins that link adhesion molecules at adherens junctions and desmosomes to the myocyte cytoskeleton. Among the genes implicated in these diseases are those encoding desmoplakin, plakoglobin and plakophilin-2. These mutations have both dominant and recessive patterns of inheritance and are associated with clinical phenotypes of arrhythmogenic right ventricular cardiomyopathy (ARVC) or dilated cardiomyopathy, with or without hair and skin abnormalities (Protonotarios & Tsatsopoulou, 2004). One common feature of the cell-cell junction cardiomyopathies

is a high incidence of syncope, ventricular arrhythmias and sudden cardiac death. This observation suggests that alterations in intercellular adhesion caused by defects in cell-cell mechanical junctions may create anatomic substrates that are particularly conducive to the development of lethal ventricular arrhythmias.

Our work in this area has focused on the hypothesis that defective mechanical linkage in the cell-cell junction cardiomyopathies causes remodeling of gap junctions, which in turn can give rise to conduction abnormalities that contribute to the high incidence of sudden death in these patients (Saffitz, 2005). As shown in Figure 1, this hypothesis provides a mechanistic link between contractile dysfunction and electrical dysfunction in the cell-cell junction cardiomyopathies. As briefly presented below, we have tested this hypothesis through multiple approaches involving analysis of tissues from patients, characterization of phenotypes in mouse models of the human diseases and *in vitro* studies to identify signaling pathways that regulate expression of mechanical and electrical junction proteins.

Remodeling of Gap Junctions in Human Cell-Cell Junction Cardiomyopathies

To test the hypothesis that defects in the adhesion junction-cytoskeleton network disrupt gap junctions, we analyzed ventricular tissues from patients with familial cardiomyopathies caused by mutations in desmosomal proteins. One of these conditions is Naxos disease, a cardiocutaneous syndrome consisting of the clinical triad of woolly hair, palmoplantar keratoderma and ARVC (Protonotarios, Tsatsopoulou & Gatzoulis, 2002). Approximately 60% of patients affected by Naxos disease present with syncope and/or aborted sudden death, and they have an annual risk of arrhythmic death of 2.3% (Protonotarios et al., 2001). Naxos disease is caused by a recessive mutation in the gene encoding plakoglobin (McKoy et al., 2000). The mutation causes a frameshift, resulting in premature termination and expression of a truncated protein lacking 56 residues at the C terminus. We characterized the distribution of cell-cell junction proteins in ventricular tissues from autopsy of Naxos disease patients using confocal immunofluorescence microscopy and observed a striking reduction in the amount of junctional signal for Cx43 (Kaplan et al., 2004b) (Fig. 2). This was confirmed by electron microscopy, which showed smaller and fewer gap junctions interconnecting ventricular myocytes. Interestingly, immunoblotting revealed that total Cx43 protein content was apparently not reduced but the highly phosphorylated P2 isoform of Cx43, which is selectively located in the junctional pool, was absent (Kaplan et al., 2004b) (Fig. 2). These observations suggest that remodeling of gap junctions in Naxos disease is not related to changes in Cx43 expression per se but, rather, to an inability to assemble and/or maintain large gap junction channel arrays. The degree of gap junction remodeling observed in Naxos disease patients is sufficient to cause conduction slowing, which could contribute to the characteristic widening of the QRS complex in the right precordial leads. Although uncoupling at gap junctions may not by itself cause arrhythmias, it could produce a substrate that promotes arrhythmias when combined with a ''second insult,'' such as the pathological changes in the right ventricle in ARVC.

We have also observed a marked decrease in the amount of Cx43 in ventricular myocytes in the heart of an 11-year-old girl with Carvajal syndrome (Kaplan et al., 2004a), a cardiocutanous syndrome characterized by woolly hair, palmoplantar keratoderma and a diffuse cardiomyopathy that is distinct from ARVC (Carvajal-Huerta, 1998). Affected children usually die before the age of 20, apparently due to both pump dysfunction and lethal arrhythmias (Carvajal-Huerta, 1998). Carvajal syndrome is caused by a recessive single-nucleotide deletion mutation in desmoplakin leading to a premature stop codon and truncation of the C-terminal desmin-binding domain (Norgett et al., 2000). These results provide further evidence that abnormal protein-protein interactions at intercellular junctions cause both contractile and electrical dysfunctions in Carvajal syndrome.

