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Review

Gap junction alterations in human cardiac disease

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Abstract

Gap junctions, assembled from connexins, 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, connexin40 and connexin45 are found in distinctive combinations and relative quantities in different, functionally specialized subsets of cardiomyocyte. Alterations of gap junction organization and connexin expression are now well established as a consistent feature of human heart disease in which there is an arrhythmic tendency. These alterations may take the form of structural remodelling, involving disturbances in the distribution of gap junctions and/or alteration of the amount or type of connexin(s) expressed. In the diseased ventricles, the most consistent quantitative alteration involves heterogeneous reduction in connexin43 expression. In the atria, features of gap organization and connexin expression have been implicated in the initiation of atrial fibrillation and, once the condition becomes chronic, gap junction alterations associated with remodelling may contribute to persistence of the condition. By correlating data from studies on the human patient with those from animal and cell models, alterations in gap junctions and connexins have emerged as important factors to be considered in understanding the pro-arrhythmic substrate found in a variety of forms of heart disease.

Keywords: Cardiac disease; Human heart; Gap junctions; Connexins; Intercellular communication

1. Introduction

Cardiovascular disease is the leading cause of death and disability in most industrialized countries of the developed and developing worlds. Arrhythmias are a common, serious and often fatal complication of many forms of heart disease. As gap junctions mediate the cell-to-cell propagation of the precisely orchestrated patterns of current flow that govern orderly contraction of the healthy heart, considerable attention has been directed to the possible role of these junctions and their component connexins in arrhythmic heart disease. There is now such a vast literature on gap junctions in relation to heart function and disease in general that comprehensive coverage of the entire field in a review of this type would not be possible. Moreover, a number of other recent reviews give a range of perspectives on these topics [1–7]. We therefore concentrate here on the nature and possible significance of the alterations in gap junction organization and connexin expression in human adult acquired heart disease, drawing selectively on studies on experimental animal and cell models where these shed useful light on the interpretation and potential significance of the alterations discovered in the human patient. Our starting point is a brief survey of gap junction organization and connexin expression in the normal heart to provide the backdrop for explaining the nature of alterations that have been identified in disease.

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

Connexin43 is the predominant connexin expressed by cardiomyocytes, occurring in abundance in adult working ventricular and atrial cardiomyocytes of all mammalian species, including human [4,8,9]. Connexin40 and connexin45 are also expressed, though in lower total quantities. Numerous studies have established that these three connexins are expressed in characteristic combinations and relative quantities in a chamber-related, myocyte-type-specific and developmentally regulated manner [4,5,8–16].

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 membrane segments that lie parallel with the long axis of the cell [17–19], with larger gap junctions typically circumscribing the disk periphery [20,21]. This and other features of gap junction organization and aspects of tissue architecture such as the size and shape of the cells combine to ensure preferential propagation of the impulse in the longitudinal axis and hence 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 [9,22], co-localized with connexin43 within the same individual gap-junctional plaques [5]. 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 [9,15,22].

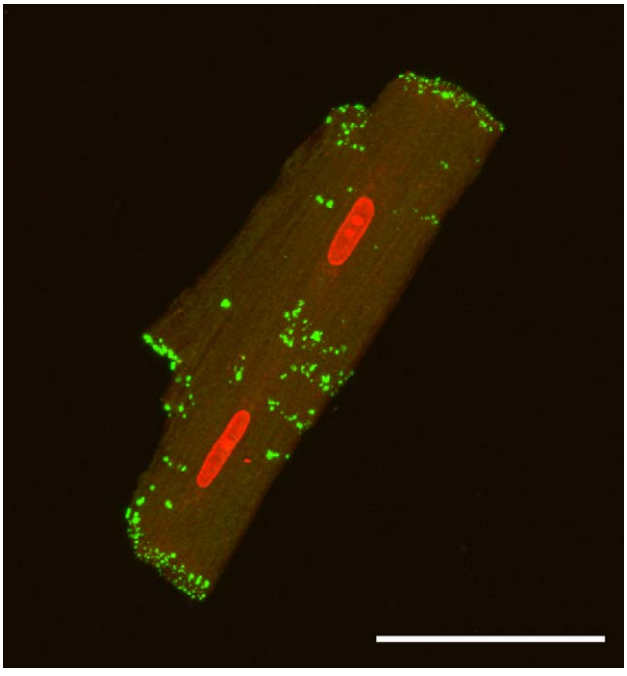


Fig. 1. An isolated ventricular myocyte labelled for connexin43 (green) illustrating localization of the gap junctions in clusters at the intercalated disks. Confocal reconstruction from serial optical sections. The nuclei are counterstained with propidium iodide (red). Bar marker = 50 μm.

