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Regulation of intercellular coupling in acute and chronic heart disease

J.E. Saffitz

Departments of Pathology and Medicine, and the Center for Cardiovascular Research, Washington University School of Medicine, St. Louis, MO, USA

Abstract

Effective pump function of the heart depends on the precise control of spatial and temporal patterns of electrical activation. Accordingly, the distribution and function of gap junction channels are important determinants of the conduction properties of myocardium and undoubtedly play other roles in intercellular communication crucial to normal cardiac function. Recent advances have begun to elucidate mechanisms by which the heart regulates intercellular electrical coupling at gap junctions in response to stress or injury. Although responses to increased load or injury are generally adaptive in nature, remodeling of intercellular junctions under conditions of severe stress creates anatomic substrates conducive to the development of lethal ventricular arrhythmias. Potential mechanisms controlling the level of intercellular communication in the heart include regulation of connexin turnover dynamics and phosphorylation.

Key words
- Gap junctions
- Cardiac hypertrophy
- Heart failure
- Arrhythmias

Correspondence
J.E. Saffitz
Department of Pathology
Box 8118
Washington University School of Medicine
660 S. Euclid Ave.
St. Louis, MO 63110
USA
Fax: +1-314-362-4096
E-mail: saffitz@pathology.wustl.edu

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Introduction

Derangements in conduction of electrical impulses play a critical pathogenic role in sudden death due to ventricular arrhythmias (1,2). In patients who have survived a previous myocardial infarction, sudden death may occur by a reentrant mechanism in which conduction slowing and unidirectional conduction block, necessary for initiation and maintenance of a reentrant circuit, typically arise in viable, but structurally altered, myocardium bordering the infarct (3-7). Despite the distinctly abnormal propagation of wavefronts at the edges of healed infarcts, intracellular recordings of resting membrane potentials and action potential upstroke velocities in myocytes adjacent to infarct scars are typically normal or nearly normal once infarct healing is complete (4,8). Thus, abnormal conduction leading to reentry in regions bordering healed infarcts is a property that emerges from the multicellular nature of diseased myocardial tissue rather than a property that resides within individual cardiac myocytes in this region. Indeed, the pathophysiologically relevant abnormality appears to be alterations in current transfer between myocytes at gap junctions.

Recent advances in understanding cardiac muscle pathobiology and the cellular and molecular biology of gap junctions have elucidated mechanisms of remodeling of intercellular electrical junctions in diseased myocardium. As a general principle, cardiac myocytes respond to overload states (e.g., increased peripheral vascular resistance in hypertension) or disease (e.g., regional myo-

cardial infarction) by undergoing hypertrophic growth. The hypertrophic response is adaptive in that it is designed to achieve a new steady state in which cardiac function is adequate to meet the demands of the prevailing conditions. As described in this brief report, current evidence suggests that during the initial, compensatory phase of the hypertrophic response, cardiac connexin expression is increased and intercellular coupling may be enhanced. As the heart undergoes a transition from compensated hypertrophic growth to a decompensated state and heart failure ensues, it appears that connexin expression is diminished and cells may become uncoupled. This may occur throughout the myocardium in diffuse heart muscle diseases such as the cardiomyopathies. Alternatively, decreased coupling may arise in restricted areas subjected to sublethal injury (e.g., myocytes at the edges of acute infarcts). In any case, alterations in connexin expression and intercellular electrical coupling may have important implications for cardiac electrophysiology and arrhythmogenesis. It appears that the normal heart has the capacity to regulate its level of intercellular coupling rapidly by multiple mechanisms involving changes in the expression, distribution, and function of gap junction channels. Any change in the three-dimensional distribution of gap junctions will affect the conduction properties of the heart in both normal and pathophysiological settings.