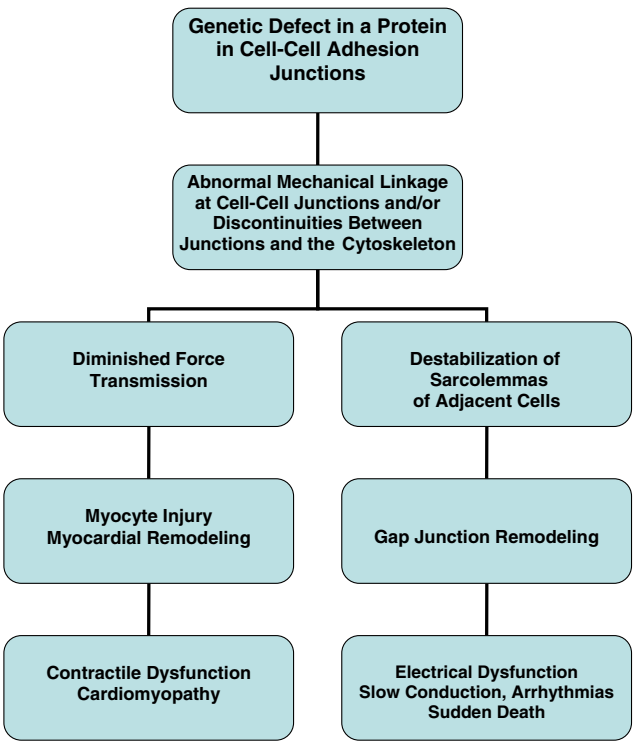

Fig. 1 A flow chart illustrating a proposed mechanism linking electrical and contractile dysfunctions in the cell-cell junction cardiomyopathies

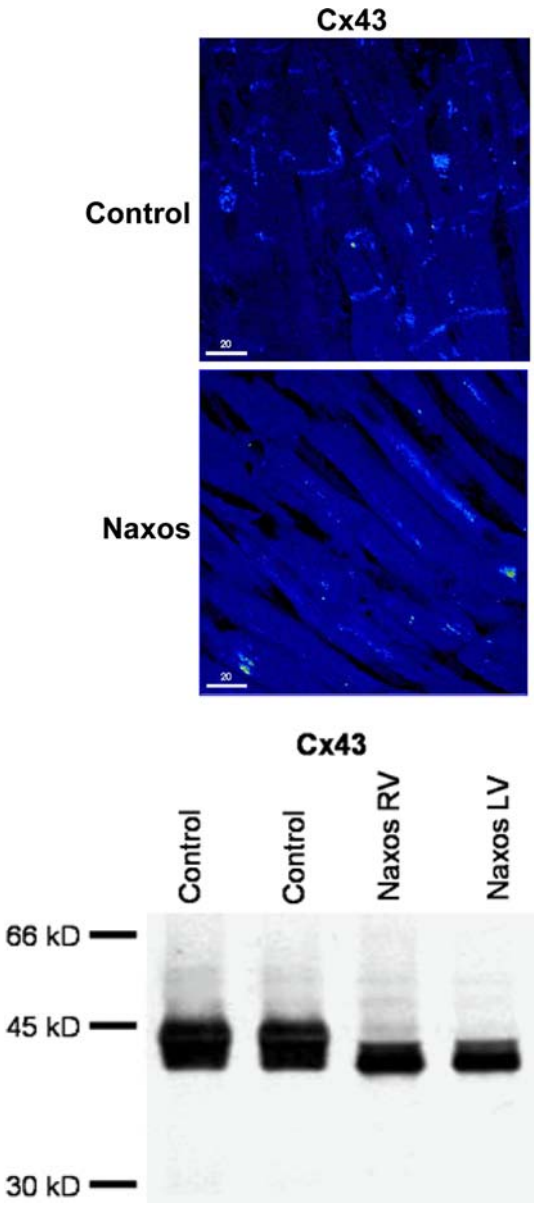

Fig. 2 Confocal microscopic images showing diminished localization of Cx43 in gap junctions and immunoblots showing abundant expression of Cx43 but absence of highly phosphorylated Cx43 in Naxos disease myocardium. Scale bar = 20 μm