The specialized cardiomyocytes of the impulse generation and conduction system are distinct from the working ventricular and atrial cells both in terms of general morphology [23] and connexin expression profiles (Fig. 2). The myocytes of the sinoatrial node, the site of impulse generation, and those of the atrioventricular node, the site at which the impulse is slowed before being routed to the ventricles, are equipped with small, sparse, dispersed gap junctions containing connexin45 [24–26], a connexin that forms low conductance channels in vitro [27–29]. 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 [25]. 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 regional differentiation within the node and species variation involving limited co-expression of connexin43 and connexin40 may also occur. For example, larger mammals, which have less need for atrioventricular nodal impulse delay, may express some connexin43 and/or connexin40 in addition to connexin45 [30]. 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 [24]. Significant gaps in our knowledge remain with respect to (i) regional variation of connexin expression patterns within the atrioventricular node and His bundle, and (ii) connexin expression within the transitional cells which are located between atrial muscle and the compact node and may be involved in the slow and fast pathways. Painstaking serial sectioning to construct three-dimensional models, combined with the use of markers to discriminate transitional cells, would facilitate further progress in these areas.

Cardiomyocytes of the His–Purkinje conduction system in most mammals, including man, prominently express connexin40, a connexin associated with high conductance

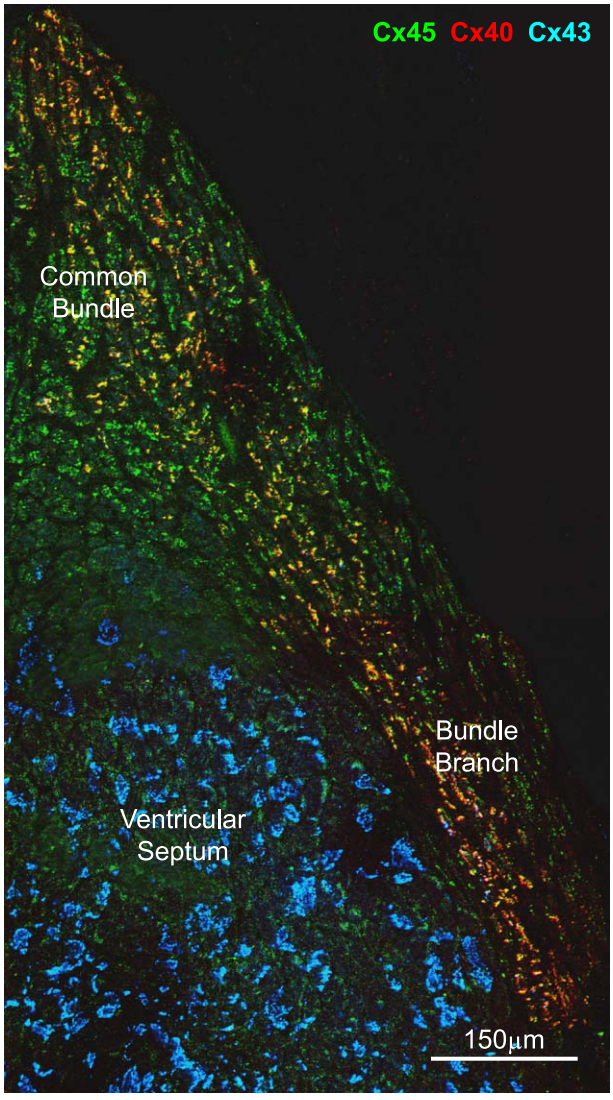


Fig. 2. Expression patterns of connexin43, connexin40 and connexin45 in different cardiomyocyte sub-types. This triple-labelled confocal montage shows part of the conduction system (common bundle and right bundle branch) which expresses connexin40 and connexin45). These two connexins are not detected in the adjacent working ventricular myocytes of the septum, which instead express connexin43. This example comes from rat heart [24].

channels [5,12,14,15,31–34]. Extensive 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 [24] but whether this feature is present in humans is unknown.

