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Is there a role for remodeled connexins in AF? No simple answers

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Introduction

Although electrocardiographically atrial fibrillation (AF) is described by well defined criteria that are mostly manifested independently of the pathophysiological origin of the arrhythmia, the pathophysiological basis for AF is extraordinarily varied and complicated. Traditionally, the electrophysiological mechanism of an arrhythmia has been defined in terms of abnormal impulse generation (automatic or triggered activity) and abnormal impulse conduction (reentry). It appears that these mechanisms are utilized either singly or in combination in AF and the specific mechanism or combination depends on the causative underlying pathophysiological event. For example, atrial fibrillation is associated with; aging, myocardial infarction, hypertension, ischemic heart disease, and genetic abnormalities or it may be classified as lone with no obvious clinical cause [1,2]. Each situation may be associated with a different electrophysiological mechanism for AF. Even within each underlying pathophysiological condition, AF may be paroxysmal, persistent that requires cardioversion, or permanent that cannot be cardioverted. Each of these types is likely to be associated with a different combination of mechanisms [3]. Furthermore, with time, as fibrillation persists electrophysiological remodeling occurs (see below) and the mechanism or combination of mechanisms may change even for a specific pathological cause. This complexity is a far cry from the seemingly simplistic view of the 20th century which regarded atrial fibrillation as being primarily a product of abnormal conduction and reentry in multiple reentrant circuits [4]. In analyzing the literature on mechanisms of atrial fibrillation it is therefore absolutely necessary to categorize clinical studies by the underlying pathophysiological causes of the AF. In further elucidating the electrophysiological mechanisms using animal models, no one model is representative of “clinical” atrial fibrillation. Hence the large number of studies in different small and large animal models which often arrived at conflicting conclusions.

The primary goal of this article is to discuss the involvement of changes in myocardial cell communication (gap junctions) as a possible cause of atrial fibrillation. Therefore, information on proposed mechanisms causing AF and the relationship of AF to abnormalities in conduction is of primary importance. Before a detailed discussion on cell coupling in AF we provide a brief overview of current concepts on mechanisms of AF. A more complete discussion can be found in a recent review [5]. In considering the electrophysiological mechanisms of atrial fibrillation, they can be subdivided into mechanisms of initiation and mechanisms of perpetuation of the arrhythmia.

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Mechanisms of Atrial Fibrillation; An Overview

Initiation of atrial fibrillation; the triggers

An increase in frequency of premature atrial beats often occurs immediately before the onset of AF [6] and may somehow initiate AF. There is an extensive literature showing that electrical activity in the atrial muscle which extends into the pulmonary veins is related to the onset and perpetuation of atrial fibrillation [7]. The mechanism for impulse initiation may be either triggered activity or reentry [8]. Both delayed and early afterdepolarizations as well as triggered activity have been found to be prevalent in single myocytes isolated from normal canine pulmonary vein myocardium as well as myocytes from pulmonary vein obtained from dogs with pacing induced heart failure [9]. Triggered activity caused by early or delayed afterdepolarizations has also been recorded in isolated preparations of pulmonary veins from experimental animals [10,11]. The evidence from *in vivo* experimental and clinical studies is more equivocal. Clinical studies have also shown that the initiator of atrial fibrillation can sometimes be in the vicinity of the coronary sinus [12]. Norepinephrine causes delayed afterdepolarizations and triggered activity in musculature inside the coronary sinus [13] suggesting that this may be an initiating mechanism for AF. Other possible sites of triggered activity caused by either delayed or early afterdepolarizations that may be related to the onset of atrial fibrillation include venae cavae, ligament of Marshall, atrial muscle and atrial muscle in the mitral valves [8].

The above mechanisms for atrial fibrillation initiation do not involve cell coupling so it would seem unlikely that characteristics of connexins at these sites are relevant. However, reentrant excitation may also occur in small circuits in pulmonary vein musculature as the mechanism for initiation of AF. The abrupt changes in fiber orientation near the pulmonary vein ostia [14] as well as properties and distributions of connexins in the veins may be responsible for the marked conduction delays in this region [15]. The decreased upstroke velocity and shorter action potential duration of pulmonary vein musculature also favor reentry [16]. Reentry in isolated pulmonary veins has been mapped with optical recordings [17,18].

