Remodelling of gap junctions and connexin expression in diseased myocardium

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Gap junctions form the cell-to-cell pathways for propagation of the precisely orchestrated patterns of current flow that govern the regular rhythm of the healthy heart. As in most tissues and organs, multiple connexin types are expressed in the heart: connexin43 (Cx43), Cx40 and Cx45 are found in distinctive combinations and relative quantities in different, functionally-specialized subsets of cardiac myocyte. Mutations in genes that encode connexins have only rarely been identified as being a cause of human cardiac disease, but remodelling of connexin expression and gap junction organization are well documented in acquired adult heart disease, notably ischaemic heart disease and heart failure. Remodelling may take the form of alterations in (i) the distribution of gap junctions and (ii) the amount and type of connexins expressed. Heterogeneous reduction in Cx43 expression and disordering in gap junction distribution feature in human ventricular disease and correlate with electrophysiologically identified arrhythmic changes and contractile dysfunction in animal models. Disease-related alterations in Cx45 and Cx40 expression have also been reported, and some of the functional implications of these are beginning to emerge. Apart from ventricular disease, various features of gap junction organization and connexin expression have been implicated in the initiation and persistence of the most common form of atrial arrhythmia, atrial fibrillation, though the disparate findings in this area remain to be clarified. Other major tasks ahead focus on the Purkinje/working ventricular myocyte interface and its role in normal and abnormal impulse propagation, connexin-interacting proteins and their regulatory functions, and on defining the precise functional properties conferred by the distinctive connexin co-expression patterns of different myocyte types in health and disease.

1. Introduction

Gap junctions are clusters of transmembrane channels which, by directly linking the cytoplasmic compartments of neighbouring cells, form conduits for direct intercellular communication (review1). In cells in general, gap-junctional intercellular communication plays a key role in tissue homeostasis and regulation of growth, development, and differentiation. In the heart, gap junctions mediate electrical coupling between cardiac myocytes, forming the cell-to-cell pathways for orderly spread of the wave of electrical excitation responsible for synchronous contraction (reviews2,3). The normal heart rhythm thus depends fundamentally on the coupling of cardiac myocytes by gap junctions. Disturbances of the normal cardiac rhythm (arrhythmias) are a common, serious and often fatal complication of many forms of heart disease. Abnormalities in the active membrane properties responsible for the action potential play a central part in the genesis of arrhythmia, but it is now recognized that gap junctions and their component connexins also play an important role. Here, we examine the role of gap junctions in heart disease, focusing in particular on the nature and functional significance of the alterations to gap junction organization and connexin expression referred to as ‘gap junction remodelling’. To provide the background from which disease-related alterations can be interpreted, we start with a summary of the principal features of gap junctions and connexin expression in the normal adult heart.

2. Gap junction organization and connexin expression in the normal adult heart

Gap junctions in the heart vary enormously in size, from tens of thousands to fewer than ten channels. Each gap junction...
The myocytes of the impulse generation and conduction system, connexin43 (Cx43), Cx40, and Cx45. Although Cx43 predominates in the heart as a whole, it is typically co-expressed in characteristic combinations and relative quantities with Cx40 and/or Cx45 in a chamber-related and myocyte-type-specific manner.6,7 Although a few other connexins have been reported in cardiac tissue, these are minor components, species variants, or, where early reports have not been confirmed, are likely to be the product of mistaken interpretation. Figure 1 gives an overview of the typical connexin expression patterns of the normal adult mammalian heart.

The working (contractile) myocytes of the ventricle are extensively interconnected by clusters of Cx43-containing gap junctions (Figure 2). The gap junctions are organized, together with two types of adhesion junction—fasciae adherentes junctions and desmosomes—at the intercalated discs (Figure 3). The intercalated disc has a characteristic irregular, step-like structure, exquisitely specialized for the task of integrating cell-to-cell electro-mechanical function. Fasciae adherentes junctions, which transmit mechanical force from cell to cell, are situated in the vertical ‘steps’ of the disc, linking up the myofibrils of adjacent cells in series. Desmosomes, often likened to ‘press studs’ between cells, form attachment sites for the desmin cytoskeleton, and are found predominantly in the intervening horizontal portions of the disc. Most of the gap junctions are also found in these horizontal segments, often with larger junctional plaques at the disc periphery. Atrial myocytes similarly have abundant gap junctions, but in contrast to their ventricular counterparts, in most mammalian species (including humans) these junctions are constructed from both Cx43 and Cx408 and are organized in less well demarcated intercalated discs (Figure 4). In both ventricular and atrial human myocardium, Cx45 is detected in low quantities, with higher levels in the atria than the ventricles.7