Despite the overall features of connexin expression common to many mammalian species outlined in the foregoing account, the existence of species differences should not be overlooked. Notable amongst these differences are the lack of connexin40 expression reported in rat atrial muscle and in the guinea pig conduction system [33,35]. The ever wider use of transgenic animals for investigating the role of connexins in cardiac function (see review by Gros, this spotlight issue, for full discussion of this topic) focuses on the need for a more detailed knowledge of such species-specific patterns. In particular, 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 [36,37]. One especially important gap to fill concerns the connexin expression patterns of the human impulse generation and conduction system, which still remain largely unknown.

3. Alterations in gap junctions and connexin expression in heart disease

The established role of gap junctions as the cell-to-cell pathways for the orderly spread of current flow required for 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 [38,39]. Arrhythmogenesis is, of course, multifactorial in origin, involving an interplay between gap-junctional coupling, membrane excitability and cell and tissue architecture [40–42]. 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. It thus needs to be borne in mind that alterations to gap junction organization and connexin expression in diseased human myocardium represent just one potential facet of a constellation of factors that may contribute to pro-arrhythmogenic substrates.

3.1. Ventricular myocardium in disease

Two principal gap junction-related alterations have been reported in the diseased ventricle: disturbances in the distribution of gap junctions and reduced levels of their major component, connexin43.

Disturbance of the normal ordered distribution of connexin43 gap junctions was first reported in the myocardial zone bordering infarct scar tissue in the ventricles of patients with end-stage ischaemic heart disease [38]. 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 (Fig. 3). Electron microscopy reveals that both true laterally disposed gap junctions that connect adjacent cells, and annular profiles of

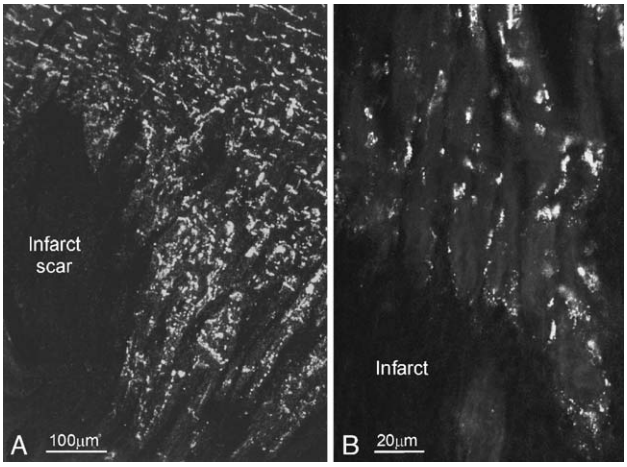


Fig. 3. Disordered distribution of connexin43 gap junctions in border zone ventricular myocardium facing infarcted tissue. (A) From a patient with ischaemic heart disease [39]; (B) from a 4-day infarct in rat ventricle [43]. In both panels, myocardium is seen approaching the (connexin43-immunonegative) infarct from the top of the field. In (A), the upper to mid-right area of the myocardium shows short lines of immunolabelling at intervals, representing linear rows of gap junctions organized in well-ordered intercalated disk arrays 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 in A) where many of the immunolabelled gap-junctional spots appear distributed in disordered fashion along the lateral borders of the myocytes. (B) Shows an example of the changes in connexin43 gap junctions observed following experimental infarction in the rat. At 4 days post-infarction, the ends of the myocyte facing the infarct develop cytoplasmic processes, with connexin43 gap junctions and adhesive junctions distributed along lateral boundaries [43].

apparently internalized and hence non-functional gap-junctional membrane, contribute to the dispersed connexin43 immunolabelling patterns in these human infarct border zone myocytes [38]. Gap junction disarray of this type occurs not only in association with established infarct scar tissue in human heart (Fig. 3A), but has been shown in experimental animals to be initiated rapidly after ventricular ischaemia and infarction [43,44] (Fig. 3B). At 4 days post-infarction in a canine model, border zone gap junction dissarray extending across the epicardial layer has been shown to correlate spatially with the central common pathway of figure-of-eight reentrant circuits [45]. Other features of remodelling, reported at 3–10 weeks post-infarction in the canine ventricle, include reduction in the size and the number of gap junctions per unit length of intercalated disk, and fewer side-to-side connections between myocytes (with relative preservation of end-to-end contacts), alterations that could potentially increase transverse axial resistivity [46].