Taken together, our studies of these and other human cell-cell junction cardiomyopathies have led to two major conclusions: (1) remodeling of gap junctions is a consistent and prominent feature of the cell-cell junction cardiomyopathies and (2) specific patterns of abnormal localization of mechanical junction proteins at intercalated disks correlate with cardiomyopathy disease phenotypes.

Mouse Models of Human Cardiomyopathies

To further test the hypothesis that defective linkage between adhesion junctions and the cytoskeleton leads to remodeling of gap junctions, we characterized a model of human desmin-related cardiomyopathy created by X. J. Wang in the laboratory of Jeffrey Robbins (Wang et al., 2001). We hypothesized that this model would exhibit altered desmin-desmosome interactions and, therefore, recapitulate some features of Carvajal syndrome. The model involved cardiac-specific expression of a transgene encoding a seven-amino acid deletion mutation in desmin (D7-des) which has been implicated in a human cardiomyopathy (Wang et al., 2001). These mice exhibit features of the human disease including intracellular accumulation of desmin, disruption of the desmin filament network, misalignment of myofibrils and diminished responsiveness to β-adrenergic agonist stimulation (Wang et al., 2001).

To test the hypothesis that expression of D7-des disrupts the linkage between desmosomes and the cytoskeleton and leads to remodeling of gap junctions, we characterized the expression and localization of intercellular junction proteins and searched for an electrophysiological phenotype (Gard et al., 2005). As predicted by studies of the human cell-cell junction cardiomyopathies, Cx43 signal at intercalated disks was decreased by approximately threefold in D7-des hearts due to significant reductions in both the number and mean size of individual gap junctions (Fig. 3). The amount of immunoreactive signal at intercalated disks was also reduced significantly for selected adhesion molecules and linker proteins of both desmosomes and adherens junctions, and desmin-desmosomal interactions were completely disrupted (Gard et al., 2005). Quantitative electron microscopy showed decreased gap junction density in D7-des mice, providing independent evidence of gap junction remodeling; but immunoblotting showed no reduction in the total tissue content of Cx43 and mechanical junction proteins. These observations are consistent with findings in Naxos disease, suggesting that diminished localization of cell-cell junction proteins at intercalated disks is not due to insufficient protein expression but, rather, to failure of these proteins to assemble properly within electrical and mechanical junctions. We also showed, using optical mapping, that remodeling of gap junctions in D7-des mice slows ventricular conduction (Gard et al., 2005). These results indicate, therefore, that a defect in a protein conventionally thought to fulfill a strictly mechanical function in the heart can also lead to electrophysiological alterations that may contribute to arrhythmogenesis.

Mechanisms Regulating Expression of Cell-Cell Junction Proteins in Response to Mechanical Load

To elucidate mechanisms regulating expression of intercellular junction proteins, we developed an *in vitro* system

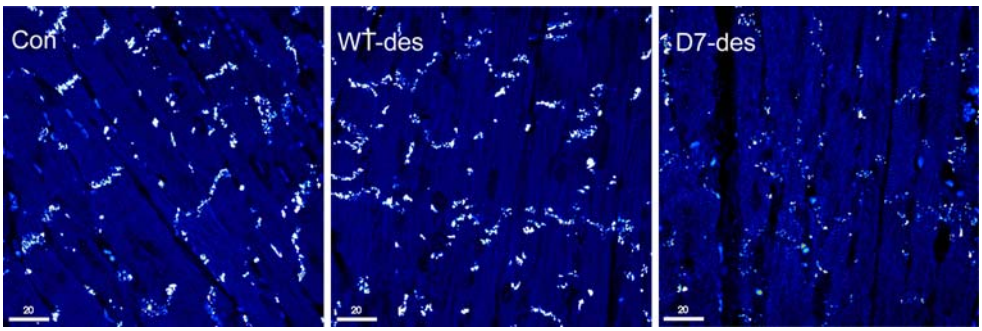

Fig. 3 Representative confocal immunofluorescence images showing the amount of Cx43 immunoreactive signal at cell-cell junctions in left ventricular myocardium from a nontransgenic control mouse (*Con*), a transgenic mouse expressing wild-type desmin (*WT-des*) and a transgenic mouse expressing D7-des. Scale bar = 20 μm

