Remodelling of gap junctions and connexin expression in heart disease

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Abstract

Different combinations and relative quantities of three connexins—connexin43, connexin40 and connexin45—are expressed in different subsets of cardiomyocyte. In the healthy heart, gap junctions assembled from these different connexin combinations form the cell-to-cell pathways for the precisely orchestrated patterns of current flow that govern the normal heart rhythm. Remodelling of gap junction organization and connexin expression is a conspicuous feature of human heart disease in which there is an arrhythmic tendency. This remodelling may take the form of structural remodelling, involving disturbances in the distribution of gap junctions (i.e., disruption of the normal ordered pathways for cell-to-cell conduction), and remodelling of connexin expression, involving alteration in the amount or type of connexin(s) present. Most notable among quantitative alterations in connexin expression is a reduction in ventricular connexin43 levels in human congestive heart failure. By correlating data from studies in experimental animal models, gap junction and connexin remodelling emerges as a factor to be considered in understanding the pro-arrhythmic substrate characteristic of many forms of heart disease. However, our knowledge of the functional correlates of the specific patterns of multiple connexin expression found in different regions of the heart in health and disease remains rudimentary, and the development of new experimental cell models heralds advances in this area over the next few years.

Keywords: Cardiac disease; Human heart; Gap junction; Connexin; Intercellular communication

1. Introduction

Sequential contraction of the cardiac chambers depends on orderly spread of the wave of electrical excitation from one cardiomyocyte to the next, throughout the heart. The pathways enabling this cell-to-cell current flow are formed by the gap junctions that link individual myocytes into a functional syncitium. Cardiac gap junctions vary in size, abundance, distribution and connexin make-up according to their location within the different myocardial tissues of the heart. Moreover, alterations in the organization of gap junctions and expression of connexins accompany the pathogenesis of heart disease. The idea has thus grown that specific features of gap junction organization and connexin expression may be central to the electromechanical function of the heart in health and disease. In this review, we focus on the nature and possible significance of remodelling of gap junctions and connexin expression in human adult acquired heart disease, set within the context of knowledge on the normal heart, and drawing on selected correlative studies on animal and cell models.

2. Gap junctions and connexin expression in cardiomyocytes of the normal heart

An understanding of gap junction organization and connexin expression in the normal heart is a prerequisite to the interpretation of altered expression patterns or remodelling processes that may occur in cardiac disease. As widely documented in the literature, connexin43 is the predominant connexin expressed by cardiomyocytes, occurring in abundance in adult working ventricular and atrial cardiomyocytes of all mammalian species, including human [1–3]. Connexins 40 and 45 are also expressed in lower overall quantities, though in specific localized sites the expression of either of these connexins may exceed that of connexin43. Numerous studies have demonstrated that these are not random variations but that the three connexins are expressed...
in characteristic combinations and relative quantities in a chamber-related, myocyte-type-specific and developmentally regulated manner [1–10].

The working (contractile) cardiomyocytes of the ventricle are extensively interconnected by clusters of connexin43-containing gap junctions located at the intercalated disks (Fig. 1). The intercalated disks of working ventricular myocardium have a step-like configuration, with the gap junctions situated predominantly in the “horizontal”-facing segments of these steps rather than the vertical segments [11–13], and particularly large gap junctions typically circumscribing the disk periphery [14]. Features of gap junction organization such as this, together with aspects of tissue architecture such as the size and shape of the cells, combine to encourage preferential propagation of the impulse in the longitudinal axis, thus contributing to the normal pattern of anisotropic spread of the impulse of healthy ventricular myocardium.

Atrial cardiomyocytes are slender cells compared with their ventricular counterparts, with shorter, less elaborate intercalated disks. The gap junctions of atrial myocytes of most mammalian species, including humans, contain abundant connexin40 [3,15], co-localized with connexin43 within the same individual gap-junctional plaques [9]. Working ventricular myocytes, by contrast, normally lack detectable connexin40. In both ventricular and atrial human working myocardium, connexin45 is present in very low quantities, with slightly higher levels in the atria than the ventricles [3,10,15].