Enhanced connexin expression and intercellular coupling in the acute hypertrophic response

In response to a moderate increase in load, cardiac myocytes undergo 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. A detailed understanding of specific signal transduction pathways and molecular mechanisms regulating changes in gap junction protein expression and function during the hypertrophic response is lacking. However, results from both *in vitro* and *in vivo* studies suggest that, in general, hypertrophic growth is associated with increased expression of cardiac connexins. For example, we have observed that long-term (24 h) exposure of neonatal rat ventricular myocyte cultures to a membrane permeable form of cAMP, a potential chemical mediator of cardiac hypertrophy, increases the total content of connexin43 (Cx43) by ~2-fold and increases the number of gap junctions between cells (9). This increase in Cx43 expression and in the number of gap junctions is associated with a gain of function as demonstrated by optical mapping of impulse propagation in cultured myocytes grown in sheets or strands (9). For example, in myocytes grown in sheets, we observed that conduction velocity increased significantly from 30.7 ± 5.4 cm/s in control cultures to 39.6 ± 5.4 cm/s in cultures incubated with cAMP ($P<0.001$). There was no corresponding change in the excitability of individual cells as reflected by measurements of dV/dt_{max} (158 ± 12.8 V/s, treated; 156.5 ± 16.3 V/s, control). Thus, more rapid conduction in cells incubated with cAMP was related primarily to enhanced intercellular coupling due to increased numbers of functional gap junction channels.

In other experiments, we observed that cultured neonatal rat ventricular myocytes exposed for 24 h to angiotensin II, another chemical mediator strongly implicated in the hypertrophic response, also exhibited a 2-fold increase in Cx43 content and an increase in the number of gap junction profiles (10). However, it appears that the increases in Cx43 content and the number of intercellular junctions arise by different mechanisms in cardiac myocytes exposed to cAMP or angiotensin II. Metabolic labeling studies

revealed that the rate of Cx43 synthesis was increased by 2- to 3-fold in cardiac myocytes exposed to angiotensin for 24 h (10) (Figure 1). In contrast, exposure to cAMP did not increase Cx43 synthesis (Figure 1) even though the total tissue content of Cx43 and the number of gap junctions increased to the same extent in response to either cAMP or angiotensin II. These results suggest that cAMP increases the total tissue content of Cx43 by regulating connexin degradation, whereas increased Cx43 levels in cells treated with angiotensin II may result mainly from enhanced synthesis.

Remodeling of conduction pathways during early, compensatory responses to increased load *in vivo* may also be an active process involving up-regulation of connexin expression and rearrangements of gap junction distribution. Enhanced connexin expression has been observed during early stages of hypertrophy induced by renovascular hypertension in guinea pigs (11). Expression of Cx40 is enhanced in Purkinje fibers of the rat when hypertrophy is induced by hypertension (12). Up-regulation of connexins in hypertensive hypertrophy may be another example of enhanced connexin expression during early, adaptive hypertrophic growth in the heart.

Alterations in connexin expression and distribution as a feature of myocytolytic degeneration in heart failure