The myocytes of the impulse generation and conduction system are quite distinct from the contractile ventricular and atrial cells, both morphologically and with respect to their connexin expression profiles.9–11 Myocytes of the sinoatrial and atrioventricular (AV) nodes characteristically have small, dispersed gap junctions composed of Cx45 and, in the case of the mouse, also Cx30.2, connexins that form low conductance channels in vitro.10–13 These features suggest relatively poor coupling which, in the AV node may contribute to slowing of impulse propagation, thus ensuring sequential contraction of the cardiac chambers. Within the AV node, complex, compartmentalized connexin expression patterns are apparent; three-dimensional reconstruction of the rabbit AV node, for example, reveals that while the compact node and transitional cells predominantly express Cx45, the His bundle, lower nodal cells and posterior nodal extension co-express Cx45 with Cx43.14 Downstream from the His bundle, the conduction system myocytes of most mammals, including man, prominently express Cx40.15,16 Cx43 becomes abundant in the more distal portions of the system6,11,15 while Cx45 is expressed continuously from the AV node to the ends of the Purkinje fibres.11

Though these features of connexin expression are common to most mammalian species, there are notable exceptions. For example, Cx40 is not detectable in rat atrium or the guinea pig conduction system.6,17 Species variations in co-expression patterns within the AV node are apparent between large and small mammals, possibly reflecting differences in the need for impulse delay according to

Figure 1 Summary of the typical connexin expression patterns of the mammalian heart.

Figure 2 Characteristic distribution pattern of Cx43 gap junctions in ventricular myocardium. (A) Longitudinal section from rat left ventricle. The Cx43 gap junctions appear in rows, corresponding to edge-on viewed intercalated discs. Inset shows a single intercalated disc viewed face-on from transversely sectioned human myocardium. Note larger gap junctions at the periphery of the disc. (B) The presence of multiple discs of different size is best appreciated in views of isolated myocytes (in this example, from rat). The steps of the disc are indicated by the white line. Note that some apparently isolated gap junctions at the lateral surface (indicated by spot on the line) can be considered as components of extended intercalated discs.
Figure 3  Organization of junctions at the intercalated disc, as seen by thin-section electron microscopy. In low magnification view (A), the step-like features of the discs are clearly seen (right end of cell and arrowheads). If we take, at higher magnification, an area from within the disc like that enclosed by the box, the three junction types are visible (B). Fasciae adherentes occupy electron-dense vertical plicate zones of the disc; gap junctions and desmosomes mainly the lateral-facing zones. In this example, the ends of the gap junction contact a fascia adherens (arrows), though in many instances non-junctional intercalated disc plasma membrane separates the junctions from one another [(A) from Severs, N.J. et al J Ultrastruct Res 1982;81:222–239; reprinted with permission from Elsevier; (B) from Severs, N.J. BioEssays 2000;22:188–199].

Figure 4  Variations in the pattern of organization of gap junctions in atrial myocardium, as seen by Cx43 labelling in dissociated groups of rat atrial myocytes. While clusters of gap junctions in classic step-like and straight-end intercalated disc configurations are present [lines, left side of cells in (A) and (B)], the junctions often appear spread laterally (asterisks). Gap junction distribution can thus range from largely end-to-end (C) to predominantly side-to-side (D).
heart size. That Cx31.9 is present in the human AV node has been inferred from the presence of its orthologue, Cx30.2, in the mouse AV node, but comprehensive investigation shows no evidence for such a localization (unpublished observations, in collaboration with K. Willecke and M. Kreuzberg, University of Bonn). Such species differences in connexin expression, and those of cardiac function, need to be borne in mind when extrapolating functional data from transgenic mice to the human.