A strikingly similar lateralization of connexin43 gap junctions to that observed in human infarct border zone myocytes occurs in experimentally induced hypertrophy of the right and left ventricle of the rat [47,48], which, in the former, has been shown to correlate with reduced longitudinal conduction velocity [47]. This feature is not generally apparent in ventricular hypertrophy associated with coronary heart disease in patients undergoing by-pass operations, though focal disordering of connexin43 gap junctions is found in small areas of the explanted ventricle in transplant patients with heart failure due to idiopathic dilated cardiomyopathy and myocarditis, as well as ischaemic heart disease [49]. More widespread spectacularly disordered arrangements of ventricular connexin43 gap junctions are an inevitable consequence of the haphazard myocyte organization characteristic of human hypertrophic cardiomyopathy, the most common cause of sudden cardiac death due to arrhythmia in young adults [50].

A less drastic form of structural remodelling is associated with hibernating myocardium in the human ventricle [51]. The term "hibernating myocardium" denotes a condition in patients with coronary artery disease in which a region of myocardium shows impaired contraction but retains the capacity to recover contractile function after coronary artery by-pass operation [52,53]. In hibernating myocardium, the large connexin43 gap junctions typically found at the periphery of the intercalated disk are smaller in size, and the overall amount of connexin43 immunolabelling per intercalated disk is reduced, compared with normally perfused (and reversibly ischaemic) segments of the same heart [51]. These observations first highlighted the possibility of a link between connexin43 gap junction alterations and impaired ventricular contraction in human heart disease.

Apart from disturbances in gap junction organization, as the findings in hibernating myocardium suggest, connexin expression may also be altered in human heart disease. The most consistently observed alteration in ventricular connexin expression involves down-regulation of connexin43 (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 reduction of connexin expression is seen irrespective of whether heart failure is due to idiopathic dilated cardiomyopathy or ischaemic heart disease. Quantitative immunoconfocal microscopy suggests that such reduction is not confined to these two causes of heart failure, but also occurs in that due to myocarditis [49]. The reduction in ventricular connexin43 appears to develop long before terminal heart failure, at least in ischaemic heart disease, as indicated by reduced connexin43 levels determined by immunoconfocal analysis of tissue from patients undergoing coronary artery by-pass operation [55]. In line with this finding, in a transgenic mouse model of juvenile dilated cardiomyopathy, reduced connexin43 and conduction defects become apparent at 4 weeks after birth, with contractile dysfunction and heart failure not following until 12 weeks [56]. Reduced levels of connexin43 may occur even with very brief episodes of ischaemia and reperfusion, such as those used in animal models of preconditioning (i.e., brief, repetitive episodes of ischaemia), though this appears to be a temporary effect [44].