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 perfor-

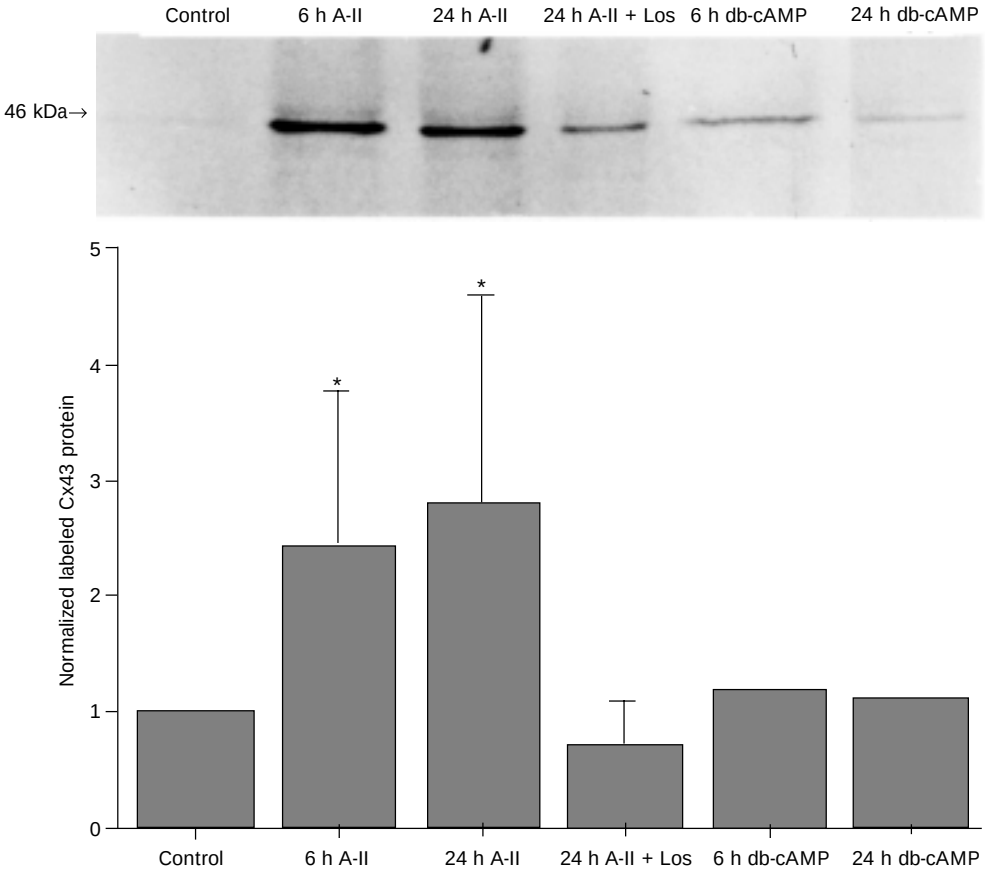

Figure 1 - The rate of connexin43 (Cx43) synthesis increases in cultured neonatal rat ventricular myocytes incubated with angiotensin II (A-II) but not cAMP. The upper panel shows an immunoprecipitation assay of radiolabeled Cx43 isolated from cultures incubated with [^{35}S]-methionine during the final 2 h of a 6- or 24-h treatment interval with A-II or dibutyryl cAMP (db-cAMP). As shown in the lower panel, the amount of radioactivity incorporated into Cx43 increased significantly in cultures treated with A-II compared with control cultures (means ± SD; *P<0.03) which were incubated with [^{35}S]-methionine in the absence of A-II or db-cAMP. The effects of A-II were blocked by the AT-1 receptor blocker losartan (Los). In contrast, there was no increase in radioactivity incorporated into Cx43 in cultures exposed to db-cAMP for 6 or 24 h. Thus, accumulation of Cx43 induced by cAMP appears to be due to diminished degradation rather than enhanced synthesis. Reprinted from Ref. 10 with permission.

mance. This phenotypic transformation is an adaptive process on the part of a seriously injured cardiac myocyte whose viability is threatened. The cell undergoes changes designed to ensure its survival but the inevitable consequence is diminished function. This is manifested clinically as congestive heart failure. The range of specific molecular and biochemical changes during this phenotypic transformation is undoubtedly vast. Alterations in multiple cellular regulatory processes play a role, including a significant shift in expression of enzymes responsible for energy metabolism from those using fatty acid substrates to those using glucose. Among the most prominent of these changes appears to be a reduction in the content of subcellular organelles that support contraction, especially sarcomeres. As a component of this response, however, other organelles including gap junctions also undergo marked alterations. The morphological picture of the myocyte survival response is nonspecific. 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 (13-15), as well as a reduction in gap junction protein expression and loss of large gap junctions (16-18). These stereotypical morphological changes, referred to as myocytolysis, are universally observed, at least to some extent, in dysfunctional ventricular wall segments in patients with chronic ischemic heart disease (18) and in diverse forms of cardiomyopathy in patients with heart failure (13-15,19).