3. Mutations in connexin-encoding genes

Although our primary focus here is on gap junction remodeling and arrhythmia in acquired adult heart disease, we should not overlook the possible consequences of defects in gap-junctional communication as a potential cause of developmental malformations of the heart. As in non-excitable tissues, cardiac myocyte gap junctions have the potential to act as pathways for the direct passage of signaling molecules and ions from cell to cell, and that they may do so during cardiac morphogenesis is suggested by the presence of developmental malformations in connexin knockout mice.19–22 Reports that mutations of the Cx43 gene (GJA1) affecting phosphorylation sites in the Cx43 carboxy tail are associated with complex cardiac malformations and viscerotrautal heterotaxy21 or hypoplastic left heart syndrome24 generated major interest. Subsequent studies, however, failed to confirm this link 25,26 though as these did not examine cardiac tissue, the possibility remains that somatic mutations may explain some of the original findings.23 A range of mutations in the Cx43 gene are, however, clearly associated with oculodentodigital dysplasia, a developmental abnormality affecting the limbs, teeth, face, and eyes, and, in some instances, also the heart.27–29 Chromosomal deletion that includes the Cx40 gene (GJA5) has been reported in a minority of cases of congenital heart disease involving anomalies of the aortic arch.30 Heterozygous somatic missense mutations and polymorphisms within the gene’s regulatory region31,32 have also been linked to atrial fibrillation (AF).

4. Disease-related remodelling of gap junctions and connexin expression

That alterations in gap junction organization and connexin expression contribute to abnormal impulse propagation and arrhythmia in acquired adult heart disease has gained progressively wider acceptance (reviews2,3). In any discussion of this topic, however, it is important to bear in mind that arrhythmias are multi-factorial in origin, involving an interplay between gap-junctional coupling, membrane excitability, and cell and tissue architecture.33 Gap-junctional coupling will itself be determined by a number of factors, including the amount and types of connexin expressed, the size and distribution of gap-junctional plaques, the proportion of each connexin assembled into functional junctions, and the gating and specific connexin make-up of individual gap-junctional channels.

4.1 Acute cardiac ischaemia

Acute focal cardiac ischaemia resulting from sudden occlusion of a coronary artery triggers major alterations in impulse formation and propagation. Action potential upstroke velocity decreases, conduction transiently increases and then slows, and changes in refractory period ensue, resulting in localized conduction block and re-entry arrhythmia. The changes are not uniform across the affected region, and ectopic foci arise within the ventricle. Although the effects of acute ischaemia on human cardiac gap junctions are difficult to investigate directly, studies on experimental animals demonstrate rapid dephosphorylation of Cx43, electrical uncoupling and alteration in the distribution of gap junction immunolabel to the sides of the myocyte (referred to as ‘lateralization’).34,35 Immunolabelling using antibodies directed to different phosphorylation sites on the Cx43 molecule suggests that dephosphorylated Cx43 is associated with the laterally distributed gap junction label, while those gap junctions that remain in the classic, polar intercalated disc orientation contain phosphorylated Cx43.35 While the altered distribution of gap junction label, as viewed by immunofluorescence microscopy in these studies, is striking, it remains unclear what proportion of the lateral signal is attributable to potentially functional gap junctions connecting side-by-side myocytes, and what proportion represents vesicles of gap-junctional membrane that are internalized as a result of stress.

The adverse effects of ischaemia on gap junctions are reduced with ischaemic preconditioning.36–38 Roles for the gap junction and Cx43 other than those related to cell-to-cell electrical coupling and arrhythmia have been put forward in interpreting findings on ischaemia, preconditioning and ischaemia-reperfusion. Transient opening of Cx43 hemichannels that lie free in the plasma membrane, external to gap junctions, has been implicated in cell swelling, ATP release, and loss of membrane potential during ischaemia,39,40 though the presence of pannexin rather than connexin hemichannels has been suggested as an alternative explanation for such phenomena.41 Gap-junctional mediated passage of ionic/molecular signals appears responsible for the spread of the ischaemia-reperfusion injury from myocyte to myocyte that leads to rigour contracture and cell death,42 an idea supported by the observation that reduction of gap junction coupling (using heptanol) prior to an ischaemic insult results in significantly reduced infarct size.43 Cx43 trafficked to mitochondria (rather than that located in functional gap junctions) has been invoked as central to the part played by Cx43 in preconditioning, and a number of candidate mechanisms for this has been proposed (review44). The benefit of preconditioning on infarct size is apparently abolished in heterozygous Cx43 knockout mice which express half the normal level of Cx43,45 yet coronary occlusion in these mice reportedly leads to smaller infarcts than in their wild-type counterparts.46 This discrepancy may be related to the timing of infarct size measurement.