Perpetuation of atrial fibrillation

Many initial AF episodes have short durations of seconds or minutes and are asymptomatic [6,19] However, with time, AF eventually may become long lasting or persistent, hence the phrase “atrial fibrillation begets atrial fibrillation” [20]. The mechanisms that lead to this perpetuation (persistence) of AF may not be the same as the triggering mechanisms, although triggering mechanisms may still assist in perpetuation. Reentry is the most likely mechanism for perpetuation and hence the importance of assessing the role of intercellular communication. The forms that the reentry may take are several, single driving rotors with fibrillatory conduction or multiple reentrant circuits and it is likely that each may be important under different pathological conditions.

Multiple reentrant wavelets may be involved in the perpetuation of AF. The proposal of Moe and Abildskov [21] is that AF is the result of randomly propagating multiple wavelets that interact in complex ways determined mainly by heterogeneities in atrial refractoriness [21–23]. This concept fit nicely with the leading circle mechanism for reentry [24] in which a functional circuit establishes itself in accord with the wavelength of the impulse, slow conduction and short refractory periods providing short wavelengths and small circuits. The size of the wavelengths in conjunction with the size of the atria dictates the number of circuits that occur simultaneously. The combination of these small circuits and large dilated atria may favor the occurrence of multiple reentrant circuits caused by these mechanisms. The role of altered cell coupling in contributing to short wavelengths is discussed later.

A discrete rapidly firing focus may also cause AF. The concept is supported by the finding of circumscribed regions initiating impulses at high frequencies with a gradient of decreasing frequencies distant from the highest frequency region [25,26]. Mapping from the high frequency region has shown centrifugal spread and in some instances AF cycle length has prolonged during radiofrequency delivery to the high frequency region (27). A likely mechanism for high frequency activity in a focus is spiral wave reentry [28]. Because of the rapid rate, impulses propagating away from the high frequency source conduct irregularly and inhomogeneously. The pattern of conduction gives rise to the typical low amplitude irregular activity on the ECG and is therefore called fibrillatory conduction. The location of the focus in a cholinergic sheep model of AF has been found in the posterior left atrium near the junction with the pulmonary veins with a left to right frequency gradient, although other atrial regions, including the right atrium have been found to be driver sources with other causes of AF [28]. Alterations in cell coupling may enable such reentry to occur on a microscale.

Remodeling of Atrial Myocardium in AF; Sarcolemmal Ion Channels

The necessary conditions to sustain reentry from either multiple wavelets or single high frequency rotors with fibrillatory conduction are provided by remodeling of atrial structure, myocyte sarcolemmal ion channels and intercellular communication among myocytes. Remodeling in its broadest sense implies macroscopic structural changes in the atria such as dilation and/or fibrosis [29,30,31] as well as microscopic and molecular changes in structure and function of myocytes related to altered protein synthesis of ion channel constituents.

Remodeling of sarcolemmal ion channels leads to changes in atrial action potentials. Primary changes in action potentials include an acceleration of repolarization, shortening of refractory periods and a deficiency in the ability of repolarization time course to adapt to rate changes [5]. Shortening of repolarization and refractory periods causes decrease of the wavelength and allows small reentrant circuits. Inhomogeneous remodeling promotes fibrillatory conduction, inhomogeneous slow conduction and block. Changes in intracellular ions resulting from the initial short periods of rapid activity that occur before atrial fibrillation becomes permanent appear to be primary stimulators for ion channel remodeling. Much of the remodeling can be attributed to abnormalities in calcium handling. Periods of rapid activity are likely to result in intracellular calcium loading eventually leading to reduced ICa_L amplitude [32]. In addition to the reduced ICa_L decreasing the action potential plateau and duration, elevated Calcium may effect control of protein synthesis.. The hyperphosphorylation of the ryanodine receptor (RyR2) that results in leaky RyR2 channels in AF may also contribute to the occurrence of delayed afterdepolarization triggered activity which may be involved in perpetuation of AF [33]. Inward sodium current may also be decreased, contributing to slow non uniform conduction [5,34] although this has not been found in all studies of atrial fibrillation [35]. Outward K currents are also remodeled [5]. I_{to} is downregulated [5,35,35a] although the functional importance of this change is not clear. IK_r or IK_s may or may not be altered [5, 36]. Several studies have reported increased background inward-rectifier current, attributed to IK_1 , in AF [36,37,38] that may contribute to the reduced action potential duration [39]. Atrial remodeling in experimental heart failure is distinctly different from atrial tachycardia remodeling [39]. Action potential duration is little changed, IK_1 is unchanged and IK_s is reduced, in contrast to atrial tachycardia remodeling in which they are increased and unchanged, respectively. In addition, atrial Na/Ca exchange is increased in atria from failing hearts, potentially promoting triggered activity [40].