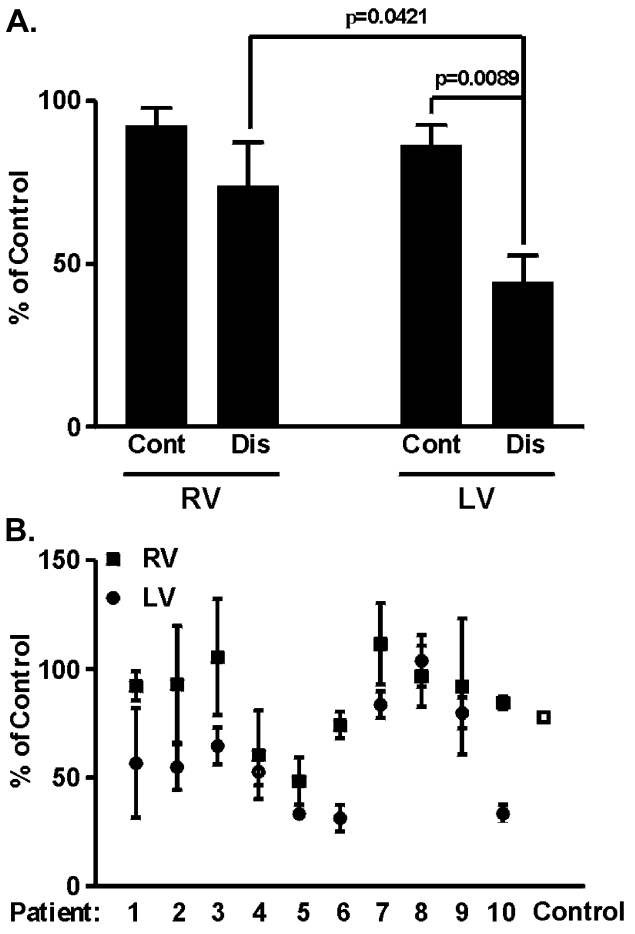


Fig. 4. Data illustrating heterogeneous reduction of ventricular connexin43 levels in human heart disease. The Western blot analysis (A) shows significant reduction in connexin43 in samples of failing ventricle from explanted hearts of patients undergoing cardiac transplantation. Note significant reduction of connexin43 levels in failing left ventricle compared with control samples (cont) from normal hearts (intended for transplantation but not used for technical reasons). That the reduction in connexin43 does not uniformly affect all regions of the heart is shown in the Northern blot data in B. Multiple samples taken from the same diseased heart in many (though not all) instances reveal a considerable spread of values, shown by the large standard deviations. In control hearts, however, the standard deviation is very small (unfilled square). From Ref. [54].

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 or coupling, but do not provide information on the quantity of functional (open) channels. Furthermore, computer modelling studies predict that reductions of up to 40% in gap junction content (without change in junction size) would not have a significant effect on conduction velocity [6]. On this basis, a reduction of connexin43 in the diseased ventricle, if it were the only change occurring, would not, per se, be of functional relevance. On the other hand, in view of the complex relationship between passive and active membrane properties [40,57,58], the multiplicity of structural and functional alterations in the diseased heart and the assumptions inherent in computer modelling, in vivo extrapolation of the effects of a single change (i.e., reduced connexin43 levels), in isolation from other factors, may not give the full picture. Studies on experimental animals and the intact heart are therefore also instructive in gaining further insight.

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% [59], 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 [60] even though there may only be a modest reduction in conduction velocities [61,62]. In the failing human ventricle, considerable variation is apparent in the extent of connexin43 reduction between and, in particular, within hearts (Fig. 4), 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 in the diseased human heart 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. Inhomogeneous wave front propagation could, in turn, lead to asynchronous myocyte contraction and poor ventricular force development.

A neat experimental demonstration that heterogeneity of cardiac connexin43 expression is indeed linked to disturbances in electromechanical function comes from work by Gutstein et al. [63] using chimeric mice created from connexin43-deficient stem cells and blastocysts. This approach was designed to give patchy expression of connexin43, mimicking to a degree the features found in human pathological specimens. The resultant experimental mice were demonstrated to have both abnormal conduction and contractile dysfunction, as originally hypothesized in the human studies [38,51,54]. Thus, the possibility that spatially heterogeneous connexin43 down-regulation of the magnitude and nature observed in the diseased human ventricle could similarly predispose to arrhythmia and contractile dysfunction remains open to further debate.

Apart from alterations in connexin43 level, rapid dephosphorylation of connexin43 and translocation of connexin43 from gap junctions into the cytosol has been reported when electrical uncoupling is induced by acute ischaemia in the Langendorff-perfused rat heart [64]. These processes are reversible upon reperfusion [64] and substan-

tially reduced with ischaemic preconditioning [65,66]. In transgenic mice expressing half the normal level of connexin43, preconditioning apparently does not afford protection from prolonged ischaemia as it does in mice with the normal level of connexin43 [67]. However, while this last study found that infarct size was not reduced by ischaemic preconditioning in the transgenic animals, another report has concluded that these animals do develop smaller infarcts after coronary ligation than their wild-type counterparts [68]. For further discussion of the topic of preconditioning and gap junctions, see the review by Gerd Heusch in this spotlight issue.

Ethical considerations preclude corresponding studies of the short-term effects of ischaemia in the human heart. However, during cardiopulmonary bypass, the human heart may be subject to stress resembling the challenge of ischaemia. The accessibility of right atrial appendage samples during cardiac surgery has enabled investigation of temporal changes in connexins and gap junctions during cardiopulmonary by-pass which may reflect changes in the heart as a whole (i.e., including the ventricles which, for ethical reasons, cannot be sampled) [69]. Connexin43 expression and gap junctions appear reduced during cardiopulmonary by-pass, with coronary artery disease patients showing a greater reduction than other patients. This suggests that, despite the application of hypothermia and cardioplegic solution, protection of the heart may in some instances be inadequate during the operation, especially in patients with coronary artery disease. Whether these changes are sufficient to contribute to the common occurrence of post-operative ventricular dysfunction is unknown [69].