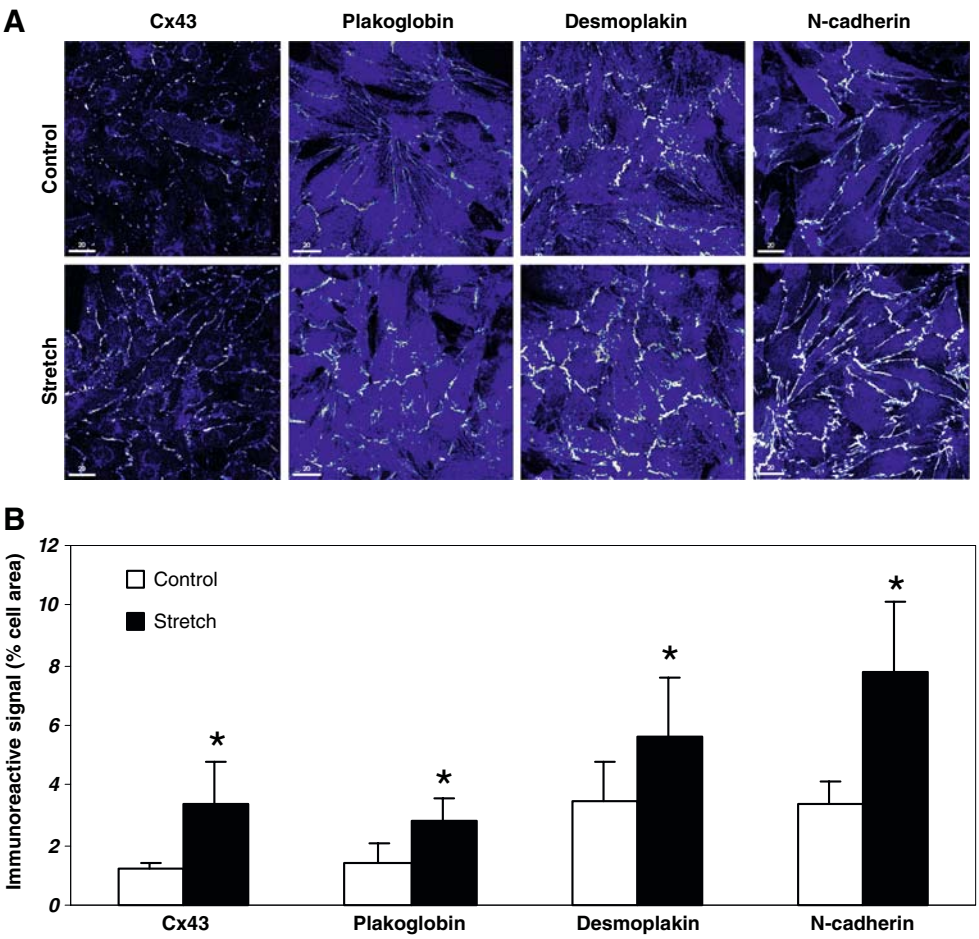

Fig. 4 Representative confocal immunofluorescence images (**A**) and quantitative confocal microscopic data (**B**) showing the effects of stretch on expression of Cx43 and the mechanical junction proteins plakoglobin, desmoplakin and N-cadherin. *$p < 0.05$ compared with control. Scale bar = 20 μm