The specialized cardiomyocytes of the impulse generation and conduction system are morphologically quite distinct from the working ventricular and atrial cells [16]. The myocytes of the sinoatrial node, the site of impulse generation, and those of the atrioventricular node (Fig. 2), the site at which the impulse is slowed before being routed to the ventricles, are equipped with small, sparse, dispersed gap junctions containing connexin45 [17–19], a connexin that forms low conductance channels in vitro [20–22]. These gap junction features of nodal myocytes suggest relatively poor coupling, a property which in the atrioventricular node is linked to slowing of conduction and hence sequential contraction of the atria and ventricles. In the sinoatrial node of the rabbit, the connexin45-positive sinoatrial node is delineated from the surrounding atrial myocardium by a connective tissue layer, except at a restricted zone of connexin45/connexin43 co-expression at the nodal/crista terminalis border. These features may contribute to the ability of the sinoatrial node to drive the large mass of surrounding atrial tissue while remaining protected from its hyperpolarizing influence, with the zone of connexin45/connexin43 co-expression possibly serving as the exit route for the impulse into the atrial tissue [18]. Whether similar features occur in the human sinoatrial node is unknown.

Although connexin45 is the predominant atrioventricular nodal connexin, common to all mammalian species so far examined, some species variation involving limited co-expression of connexins 40 and 43 may occur. In particular, there are indications that larger mammals, which may have less need for atrioventricular nodal impulse delay, express connexin43 and/or connexin40 in the atrioventricular node in addition to connexin45 [23]. In the rodent, the spatial pattern of expression of connexin45 reveals that the atrioventricular node and His bundle form part of an elaborately extended central conduction system circumscribing the atrioventricular and outflow junctional regions [17], though the extent of this feature in other species is yet to be determined.

In addition to connexin45, cardiomyocytes of the His-Purkinje conduction system in most mammals, including man, express connexin40, a connexin associated with high conductance channels [6,8–10,24–26]. Prominent immunolabelling for this connexin, in the form of large, abundant gap junctions, correlates with the fast conduction properties of the bundle branches and Purkinje fiber system which facilitate rapid distribution of the impulse throughout the working ventricular myocardium. In rodents, connexin45 is co-expressed with connexin40 in a central zone of the bundle branches and Purkinje fibers, enveloped by an outer zone in which only connexin45 is found [17]. Again, whether this feature is present in humans is at present unknown.

New opportunities for investigating the role of the three connexins in cardiac function came with the development of transgenic animals in which expression of specific connexins may be ablated, or one connexin type is substituted for another. From the perspective of analysing the electrophysiological aspects of connexin function in the adult heart, however, this approach can have limitations arising from a

![Fig. 1. Reconstruction of serial optical sections through an isolated ventricular myocyte labelled for connexin43 (green) illustrating localization of the gap junctions in clusters at the intercalated disks. The nuclei are counterstained with propidium iodide (red). Bar marker =100 µm.](image-url)
critical need for some connexin types during development. The first connexin knock-out studies in mice demonstrated that absence of connexin43 was compatible with survival to term but the animals died shortly after birth owing to obstruction of the right outflow tract [27], a developmental abnormality resulting from defective migration of neural crest cells [28,29]. To determine the effects of lack of connexin43 on the cardiomyocyte after birth, Gutstein et al. [30] generated mice with cardiac-specific knock-out of Cx43, thereby circumventing the non-cardiac myocyte related developmental defect [29]. The resultant mice showed marked reduction of ventricular conduction velocity and developed lethal spontaneous ventricular arrhythmia by 2 months of age, indicating that cardiomyocyte connexin43 is essential for function of the more mature ventricle but not for the early neonatal ventricle where the low amounts of connexin45 still present are apparently sufficient to maintain impulse propagation. Whether the progressive spatial remodelling of gap junctions into intercalated disk clusters that takes place during the postnatal period [31,32] also contributes to increased susceptibility to arrhythmia in these mice is unknown.