4.2 Altered gap junction distribution and reduced connexin43 expression in diseased ventricle

‘Lateralization’ of Cx43 gap junction label is a prominent feature of the border zone of surviving myocytes around infarct scar tissue in the human ventricle.47 a finding that pre-dated the descriptions of this phenomenon in animal models of acute infarction. Electron microscopy reveals that both laterally disposed gap junctions connecting
adjacent cells, and internalized (non-functional) gap-junctional membrane, contribute to this abnormal pattern. A similar change, found in some rat models of ventricular hypertrophy, correlates with reduced longitudinal conduction velocity, a potentially pro-arrhythmic change. At 4 days post-infarction in a dog model, lateral gap junction label in the extended infarct border zone has been correlated spatially with electrophysiologically identified figure-of-eight re-entrant circuits. Gap-junctional changes distant from the infarct scar tissue, in particular reduction in the size and the number of gap junctions per unit length of intercalated disc, and fewer side-to-side connections between myocytes, have been described as longer term remodelling events in dog myocardium. Smaller areas of gap junction disarray than those found at the infarct border zone have been reported in end-stage human heart failure due to idiopathic dilated cardiomyopathy and myocarditis, and in the ventricles of patients with compensated hypertrophy due to valvular aortic stenosis. In decompensated hypertrophy from the same cause, patches are seen in which the otherwise normally arrayed gap junctions are fewer or absent. These findings emphasize that even in the absence of infarcts, Cx43 gap junction distribution becomes heterogeneous in disease. Cx43 gap junction arrangement is particularly disordered in hypertrophic cardiomyopathy, the most common cause of sudden cardiac death due to arrhythmia in young adults; here the abnormal gap junction distribution is dictated by the haphazard myocyte organization characteristic of this condition.

A rather different form of gap junction remodelling is associated with 'hibernating myocardium' in patients with ischaemic heart disease. The term 'hibernating myocardium' refers to regions of ventricular myocardium that do not contract properly but which recover after normal blood flow is restored following coronary artery by-pass surgery. In hibernating myocardium, the large Cx43 gap junctions typically found at the periphery of the intercalated disc are smaller in size, and the overall amount of immunodetectable Cx43 per intercalated disc is reduced, compared with normally perfused myocardial regions of the same heart. These findings were the first indication that Cx43 gap junction remodelling contributes to impaired ventricular contraction, in addition to arrhythmia, in human ischaemic heart disease.

Apart from alterations in Cx43 gap junction organization, marked reduction in ventricular Cx43 transcript and protein levels typify the hearts of transplant patients with end-stage congestive heart failure. This Cx43 reduction occurs irrespective of whether heart failure is due to ischaemic heart disease, idiopathic dilated cardiomyopathy, or aortic stenosis. The reduction in Cx43 is spatially heterogeneous and develops progressively during the course of disease, as indicated by the pattern of change observed in pressure-overloaded hearts with aortic stenosis and its presence in non-failing ventricles of patients with ischaemic heart disease.

4.3 Does reduced connexin43 contribute to ventricular arrhythmia?

A critical question is whether the reduced Cx43 in the diseased human ventricle contributes to the arrhythmic substrate. It is commonly assumed that such reductions in Cx43 inexorably result in slowed conduction, thereby rendering the ventricle more susceptible to re-entry arrhythmia. On this basis, therapeutic intervention to improve coupling is sometimes proposed. However, theoretical and experimental cell models show that action potential propagation can fail in well coupled cells if these form a large mass ('sink') receiving a limited amount of depolarizing current from a smaller 'source' (i.e. a 'source/sink mismatch'); under these conditions, reducing coupling in the 'sink' can actually overcome a conduction block. Thus, reduction of Cx43, if of a magnitude sufficient to decrease coupling, could form part of a protective response that increases the safety of conduction in the diseased ventricle.