Remodeling of Atrial Myocardium in AF; Intercellular communication

Despite the different possible forms that reentry might take during AF e.g. single rotors or multiple circuits in the left or right atria, or pulmonary veins, the size of the circuits is undoubtedly quite small requiring very short wavelengths. Although the electrical remodeling

of sarcolemmal ion channels during AF leads to short duration action potentials with short refractory periods and perhaps a decrease in sodium current, these changes alone might not be sufficient to provide the substrate needed to sustain reentry in single or multiple small circuits. Furthermore, the time course of occurrence of sarcolemmal ion channel remodeling does not parallel the appearance of sustained AF in a goat model but rather occurs much earlier [41] further supporting the hypothesis that electrical remodeling of ion channels alone are not sufficient to cause sustained AF. An additional mechanism that may be necessary is the remodeling of gap junctional cell coupling.

Gap Junctions in Atrial Fibrillation

Structure and function of gap junctions

Atrial myocytes are electrically coupled through gap junctions that are made up of proteins from the connexin (Cx) family. Connexins are four transmembrane domain proteins which oligomerize in the Golgi apparatus of cells to form a hemichannel (connexon). These connexons then traffic out to the myocyte sarcolemma becoming localized at the intercalated disk. Here they bind head-to-head across the extracellular space with connexons from the adjacent cell. Once the interaction is complete the channels then open forming a direct cytoplasmic continuity between the cells. These channels show only mild specificity and generally transfer any ion or molecule under approximately one kilodalton in size [42]. Thus it is a low resistance pathway for an action potential to spread from myocyte to myocyte.

There are 21 isoforms of connexin in the human genome, five of which are found in the heart (Cx31.9, Cx37, Cx40, Cx43, and Cx45) [43]. Connexins have been sub-grouped based on amino acid sequence homology into 3 main types, the alpha, beta, and gamma subgroups [43]. The major (most abundant) myocyte connexins (Cx40 and Cx43) fit into the alpha category while the minor or less abundant connexins (Cx31.9 and Cx45) do not fit into either of the major subgroups. The functional significance of this subdivision may relate to the regulation of these connexin subgroups at the cellular level [44] thus allowing for differential regulation in both normal and pathophysiological changes within myocytes. Due to the fact that connexin channels formed by different isoforms of connexin have different physiological properties, the presence of multiple connexins within a single tissue may confer differing levels of regulation of cell-cell coupling.

Ventricular myocytes contain primarily Cx43, although small levels of Cx45 have also been reported [43]. The specialized conduction system contains Cx31.9, Cx40 and Cx45, the isoforms vary in different regions. Cx37 is found in the vascular endothelium [43]. The most connexin heterogeneous tissue within the heart is atrial myocardium making it very difficult to sort out the effects of remodeling as described below. The atrium contains Cx40, Cx43, and Cx45 (Figure 1) [43,45,46]. Cx40 is expressed in high levels, 2 to 3 fold higher in the right atrium than the left [46]. It has been shown that the normal Cx40 distribution may also be heterogeneous with areas containing large amounts of Cx40 adjacent to others containing minimal or no Cx40 although heterogeneities have not been precisely quantified [47–49]. The variable expression is likely to lead to inaccuracies in quantification of Cx40 in studies on remodeling in AF since only small samples of right atrial myocardium have been analyzed in most studies. Like the other alpha connexins, Cx40 gap junctions have a relatively high unitary conductance (200 pS) [42]. It is a mildly voltage dependent channel with conductance being pH dependent [42]. These properties may be important for changes in gap junction function during AF as described later. While there have been few studies of protein partners for Cx40, the amino acid sequence of the carboxyl terminal domain is suggestive of regulation by kinases and phosphatases (see below), thus altered interactions of connexins with regulatory proteins may also be involved in remodeling. Unlike Cx40, Cx43 is distributed homogeneously throughout the right and left atria and reaches levels similar to Cx40 in the right atrium [46].