Knock-out of connexin45 in mice results in death at about embryonic day 10, demonstrating that expression of this connexin is vital for development [33,34]; a cardiac-restricted knock-out of this connexin will thus be required to help determine cardiac specific functions. Connexin40 knock-out is, however, compatible with survival, but leads to impairment of conduction through the conduction system [35–39]. Residual ability of the His-Purkinje system to support conduction in the absence of connexin40 is attributable to the presence of connexin45 which, unlike con-

Fig. 2. Confocal micrographs showing the distribution of connexins 45 and 40 in the atrioventricular node of a heart from a 3-month-old mouse, cut in the plane shown in the adjacent diagrammatic representation of the conduction tissues (A). Connexin45 (B) is distributed throughout the whole nodal area, visible as bright, green spots of label, whereas connexin40 labelling (C) is confined to the distal part of the node. Colocalization of the two connexins (D) highlights their relative spatial patterns of expression. Bar marker =125 μm.
nexin40, is distributed from beginning to end of the system [17]. Replacement of connexin43 by connexin40 or connexin32 rescues the postnatal lethality of connexin43 knock-out and overall ECG parameters of the heart are unaltered, though the mice are prone to spontaneous ventricular arrhythmia [40]. Thus, one connexin type may substitute functionally for another to quite a remarkable degree, though some abnormalities arise, indicating that specific connexins have the capacity to confer subtly distinctive properties [40]. Interpretation of results from the knock-out/knock-in approach is confounded by the difficulty in establishing whether the replacement connexin is expressed at the same level as the original connexin in the wild-type animal; hence, the resultant functional properties may be due to a combination of altered expression of connexin type and amount.

Relating findings on transgenic mice to the human depends on a sound understanding of the similarities and differences of the connexin expression of these two species [41,42]. At present, significant gaps remain in our knowledge of connexin expression patterns in the human impulse generation and conduction system, and during human development.

3. Altered gap junction and connexin expression in human heart disease

Notwithstanding the remaining gaps in knowledge, the role of gap junctions as the cell-to-cell pathways for the orderly spread of current flow underlying synchronous contraction in the healthy heart led to the question being posed as to whether alterations of gap junction organization and connexin expression might contribute to abnormal conduction and arrhythmogenesis in the diseased human heart [43,44]. Arrhythmogenesis is, of course, multifactorial in origin, involving an interplay between gap-junctional coupling, membrane excitability and cell and tissue architecture [45–47]. Moreover, gap-junctional coupling is itself determined by multiple factors including, for example, channel gating and the assembly/disassembly of functional gap junction plaques, as well as potentially by the pattern, amount and types of connexin expressed. Thus, it is important to bear in mind that findings on gap junction organization and connexin expression in diseased human tissue represent one facet of one potential contributor to the arrhythmogenic substrate. In examining this topic, it is convenient to consider gap junction and connexin remodelling under two, non-mutually exclusive categories, (i) structural remodelling, which refers to alteration in the distribution or organization of gap junctions, and (ii) remodelling of connexin expression, involving changes in the amount and/or types of connexin expressed.

The most striking form of structural remodelling involves loss of the normal ordered distribution of connexin43 gap junctions in the myocardial zone bordering infarct scar tissue (Fig. 3), first reported in the ventricles of patients with end-stage ischaemic heart disease [43]. The infarct scar tissue is visualized as a patch that is immunonegative for connexin43, and connexin43 immunolabelling in the border zone myocytes is typically scattered in disordered fashion over the lateral surfaces of the cells rather than in the polar, intercalated disk arrays characteristic of normal myocardium. Electron microscopy reveals that both true laterally disposed gap junctions that contact adjacent cells, and annular profiles of apparently internalized, non-functional gap-junctional membrane, contribute to the dispersed connexin43 labelling patterns [43]. Gap junction disarray of this type occurs not only in association with established infarct scar tissue, but has been shown in infarct scar tissue from human ventricle. The immunonegative area at the bottom of this immunofluorescent image is part of an infarct scar (i). Myocardium approaches the scar tissue from the upper right. The upper to mid right area of the myocardium shows short lines of immunolabelling at intervals, representing linear rows of gap junctions organized in intercalated disks between myocytes. However, this normal ordered arrangement is severely disrupted in myocardium close to the infarct scar (mid-right through to lower part of the image) where many of the immunolabelled gap-junctional spots appear strewn along the lateral borders of the myocytes.