The mechanisms by which the expression and spatial distribution of gap junction proteins are altered in chronic heart muscle disease are poorly understood. However, strong circumstantial evidence links the generalized down-regulation of connexin expression and reduced number of intercellular junctions in chronic heart disease to development of regions of abnormal conduction which are likely to contribute to the pathogenesis of reentrant ventricular tachycardia. Dynamic changes in both connexin synthesis and degradation probably play a role in this process. Recent observations have focused attention on connexin turnover as a determinant of connexin expression levels and intercellular coupling in normal and diseased myocardium.

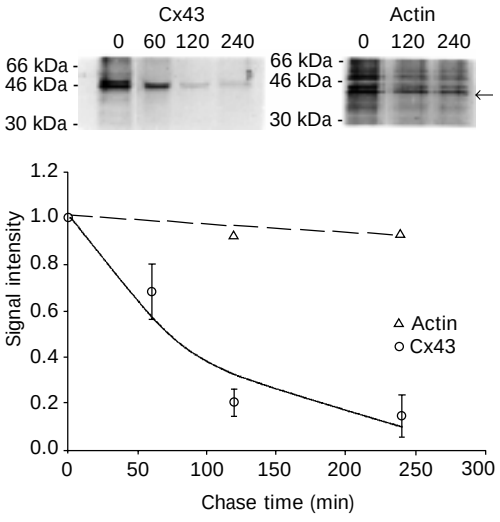

Figure 2 - Connexin43 (Cx43) and actin decay curves from pulse-chase experiments in adult rat hearts. Hearts were metabolically labeled with perfusate containing [^{35}S]-methionine, followed by chase periods of 0, 60, 120 and 240 min (N = 4 at each time point for Cx43). Radiolabeled Cx43 or actin immunoprecipitated from heart homogenates was separated on 12.5% SDS-PAGE gels and detected by autoradiography. Representative immunoprecipitation assays are shown. Actin was immunoprecipitated as a band at ~43 kDa (arrow). Cx43 and actin signals were quantified by densitometry and normalized to unchased values (given a value of 1.0). Background gray scale densitometry values were subtracted from each specific band to quantify signal intensity at each time point. A greater background level in the 0-min lane in the actin preparation accounted for the appearance of a greater signal intensity in the actin band at 0 min compared with 120- and 240-min time points. The decay constant, k (derived from the equation $y = e^{-kt}$), was used to calculate Cx43 half-life. Reprinted from Ref. 24 with permission.

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 cellular proteolytic systems as a fundamental mechanism in myocytolysis (20). Laird and co-workers (21) reported rapid turnover of Cx43 in cultured neonatal myocytes and this observation has been confirmed (22). Laing and co-workers (23) have characterized mechanisms of Cx43 degradation in primary cultures of neonatal rat ventricular myocytes using specific inhibitors of lysosomal and

proteasomal pathways. They reported that Cx43 is degraded by both pathways and have provided evidence that polyubiquitinated Cx43 is targeted to the proteasome (23). Recently, we characterized connexin turnover dynamics in the adult heart to elucidate its potential role in remodeling of gap junctions (24). We measured connexin turnover kinetics using metabolic labeling and pulse-chase strategies and characterized the proteolytic pathways involved in Cx43 degradation in intact, isolated perfused adult rat hearts. We observed that metabolically labeled Cx43 disappears from adult rat hearts with a half-life of ~1.3 h (Figure 2), equivalent to the turnover rate observed in neonatal rat ventricle myocytes grown in tissue culture (24). When hearts were perfused with specific inhibitors of either lysosomal or proteasomal proteolysis, significant increases in Cx43 content were observed. Thus, both pathways appear to degrade Cx43 in the adult heart as well as in cultured neonatal rat myocytes. However, much of the accumulated Cx43 in hearts perfused with lysosomal inhibitors consisted of phosphorylated isoforms, whereas nonphosphorylated Cx43 accumulated selectively in hearts perfused with a specific proteasomal inhibitor (24) (Figure 3).