Cx43 channels have a unitary conductance between that of Cx45 and Cx40 (~120 pS) and is also both voltage and pH dependent [42]. Cx43 channels are regulated by a number of signal transduction cascades, particularly those that alter the phosphorylation state of the protein [50] that may be involved in remodeling. Along with these forms of regulation there are now a plethora of studies indicating that Cx43 is under stringent control by a variety of protein partners [51]. Cx43 has also been reported to regulate Cx40 [52], an interaction that may play a role in both normal and pathological atrial function if one of these connexins changes during remodeling. Cx45 is a lower conducting isoform (30 pS) when compared with the other atrial connexins [42]. Due to the difficulty in detecting this connexin (in part due to high cross reactivity of some of the Cx45 antibodies) it has been difficult to determine how much Cx45 is in either normal or pathological atrial tissue although the levels appear to be much lower than either of the other two atrial connexins [46]. The impact of Cx45 on normal function or in atrial fibrillation is not known since it has not been measured in most studies (but see [47]).

The gap junctions in the atrial myocardium of the thoracic veins (vena cavae and pulmonary veins) are comprised of Cx40 and Cx43 [15,53]. Level of Cx43 are similar to that found in the atria but Cx40 levels within the pulmonary veins are lower than that found in the atria [53]. This heterogeneous distribution may contribute to slow conduction in the veins that could lead to reentry.

Unlike the ventricles which predominantly have a single connexin isoform, Cx43, the presence of multiple connexin isoforms in the atria may result in many different types of gap junction channels with different regulatory and physiological characteristics, making it difficult to examine the remodeling process. The channels formed from these connexins may be homomeric and homotypic, that is each hemichannel may form from one connexin isoform (homomeric) and then interact with another homomeric connexin of the same kind in an adjacent cell (homotypic) [42]. Thus atrial myocytes may be connected by gap junctions comprised either of Cx40 alone, Cx43 alone or Cx45 alone (Figure 2). The channels may also be homomeric but heterotypic. Added confusion comes with the fact that homomeric Cx40 connexons might be coupled to homomeric Cx43 or Cx45 connexons. Channels may also be heteromeric and heterotypic [42]. Heteromeric means that a single connexon may be composed of different connexins such as Cx40 and Cx43 in atria, and coupled to another connexon comprised of different connexins. Cx40 and Cx43 colocalize in intercalated disks between atrial myocytes [48,54]. These two proteins have been demonstrated to interact in the formation of heteromeric/heterotypic gap junction channels *in vitro* [55,56]. A convenient model for the study of these interactions has been a neonatal rat aortic smooth muscle cell line, that naturally coexpresses both Cx40 and Cx43 [57]. Cotrell and Burt have stated that “In theory, if a cell type expresses both Cx40 and Cx43 isoforms, and if they are free to associate in a random fashion to form connexons, the cell could contain at least 14 different connexons, 12 heteromeric and 2 homomeric. If each of these 14 connexons can dock with all of the others, then pairing two such coexpressing cells could result in as many as 196 different channel types, more if the connexons are allowed to rotate relative to one another” [57]. It is not known how many such channel types might occur in normal atrial myocardium. Furthermore it would be difficult to predict the behavior of each channel type since it might not reflect the majority composition of its constituents. In canine atrial cell, pairs a range of gap junctional unitary conductances has been reported suggesting interaction between different connexins (heteromeric, heterotypic) involving Cx40, Cx43 and Cx45, rather than discrete peaks as would be expected from two populations of channels each of a single connexin type (homotypic Cx43 and Cx40 channels) [58]. It can therefore be readily understood why the functional significance of data on alterations in connexins accompanying atrial fibrillation is difficult to interpret.