in collaboration with André Kléber in which monolayers of neonatal rat ventricular myocytes are grown on silicone membranes and subjected to uniaxial pulsatile stretch (Zhuang et al., 2000). Imposition of this mechanical load rapidly induces a hypertrophic response, which can be rigorously quantified and characterized. An important feature of this response is a marked increase in Cx43 expression and enhanced intercellular coupling. After only 1 h of stretch (110% of resting length at 3 Hz), expression of Cx43 is increased by approximately twofold, resulting in a significant increase in both the number of gap junctions and the velocity of impulse propagation (Zhuang et al., 2000; Pimentel et al., 2002). We have shown previously that upregulation of Cx43 expression is mediated by stretch-induced secretion of vascular endothelial growth factor (VEGF), which acts in an autocrine fashion (Pimentel et al., 2002). Incubation of cells with exogenous VEGF for 1 h increases Cx43 expression by an amount roughly equal to that seen after 1 h of pulsatile stretch. In addition, stretch-induced upregulation of Cx43 expression can be blocked by stretching cells in the presence of anti-VEGF or anti-VEGF receptor antibodies.

To determine whether stretch-induced formation of new gap junctions requires concomitant assembly of new mechanical junctions, we measured changes in mechanical junction protein expression in cells subjected to stretch

(Yamada et al., 2005). The amounts of plakoglobin, desmoplakin and N-cadherin at cell-cell junctions all increased by at least twofold in myocytes subjected to 1 h of pulsatile stretch (Fig. 4). However, stretch-induced secretion of VEGF played no role in this process. Addition of exogenous VEGF does not affect expression of mechanical junction proteins nor is stretch-induced upregulation of these proteins blocked by anti-VEGF antibodies. Rather, intracellular signaling pathways mediated via activation of focal adhesion kinase (FAK) appear to be responsible. This was demonstrated by infecting cardiac myocytes with an adenovirus containing FRNK, a green fluorescent protein-tagged dominant-negative inhibitor of FAK-dependant signaling, and then subjecting cells to pulsatile stretch (Yamada et al., 2005). FRNK blocked stretch-induced upregulation of both electrical (Cx43) and mechanical (N-cadherin, desmoplakin and plakoglobin) junction proteins. Addition of exogenous VEGF upregulated expression of Cx43, but not mechanical junction proteins, in FRNK-infected cells. Conditioned medium removed from uninfected cells after 1 h of stretch also increased Cx43 expression when added to nonstretched cells, and this effect was blocked by anti-VEGF antibodies; however, stretch-conditioned medium from FRNK-infected cells had no effect on Cx43 expression. Thus, stretch-induced secretion of VEGF requires activation of FAK. Finally, the src kinase inhibitor PP2 blocked stretch-induced upregulation of mechanical junction proteins but not Cx43 (Yamada et al., 2005). These results indicate that mechanical load regulates expression of both electrical and mechanical junction proteins but by disparate mechanisms. Cx43 expression is regulated by autocrine actions of chemical mediators secreted during stretch, whereas adhesion junction proteins are regulated by intracellular mechanotransduction pathways initiated via FAK and dependent on downstream activation of src kinase.

Conclusions

Cardiac myocytes have the ability to increase or decrease their level of coupling at gap junctions in response to changing physiological demands or stress caused by injury or disease. The molecular mechanisms responsible for gap junction remodeling are not fully understood and probably involve multiple events including changes in connexin trafficking and assembly into functional channels, changes in channel gating and changes in connexin synthesis and degradation rates. Remodeling of gap junctions in the cell-cell junction cardiomyopathies appears to be related to problems with assembly and/or maintenance of large gap junction plaques. It must also be considered, however, that Cx43 gene expression may be altered in the cell-cell junction cardiomyopathies. Plakoglobin and other members of the catenin family fulfill both structural and nuclear signaling roles (Conacci-Sorrell, Zhurinsky & Ben-Ze'ev, 2002). Disease-related mutations may shift the relative proportions of these proteins within junctional and cytosolic pools, which in turn could affect nuclear signaling mediated by plakoglobin, β-catenin or other related proteins. Thus, it is possible and perhaps even likely that both altered mechanical integrity and altered nuclear signaling underlie the pathogenesis of contractile and electrical dysfunctions in heart muscle diseases caused by mutations in cell-cell junction proteins.

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