Less is known about alterations in expression of connexins other than connexin43 in the human ventricle. However, the overall level of connexin40 transcript is increased in the ventricles of patients with congestive heart failure due to ischaemic heart disease 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 [32]. The significance of this expanded zone of connexin40 expression is unclear; whether it represents a compensatory response (e.g., improving depolarization from the conduction tissues in the face of declining connexin43 levels), or whether it exacerbates heterogeneity of impulse propagation between adjacent regions of myocardium (perhaps increasing susceptibility to arrhythmias) is unknown.

3.2. Atrial myocardium in disease

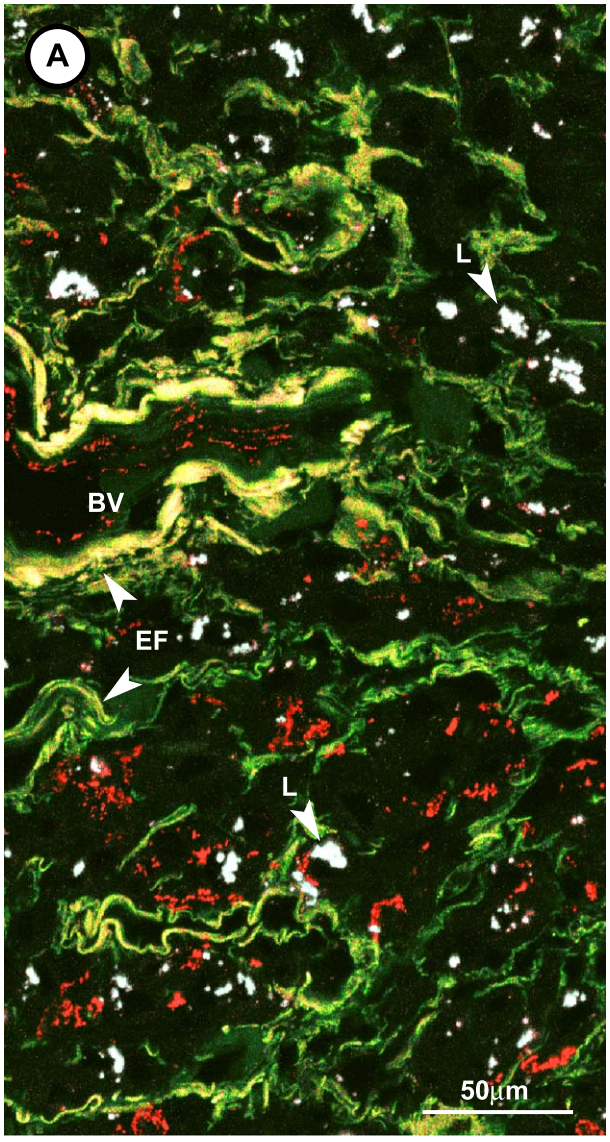
Arrhythmia commonly afflicts the atria in the form of atrial fibrillation, a condition in which wavelets of electrical activity propagate in multiple directions leading to disorganized depolarization and ineffective atrial contraction [70]. The condition is associated with progressive electrical, contractile and structural remodelling, including altered cell size and mitochondrial shape, loss of sarcomeres and perinuclear accumulation of glycogen [71]. These changes, resulting from atrial fibrillation itself, exacerbate the condition, so that once established it tends to persist [72]. Alterations of gap junctions and connexin expression, in particular that involving connexin40, are reported to feature in the remodelling process. Studies on human atrial samples have variously reported a net increase [73] or decrease [74,75] in connexin40 expression in patients suffering chronic atrial fibrillation, with redistribution of connexin40 labelling to predominate at the lateral borders of the myocytes [73,74]. It has been hypothesized that this last change might result in dispersion and heterogeneity in the anisotropy of conduction, contributing to perpetuation of re-entrant pathways and thus of atrial fibrillation itself. Other junctional proteins such as N-cadherin and desmoplakin are reported to show similar changes in distribution to those of connexin40 [74], suggesting a spatial association between gap junctions and adhesive junctions during their re-organization at the cell surface, as occurs in the maturing heart [76,77].