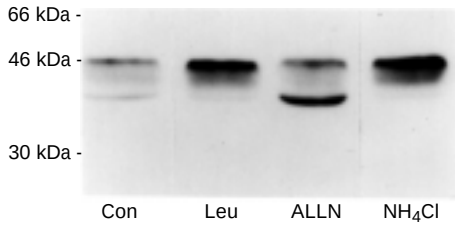

Figure 3 - Immunoblot analysis of Cx43 content in hearts perfused for 4 h with either buffer alone (Con) or protease inhibitors including leupeptin (Leu), ALLN, or NH_4Cl. Aliquots of total protein (30 µg) were separated by SDS-PAGE and probed with a rabbit polyclonal anti-Cx43 antibody. The total amount of Cx43 signal was increased in hearts perfused with all protease inhibitors. However, in hearts treated with the lysosomal inhibitors (leupeptin or NH_4Cl), there was selective accumulation of phosphorylated isoforms of Cx43 which migrate at apparent molecular mass of 44-46 kDa. In contrast, perfusion of hearts with the proteasomal inhibitor ALLN caused accumulation of nonphosphorylated Cx43 which migrates at 41 kDa. Reprinted from Ref. 24 with permission.

These results suggest that even in the normal adult heart, in which ventricular myocytes may be stably interconnected under basal conditions, the proteins that form gap junction channels turnover at a high rate and are apparently degraded by multiple proteolytic pathways. The high through-put 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, constitutively active synthetic pathways could be counter-balanced by regulated degradation pathways that determine final channel number. Immunohistochemical studies of Cx43 signal in control hearts or hearts perfused with either proteasomal or lysosomal pathway inhibitors revealed that under conditions in which either phosphorylated or nonphosphorylated isoforms of Cx43 accumulated, enhanced immunoreactive Cx43 signal occurred at sites

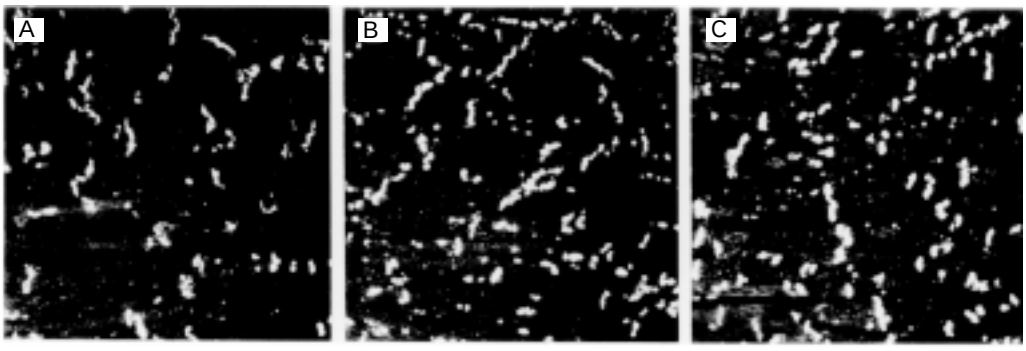

Figure 4 - Representative laser scanning confocal images of Cx43 immunofluorescence in a control heart (A) or in hearts perfused for 4 h with the proteasomal inhibitor ALLN (B) or the lysosomal inhibitor NH_4Cl (C). Increased signal is apparent at sites of intercellular apposition. Reprinted from Ref. 24 with permission.

of apparent gap junctions (24) (Figure 4). These results suggest that Cx43 may undergo dephosphorylation while still residing within gap junctions at the cell surface. The relationship between alterations in Cx43 phosphorylation and its subsequent degradation are not fully understood. Recently, we have observed that in response to acute myocardial ischemia, Cx43 undergoes a dramatic loss of phosphorylation with a time course that reflects the development of electrical uncoupling (25). These results add further weight to the hypothesis that regulation of connexin phosphorylation may be a fundamental means by which cells control the level of intercellular coupling and their expression levels of connexins during basal and pathophysiological conditions. Interventions with specific drugs targeted to connexin degradation pathways may limit anatomic changes that increase the risk of developing reentrant arrhythmias.

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