The mammalian heart, however, has a considerable surplus of gap junctions, and computer modelling predicts that even substantial reductions in gap junction content make relatively little difference to propagation velocity. Apparently in keeping with these theoretical predictions, the magnitude of the Cx43 reduction associated with sudden cardiac death due to arrhythmia in the cardiac restricted Cx43 knock-out mouse is in the order of 90%, much greater than the average reduction of ~50% observed in the failing human ventricle. However, this average disguises considerable spatial heterogeneity in the extent of the reduction, some regions of some diseased hearts reaching a reduction of >90% of control values. That heterogeneity in Cx43 expression is critical both to abnormal impulse propagation and contractile dysfunction is elegantly demonstrated experimentally in a chimaeric mouse model generated to give patches of myocardium lacking Cx43. Heterogeneity of gap junction distribution combined with reduced Cx43 levels appears to act co-operatively to create an arrhythmogenic substrate at less severe levels of overall gap junction reduction than predicted in theoretical models. In selectively bred cardiac-restricted knock-out mice, a 59% reduction in Cx43 does not alter propagation velocity or susceptibility to arrhythmia, but when the Cx43 reduction reaches 18% of control levels and appears heterogeneous, propagation velocity is slowed by 50%, and 80% of the animals are inducible into lethal ventricular arrhythmias. Co-operative effects of reduced Cx43 and other factors, such as those related to acute ischaemia, may also alter the threshold at which the arrhythmic substrate is reached; a straightforward halving of the Cx43 level in transgenic mice (i.e. Cx43 '-/Cx43') is sufficient to increase the incidence, frequency and duration of ventricular tachycardias when the heart is subjected to ischaemia.

Taken together, these findings in mouse models lend considerable support to the view that the nature and extent of the Cx43 reduction in the failing human ventricle is, in practice, of sufficient magnitude to increase susceptibility to arrhythmia. It could be argued that such extrapolation from mouse to man is limited because the mouse heart cannot accommodate large re-entrant circuits; however, a heterogeneous 50% reduction in Cx43, similar to that of human heart failure, has been demonstrated to result in slowed transmural conduction and dispersion of action potential duration of a magnitude that exceeds the requirements for conduction block and re-entry in a dog model of heart failure. The results from experimental animal models thus vindicate the conclusions originally suggested from those on human cardiac disease.
4.4 Connexin45, connexin40, and the Purkinje/working ventricular myocyte interface

Our discussion so far has, however, focused solely on Cx43 to the exclusion of the other two connexins, Cx45 and Cx40. One study has reported elevated expression of Cx45 in the failing human ventricle, alongside the reduction in Cx43, thus significantly altering the Cx43:Cx45 ratio. As Cx43 and Cx45 are reported to be assembled as mixtures in the same connexon and channel, this could potentially have substantial effects on channel properties. Experimental increase of the Cx45:Cx43 ratio by over-expression of Cx45 in transgenic mice has recently been demonstrated to increase susceptibility to ventricular tachycardia and reduce gap-junctional intercellular communication.

Increased expression of Cx40 has also been found in the failing human ventricle, but this increase is regionally restricted and confined to end-stage ischaemic heart disease. The site of Cx40 up-regulation is the endocardial surface, at and adjacent to Purkinje fibres, highlighting the Purkinje/working ventricular myocyte interface or junction as a potential site of altered electrical coupling that might trigger arrhythmogenesis. In cardiac-restricted Cx43 knockout mice selected for longer term survival, reduction of coupling resulting from declining ventricular Cx43 leads to propagation of the impulse across numerous Purkinje/working ventricular myocyte junctions that normally remain dormant, thereby creating abnormal activation patterns and wave-front collisions in the ventricular myocardium. Essentially, what seems to be happening parallels some of the classic experiments of Rohr on patterned cultures, in which reduced coupling in a sink (equivalent to the ventricular myocardium) abolishes the conduction block that otherwise occurs at the boundary with a narrow source (equivalent to the Purkinje fibre).

However, the Purkinje/working ventricular myocyte interface in vivo is a more complex structure than a patterned cell culture, involving the connection of Purkinje myocytes that co-express Cx40, Cx45 and Cx43 to ventricular myocytes expressing predominantly Cx43. The connection between the two cell types is not direct, but mediated via distinctive flattened transitional cells running at an angle below the Purkinje myocytes. Examination of connexin expression patterns in the mouse reveals that Cx40 and Cx45 are co-expressed both in Purkinje myocytes and in the underlying transitional cells, and that the Cx45 expression continues down into the most superficial layer of ventricular myocytes (Figure 5). This suggests that Cx45 plays an important role in connecting up the Cx40/Cx43 Purkinje myocyte compartment to the Cx43-expressing working ventricle via the transitional cells. Further work is required to establish the precise cellular organization and nature of the connections at this critical interface. Their cellular structure appears to differ in the pig from that illustrated in Figure 5 and so there may well be differences between mouse and man.