From [44]. Bar marker =100 μm.
experimental animals to be initiated rapidly after myocardial infarction [48]. A strikingly similar lateralization of gap junctions accompanies experimentally induced hypertrophy of the right and left ventricle of the rat [49,50], and, in the former, correlates with a reduction in longitudinal conduction velocity [49]. Although no corresponding changes are obvious in hypertrophy associated with coronary heart disease in humans undergoing coronary by-pass operations, disordered arrangements of ventricular connexin43 gap junctions are prominent in human hypertrophic cardiomyopathy, the most common cause of sudden cardiac death due to arrhythmia in young adults [51]. Furthermore, in a canine model of infarction, border zone gap junction disarray extending across the epicardial layer correlates spatially with the central common pathway of figure-of-eight reentrant circuits [52].

A more subtle form of structural remodelling is associated with hibernating myocardium, a state of impaired myocardial contraction in patients with coronary artery disease [53]. The large connexin43 gap junctions typically found at the periphery of the intercalated disk are markedly smaller in size in hibernating myocardium compared with those of reversibly ischaemic regions and those of normally perfused regions of the same heart [53]. Thus, a possible link was proposed between disease-related gap junction remodelling and defective cardiac contraction. The structural remodelling of gap junctions in hibernating myocardium occurs hand-in-hand with remodelling (i.e., alteration) of connexin expression, as the overall amount of connexin43 immunolabelling per intercalated disk is reduced in the hibernating and reversibly ischaemic samples.

Down-regulation of connexin43 is the most extensively documented quantitative alteration in connexin expression in human heart disease (Fig. 4). Northern and Western blot analyses demonstrate a substantial reduction in connexin43 transcript and protein levels in the left ventricles of transplant patients with end-stage congestive heart failure [54]. This remodelling of connexin expression is prominent irrespective of whether heart failure is due to idiopathic dilated cardiomyopathy or ischaemic heart disease. Data from quantitative immunofocal microscopy of tissue from by-pass patients [55] suggest that in ischaemic heart disease, reduction in ventricular connexin43 develops long before terminal heart failure.

The possible functional significance of reduced connexin43 levels in the diseased human ventricle has been open to divergent opinions. At the outset, it is important to emphasize that total connexin levels may be regarded as indicators of the potential capacity for cell-to-cell communication, but do not provide information on the quantity of

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**Fig. 4.** (A) Typical Western blot analysis of connexin43 in heart failure patients. The upper panel shows analysis of left ventricular tissue (LV); the lower panel shows the analysis of right ventricular tissue (RV) of the same patients. The classification of the diseased samples is indicated at the top of each panel (ICM: ischaemic cardiomyopathy; DCM: idiopathic dilated cardiomyopathy). The control samples are on the left (LVC, left ventricle control; RVC, right ventricle control). Cx43=connexin43 detected on immunoreplica; Act=actin stained by Coomassie blue. The Coomassie blue staining indicates that similar amounts of proteins were run. Note that the control and right ventricular samples appear to contain more actin (and myosin, not shown) than the diseased left ventricular samples, for the same amount of protein loaded (4 µg total protein for immunoreplication and 10 µg for Coomassie blue staining) owing to fibrosis in the diseased tissue. The immunoreaction was therefore normalized to relate the connexin quantities to the myocytic component. The lower amounts of contractile protein in the diseased left ventricular samples indicate the presence of substantial fibrosis that was not obvious in the right ventricular samples. Identical samples (LVC1 and RVC1) are present in both analyses to permit gel-to-gel comparison. (B) Bar chart showing quantification of connexin43 in patients with heart failure. Data are expressed as a percentage of the left ventricle control after normalization using the actin-stained band. There is no significant difference in connexin43 levels between the ischaemic cardiomyopathy and idiopathic dilated cardiomyopathy groups; hence, these were amalgamated to give nine diseased samples (Dis). The control data (Cont) are from four undiseased hearts. On average, there is a ~50% reduction in Cx43 signal in the diseased compared to the control ventricles (unpaired t test). However, expression levels are heterogeneous and, although averaging to similar percentage reduction per heart, some lysates revealed much lower levels of Cx43 levels in some regions within the ventricle. For further details, see Ref. [54].
functional (open) channels. Furthermore, computer modelling studies predict that reductions of up to 40% in gap junction content (without change in junction size) would be unlikely to have a major effect on conduction velocity [56]. Hence, a reduction of connexin43 in the diseased ventricle may not, per se, be of functional relevance. On the other hand, in view of the complex relationship between passive and active membrane properties [45,57,58], and the assumptions inherent in computer modelling, prediction of the effects of reduced connexin43 levels in vivo is not necessarily a straightforward matter. Results of studies in the intact heart are therefore instructive in order to determine the net effects of multiple potentially interacting factors in vivo.