Studies on a goat model of pacing-induced persistent atrial fibrillation identified marked heterogeneity in connexin40 immunolabelling [78] and reduced ratio of connexin40/connexin43 as key changes [79]. However, no lateralization of the Cx40 labelling to the myocyte borders of the type observed in the human studies was noted. Whether the difference between goat and man with respect to lateralization arise from differences in species, age or manner of induction of atrial fibrillation (by pacing rather than by ‘natural’ causes) is unknown. The heterogeneity of connexin40 distribution reported as an atrial fibrillation-induced change in the goat model resembles that which is found naturally in the human atrium [22,74], though this feature may become more marked in patients with chronic atrial fibrillation [74]. As human biopsies usually come from patients over 60 years of age, the possibility exists that heterogeneity of Cx40 distribution may be age-related. It should also be borne in mind that patients with atrial fibrillation are not a uniform population, differing, for example, in the precise pattern of atrial activation and in response to therapy. Further work is needed to determine whether gap junction and connexin expression can be related to such factors.

The above studies relate to chronic or persistent atrial fibrillation in which the samples are analyzed after a substantial period of sustained fibrillation. Distinct from changes that may occur as a result of atrial fibrillation and which, once established, may help perpetuate the condition, it is also of interest to consider whether any features of gap junction organization or connexin expression may contribute to initiation of atrial fibrillation. In a substantial number

of patients, the initiating foci of atrial fibrillation are located in the proximal portions of the thoracic veins that have a sleeve of myocardium continuous with that of the atria [80,81]. Gap junctions in the myocardial sleeve of the canine superior vena cava are predominantly clustered at intercalated disks, with connexins 43, 40 and 45 commonly co-localized [82]. Areas of atypical expression have been identified, however, in which connexin43 gap junctions are diffusely distributed in a cluster of cardiomyocytes surrounded by a peripheral region of cardiomyocytes expressing predominantly tiny connexin40 gap junctions. This, together with variations in gap junction distribution and differences in assembly and spatial orientation of the myocytes, endows the myocardial sleeve with a heterogeneous structure that could potentially form a substrate for heterogeneity of coupling from which ectopic activation may be more likely [82].

Other predisposing features may occur within the atrial myocardium itself. Samples of right atrial appendage from patients in sinus rhythm undergoing coronary artery bypass show a range of connexin40 levels. Of the patients who subsequently develop post-operative atrial fibrillation, the majority have higher levels of connexin40 than those who do not develop the condition (Fig. 5) [22]. As noted above, connexin40 gap junctions are heterogeneously distributed in both groups of patients. This heterogeneity could give rise to different resistive properties and conduction velocities in spatially adjacent regions which become enhanced, and hence pro-arrhythmic, the higher the overall levels of connexin40. Whether the distribution of connexin40 gap junctions becomes lateralized (as in chronic atrial fibrillation) once post-operative atrial fibrillation has become established is unknown.



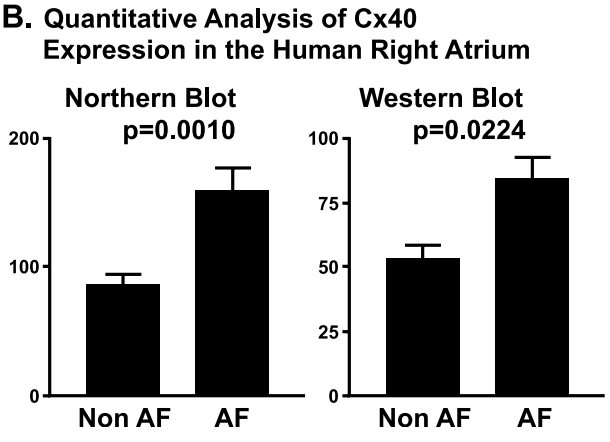


Fig. 5. Connexin40 in the human atrium. (A) shows a confocal image; connexin40 label is seen as red fluorescence, autofluorescence of elastic fibers (EF) as green, and autofluorescence of lipofuscin granules as white (L, lipofuscin). The distribution of connexin40 signal is markedly heterogeneous; the lower area of the field shows prominent labelling while the upper area shows sparse labelling. Connexin40 label is also present in the endothelial cells of intramural blood vessels (BV). (B) shows data on connexin40 levels in the right atrial appendage of patients undergoing by-pass operation; those who subsequently developed atrial fibrillation (AF) had higher connexin40 levels than those who did not (Non-AF). From Ref. [22].

4. Concluding comments

From the foregoing discussion, it is now clear that alterations in myocyte gap junctions and connexins—notably disordering in the pattern of junctional distribution and reduced levels of connexin43—do occur in the ventricle 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. It might be tempting to contemplate therapeutic approaches based on this knowledge but, from where we now stand, feasibility presents major challenges which, even if overcome, would leave serious questions of efficacy [68,83–85].

Acknowledgements

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