Figure 5  The Purkinje/working ventricular myocyte interface. (A) Cellular architecture. Purkinje myocytes link to underlying flattened transitional cells, which in turn link to the working ventricular myocardium. (B) Immunofluorescent view of Purkinje myocyte/working ventricular myocyte interface from mouse myocardium showing transversely sectioned Purkinje myocytes (P) above a longitudinally sectioned transitional cell (T). The working ventricular myocardium (WM) lies below. Connexin immunolabelling shows that whereas Cx40 (C) is abundant in the Purkinje myocytes and transitional cells, Cx45 (D) is seen in both these cell types and in the most superficial working ventricular myocytes [asterisk, (B)].
4.5 Atrial arrhythmia

The wide recognition that gap junction remodelling is a key contributor to ventricular arrhythmia has stimulated corresponding studies on atrial arrhythmia and, in particular, AF, a particularly common arrhythmia in which wavelets of electrical activity propagate in multiple directions. However, the picture to emerge is not clear-cut, with a range of disparate and seemingly contradictory findings. For example, while studies using dog models report increased Cx43 expression together with gap junction lateralization, other studies on a goat model of AF report no changes in the overall level of Cx43 but development of heterogeneity of Cx40 distribution. Studies in the human confuses the picture still further, with reports linking AF variously to increased Cx40, decreased Cx40, increased Cx43, decreased Cx43, or no change in the level of either connexin, and some reporting lateralization of gap junction distribution, whereas others claim increased heterogeneity.

How do we make sense of these apparently contradictory findings? It is important to emphasize that the published studies on animal models use different species at different ages, with different experimental protocols for induction of AF, and those on the human involve different clinical sub-sets of patients with different associated pathological factors. A key distinction has to be drawn between chronic AF, in which remodelling of gap junctions and connexin expression brought about as part of AF-related remodelling might contribute to perpetuation of the condition, and AF, in which pre-existing features of gap junctions or connexin expression may act as a predisposing factor that triggers off the arrhythmia in the first place. In each category, we must bear in mind that AF is multi-factorial in origin; hence the underlying mechanisms and relative importance of gap junctions as contributors may vary from one patient to the next. Moreover, in the course of disease, with a role for gap junctions likely assuming greater or lesser importance at different stages, and associated pathological changes such as atrial dilatation (frequently found in patients with AF who undergo cardiac surgery) may independently influence any changes in gap junctions observed.

Another contributor to the confusion stems from deficiencies of technical approach and interpretative complications. Apart from the still too common problem of lack of antibody specificity, differences in epitope detection between different antibodies to the same connexin type have a major impact on localization patterns. Claims of ‘lateralization’ and ‘heterogeneity’ of gap junction or connexin distribution as AF-related factors pose particularly tricky problems of interpretation. Normal atrial myocardium shows considerable variation in gap junction arrangement (Figure 4). While some atrial myocytes show gap junctions organized in classic intercalated disc patterns at the cell ends, others show ‘spread’ of the discs along the lateral aspects of the cell (Figure 4A–C). This ‘lateral spread’ is in part a product of the smaller diameter of atrial myocytes compared with their ventricular counterparts, and in some instances can be so pronounced in normal atrial myocardium that the predominant pattern is of laterally distributed rather than polar gap junctions (Figure 4D). ‘Lateralization’ should not be confused with ‘heterogeneity’, a term that is used to describe patches in the tissue in which gap junctions or connexin label are absent. The heterogeneity of Cx40 distribution reported as an AF-induced change in the goat model resembles that naturally seen in the human atrium. These considerations complicate development of reliable quantitative methods for assessment of differences in the distribution of connexin label between normal and AF samples.

The challenge ahead is to develop a more refined and rigorous approach to tease out precisely how gap junctions and connexins might contribute to AF in different, defined settings. To this end, a series of useful recommendations has recently been suggested by Duffy and Wit.