In transgenic mice generated to give cardiac specific loss of connexin43, the magnitude of connexin43 reduction associated with sudden death due to spontaneous ventricular arrhythmia is in the order of 86–95% [30], much lower than the average reduction found in the diseased human ventricle (~50%). On the other hand, in intact isolated hearts of transgenic mice expressing half the normal level of connexin43, experimental ischemia reportedly leads to a marked increase in incidence, frequency and duration of ventricular tachycardias [59] even though there may only be a modest reduction in conduction velocities [60,61]. In the failing human ventricle, considerable variation is apparent in the extent of connexin43 reduction between and, in particular, within hearts, some regions of some diseased hearts reaching a reduction of >90% of control values [54]. Thus, average values for the overall reduction in ventricular connexin43 disguise considerable spatial heterogeneity in the extent of the reduction. The existence of this heterogeneity could lead to exaggeration of inhomogeneities in resting potential and action potential upstroke velocity and duration, affecting individual cell excitability and refractory period, dispersion of which is a key pro-arrhythmic factor.
turbances in electromechanical function comes from the work by Gutstein et al. [62] using chimeric mice created from connexin43 deficient stem cells and blastocysts. This approach was designed to give heterogeneous expression of connexin43 (i.e., a pattern resembling that seen in heart failure), and the resultant experimental mice were demonstrated to have both abnormal conduction and contractile dysfunction, as originally hypothesized in the human studies [43,53,54]. Thus, the spatially heterogeneous connexin43 down-regulation of the magnitude and nature observed in the diseased human ventricle could similarly predispose to arrhythmia and contractile dysfunction.

Apart from reduced connexin43, the overall level of connexin40 transcript is increased in the ventricles of patients with congestive heart failure due to ischaemic heart disease (Fig. 5) but not that due to idiopathic dilated cardiomyopathy [54]. This increased connexin40 expression correlates with an increased depth of connexin40 expressing myocytes from the endocardial surface (i.e., in a position associated with and adjacent to that normally associated with Purkinje fibers) reminiscent of that reported in ventricular hypertrophy in the rat [25]. The significance of this expanded zone of connexin40 expression is unclear; one speculation is that it could represent a type of compensatory response that might conceivably improve depolarization from the conduction tissues in the face of declining connexin43 levels; another is that it could lead to heterogeneity of impulse propagation between adjacent regions of myocardium and increase susceptibility to arrhythmias.

Connexin40, apart from showing atypical site-specific expression in the ischaemic ventricle, may also contribute to arrhythmia in the atrium, specifically in post-operative atrial fibrillation (Fig. 6). Atrial fibrillation has many interacting causes, but levels of connexin40 at the upper range of normal values, as determined in samples of atrial appendage taken during coronary artery by-pass operation, are associated with an increased tendency of the patient subsequently to develop atrial fibrillation [15]. At first sight, this correlation might appear paradoxical. However, connexin40 immunolabelling in the human atrium, in contrast to atrial connexin43, typically shows a markedly heterogeneous distribution in the undiseased heart (Fig. 7). As discussed above, the balance between connexin quantity and heterogeneity of its distribution may be key factors in susceptibility to arrhythmia. In the case of the atrium, differences in resistive properties and conduction velocities due to the natural heterogeneity of atrial connexin40 may become enhanced, and hence pro-arrhythmic, the higher the overall levels of connexin40. It is important to emphasize that these