5. Co-expression of multiple connexins—counter-intuitive properties

In retrospect, it appears that a major factor contributing to the relative ease with which a link between gap junction remodelling and arrhythmia in the ventricle has been established is the presence of a single predominant connexin, Cx43. Increasing evidence suggests that co-expression of Cx40 in the presence of Cx43—as occurs in the atrium and at the Purkinje/working ventricular myocyte interface—has unexpected functional effects. Using in vitro expression systems, it has consistently been found that Cx40, when expressed alone, makes high conductance gap junction channels, and it has been widely assumed that co-expression of Cx40 with Cx43 in vivo would elevate rather than depress cell-to-cell coupling. However, in cultured neonatal atrial myocytes from heterozygous Cx43 and Cx40 knock-out mice (Cx43+/−/Cx43 and Cx40+/−/Cx40), reduction of Cx40 to half the normal level results in increased propagation velocity, and when Cx40 is ablated altogether (Cx40−/−/Cx40−), propagation velocity is higher still. Thus, progressively lowering the Cx40 content against a background of Cx43 expression unexpectedly leads to increased rather than decreased conduction. This helps make sense of previously perplexing findings, such as the positive correlation between propagation velocity and decreased proportion of Cx40 to total connexin reported in the human atrium. How these effects are mediated requires knowledge of the specific connexin make-up of the junctions and their channels at the molecular level, and how this translates into specific electrophysiological properties. Cell models, in which different multiple connexins are expressed under the control of inducible promoters and which are amenable to functional analysis, offer one approach to advance this area.

6. Cellular mechanisms of gap junction remodelling

Our understanding of the cellular mechanisms of gap junction remodelling remains fragmentary. As with all proteins, connexin expression may be regulated at the transcriptional and post-transcriptional levels. The interaction of transcription factors with their target elements to control transcript expression has advanced as our knowledge of the structure of the genes encoding Cx43, Cx40 and Cx45 has progressed. With regard to post-transcriptional regulation, a recent study has identified a role for microRNA-1 in silencing GJA1 (the Cx43-encoding gene) and KCNJ2 (K+ channel...
subunit Kir2.1) in ischaemic heart disease. MicroRNAs are endogenous non-coding RNAs that interact with target mRNAs to prevent translation; increased expression of microRNA-1 is elevated in the diseased human ventricle, and antisense blocking of this elevation prevents arrhythmia in a corresponding animal model.

Apart from connexin synthesis, regulatory mechanisms may come into play at the levels of assembly into connexons, channels and gap-junctional plaques, and during degradation. Extracellular signalling pathways leading to altered connexin expression in disease may be triggered in part by mechanical forces, and involve cAMP, angiotensin II, and growth factors such as VEGF, activated via protein kinases such as focal adhesion kinase and c-Jun N-terminal kinase (JNK). JNK activation in a transgenic mouse model leads to down-regulation of Cx43, slowing of ventricular conduction, contractile dysfunction and congestive heart failure.

A major focus of current research concerns connexin partner proteins, a series of proteins with regulatory properties that interact with the carboxy-terminal domain of Cx43 (review). This domain houses a series of functionally important sites, notably those involved in channel gating and phosphorylation (a process implicated in diverse roles including trafficking of connexins and junction assembly and degradation). Apart from c-Src and adhesion junction proteins, other connexin binding partners include tubulin, caveolins, and zona occludens-1 (ZO-1). Carboxy-terminal binding sites for tubulin and associated motor transport proteins mediate trafficking of connexins to the correct destination at the cell surface, while ZO-1 is intimately involved in regulation of gap junction size. In exogenous expression systems, blocking or abolition of the binding of ZO-1 to Cx43 leads to formation of exceptionally large, extensive gap junctions, an effect that is reversed when the capacity to interact is restored. In human ventricular myocytes, ZO-1 is localized to the intercalated disc, and a pool of this ZO-1 interacts with Cx43 gap junctions. In the failing ventricle, although an association between reduced ZO-1 and Cx43 levels has been reported, we find that ZO-1 is, in fact, consistently up-regulated, and that the proportion of Cx43 that interacts with ZO-1 is increased (Figure 6). ZO-1 appears to act by limiting the recruitment of connexons to the gap junction periphery, and in this way may contribute to reduced gap junction size and overall gap junction content in the diseased human heart.

The effects of ZO-1, however, are not mediated in isolation, but in all likelihood involve co-operative actions with other connexin interacting protein partners. Molecules classically associated with adhesion junctions, notably N-cadherin, β-catenin, γ-catenin (plakoglobin), plakophilin-2 (PKP2) and desmoplakin, are among the partner proteins that interact, either directly or via other proteins, with Cx43. It has long been known that adhesion junction proteins play a key role in the formation and stability of gap junctions, but it has only recently become apparent that mutations in or suppression of these proteins can lead to reductions in Cx43 expression and gap junction quantity of sufficient magnitude that impulse propagation is impaired. The notion of molecular 'cross-talk' between the components of the different junction types is thus now very much in vogue.

Although the precise pathways are unclear, this molecular 'cross-talk' appears to be mediated both at the level of the surface-located gap junction and within the cell. Apart from its role in binding fasciae adherentes junctions, for example, N-cadherin is implicated in trafficking and assembly of Cx43 into functional gap junctions (review). β-catenin, in addition to linking N-cadherin to actin of the contractile apparatus, operates as an effector protein in the Wnt signalling pathway; transcriptional activation via a β-catenin/Tcf nuclear complex, for example, induces Cx43 expression in cultured cardiomyocytes. In non-myocyte cells, silencing β-catenin disrupts gap junction formation, and over-expression of β-catenin leads to down-regulation of ZO-1. ZO-1 may, in fact, physically mediate the link between the cadherin/catenin complex and Cx43 (review). PKP2, as well as being a structural component of desmosomes, modulates the signalling activity of β-catenin, and loss of PKP2 leads to redistribution of Cx43 from the membrane to the cytosol. In a transgenic mouse model in which a truncated form of Cx43, lacking the carboxy-terminal domain, is expressed, there is not only an increased size of the gap junction plaques and a reduction in their overall number, but also an altered pattern of their spatial organization at the intercalated disc. The ability of protein partners to interact with the carboxy-terminal domain thus appears to be crucial not only in regulation of the size of gap junctions, but also in their correct positioning. The major task ahead is to unravel the complex interactions between these molecules and the signalling pathways involved, and how they link to the various features of disease-related gap junction remodelling in the heart.

As the concept of 'cross-talk' between connexins and adhesion junction molecules has raced ahead, basic conceptual problems have been left behind. For example, classic electron microscopy demonstrating the three types of cell junction of the intercalated disc as spatially discrete structures implies corresponding spatially discrete sites for connexins and adhesion molecules. Although associations and interactions are commonly inferred from immunofluorescence co-localization of adhesion junction proteins, ZO-1 and Cx43, this technique does not have the resolution to tell us whether apparent overlap of fluorescence signals actually represents a sufficiently close association at the molecular level to enable interaction or merely reflects a
more distant juxtaposition. We do not yet know where the adhesion junction proteins postulated to interact with Cx43 are actually located at the gap junction. Are they confined to the periphery of the gap junction at sites at which it is linked edge-to-edge with a neighbouring adhesion junction, or are they spread along the gap junction’s entire cytoplasmic face to exert more general regulatory effects? What is the hierarchy of which protein interacts with Cx43? And what protein partners correspondingly affect Cx40 and Cx45 via carboxy-terminal domain interactions? These are but few of the questions that remain to be resolved.

7. Concluding comments

It is now firmly established that remodelling of gap junction and connexin expression does consistently feature in the diseased human ventricle, and comparative studies on transgenic animal models and other experimental systems provide substantial evidence that these alterations contribute to the arrhythmic substrate in the patient. Gap junctions and connexins are thus increasingly debated as potential therapeutic targets. However, the realization that reduced coupling can increase the safety of conduction, and that increasing the Cx40:Cx43 ratio may reduce rather than increase conduction velocity, serve as important reminders that, even if some of the proposed therapeutic interventions were practicable, the functional consequences to the patient may be the opposite of those intended. Key areas of knowledge need to be consolidated to permit a more realistic assessment of the possibilities. These include determination of: (i) the precise functional properties dictated by the distinctive connexin co-expression patterns of different myocyte types in health and disease; (ii) the regulatory mechanisms underlying gap junction and connexin remodelling; (iii) the nature and role of the Purkinje/ventricular myocyte interface; (iv) the part played by gap junctions and connexins in AF; and (v) the nature and action of messenger molecules or ions involved in direct molecular signalling. A thorough understanding of gap-junctional intercellular communication in the normal and diseased human heart must also underpin strategies that aim to integrate transplanted cells with host myocardium as a potential treatment for heart failure.

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