![Fig. 6.](image)

![Fig. 7.](image)
data refer to pre-existing connexin40 levels as a risk factor in later development of post-operative atrial fibrillation, an entirely different situation from that of the alterations in gap junctions and connexins that are suggested to be induced by and hence perpetuate chronic atrial fibrillation [63–66].

4. Future directions

From the findings discussed above, it is now clear that remodelling of ventricular gap junctions—notably alteration in the pattern of junctional distribution and reduced levels of connexin43—does occur in defined categories of human heart disease, and in at least some instances similar alterations correlate with electrophysiologically identified pro-arrhythmic changes in animal models. While electrophysiological studies on transfected cells have emphasized the distinctiveness of the channel properties conferred by different connexin types, work on genetically engineered mice indicates that, at the level of the whole organ, a greater capacity arguably exists for one connexin type to substitute for the function of another than might originally have been anticipated. Despite the exciting progress of the last decade, there remain significant limitations in our attempts to define the functional significance of the distinctive connexin expression patterns observed in specific regions of the healthy heart.
and diseased human heart. To advance our understanding in this field, an in vitro model, designed to express the same combinations of connexins in the same relative quantities as those found in vivo, and which is amenable to functional analysis, could prove invaluable. To this end, we have worked on developing such a model in which the expression levels of different (multiple) connexins in transfected cells are controlled using inducible promoters.

Previous work involving transfection of HeLa cells with a single connexin has enabled determination of the properties of homomeric/homotypic [24,67,68] and homomeric/heterotypic channels [69–71], and in ascribing functions to different segments of the connexin molecule [72,73]. However, because these transfections use plasmids containing two transcription units, one for the antibiotic resistance and one for the gene of interest, this type of plasmid generates two mRNAs with no selection pressure on the gene of interest, that encoding the connexin. As a result, after a few passages of the transfectants, the expression of the connexin becomes heterogeneous. This does not constitute a problem for functional analysis of single transfectants as only a few channels are necessary to obtain measurements. However, for studies on co-expression, the heterogeneity of expression may result in major variations in the ratio of the two connexins, leading to a broad variation in the associated functional properties.

To overcome this problem of heterogeneity of expression (Fig. 8A), we physically linked the connexin and antibiotic resistance genes using an internal ribosome entry site (IRES) sequence, thereby generating bi-cistronic mRNA. Using this approach, homogeneous expression is consistently obtained throughout the culture (Fig. 8B). To make the expression of the connexins inducible, we have engineered two commercially available systems, the Tet-Off system (Clontech) and the Ecdysone system (Invitrogen), to express bi-cistronic mRNA coding for the resistance and the connexin. This strategy combines imposition of selection pressure on the (transfected) transcription factor (responsive to tetracycline and/or edysone) with ability to assess the level of induction using the antibiotic resistance. Using both these inducible systems, we now have HeLa cell lines in which the expression of connexins is adjustable (Fig. 9). In order to obtain an accurate ratio of connexin expression in the co-expressing cell lines or in the tissue that the cell lines are designed to mimic, we introduced a V5 tag at the C-terminal of the connexins. Assuming that the anti-V5 antibody reacts identically with all our tagged connexins and that the V5 antigen does not alter the binding of our specific anti-connexin antibodies to the tagged version, the V5 immunoreactivity can be used to calibrate the specific reaction with anti-connexin antibodies in Western blots.

The transfected cell models provide a novel tool to address key questions such as the influence of varying the ratio of co-expressed connexins on intracellular resistance, a major determinant of conduction velocity in myocardium. A major hope is that these models will help us to ascribe functional properties to the distinctive sets of connexin co-expression patterns that are observed in the normal and diseased heart, an aim that remains elusive despite the wealth of new data generated from transgenic approaches.

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