Remodeling of intercellular connections

During AF, remodeling of intercellular connections can theoretically take the form of quantitative changes in connections (decrease or increase) and/or changes in the location of connections. Such remodeling might be the result of fibrosis that disrupts normal gap junction connections. Although fibrosis has not been found to be associated with all pathological causes of atrial fibrillation [48], fibrosis on a microscale might not always be recognized with standard histological techniques. It invariably occurs with aging in the form of fine longitudinally oriented collagenous microsepta [59]. In atrial myocardium from elderly subjects studied *in vitro* properties of conduction are changed from uniformly to non uniformly anisotropic in which properties of conduction and refractoriness are no longer primarily dependent on sarcolemmal ion channel function. It has been proposed that these fine collagenous septae interrupt side to side connections between myocyte bundles and thus the transverse conduction velocity may be decreased to as low as 0.03 m/s (lower than values of the normal AV node) in the presence of relatively normal ionic currents. Nonuniform anisotropic properties can also cause differences in effective refractory periods in the absence of spatial differences in action potential duration that can result in conduction block and reentry of premature impulses [59]. The amount of fibrosis and nonuniform anisotropy is likely to differ markedly from one region of the atria to another. Reentrant circuits in a non uniform anisotropic medium can be very small because of the short wavelengths caused by the very slow conduction on a microscale [59]. Non uniform anisotropy in fibrillating atria has not been measured on a microscale *in vivo* in either animal models or humans.

Another mechanism for remodeling of intercellular communication is remodeling of gap junctions, either through a change in connexin quantity, distribution or function. In the ventricle, with its predominance of Cx43, remodeling data from both experimental animals and humans is relatively consistent for different pathological causes (ischemia and infarction, heart failure, cardiomyopathy). The primary changes documented have been a decrease in Cx43 quantity and a lateralization of Cx43 (e.g. occurrence of increased amounts of Cx43 on lateral sarcolemma where it is not normally present) [60,61]. Remodeling in which there is a decrease in Cx43 is associated with slowing of conduction as predicted by some transgenic mouse models with reduced Cx43 although it is apparent that a significant reduction might occur without gross conduction slowing [60]. The functional significance of even this relatively simple paradigm of changes in a single connexin (decreased total Cx43 and increased lateral membrane Cx43) is difficult to interpret. For example, in our studies on infarct border zone, there is a decrease in transverse conduction despite an increase in lateral Cx43 [61]. This has led us to propose that the Cx43 on lateral membranes might not form functional gap junction channels [62]. The lesson to be learned is that quantifying connexins is not an adequate indicator of what is happening to gap junctions and functional properties of coupling during remodeling. This problem is amplified when analyzing the data on connexin remodeling in atrial fibrillation where there are multiple connexins.

In the myocardial environment of AF there are numerous ongoing events, many of which point to potential regulators of connexins during the disease process. Hypoxia [63], inflammation [64], and fibrosis [65] all occur in the fibrillating atria and can activate intracellular cascades that might cause structural and/or functional remodeling. While the primary connexin in the atria is Cx40, most studies on regulators have focused on Cx43. Data on regulators of Cx40 are desperately needed.

Cx43 and Cx40 gap junction channels are pH sensitive (with pKa values in the range of 6.7). Thus under conditions of hypoxia and subsequent intracellular acidification loss of coupling may occur, a form of functional remodeling. It is well documented that atrial connexin isoforms do close under conditions of low pH [52]. Both Cx43 and Cx40 closure under conditions of low pH has been shown to work as a “ball and chain” mechanism [52,66]. The interaction

between the carboxyl terminal domain of Cx43 (Cx43CT) and the cytoplasmic loop is facilitated by an interaction of the non-receptor tyrosine kinase cSrc with the Cx43CT which causes a loss of interaction of Cx43 with the scaffolding protein Zonula Occludens-1 (ZO 1) [67]. This leaves the Cx43CT free for other interactions. While it is not known whether the same or similar mechanisms occur with Cx40, co-regulation of Cx40 by Cx43 has been documented [52]. Thus, in the setting of AF, tissue acidification may cause closure of Cx43 channels and decrease in conduction throughout the affected regions of the atria. Regional differences in both extracellular and intracellular pH within the fibrillating atrium would lead to heterogeneous changes.

Cx40 and Cx45 are also voltage dependent [42] leading to the possibility that gap junction channels formed by these connexins might close during the rapid rates of AF if membrane potential is partially depolarized (also functional remodeling). This might also contribute to conduction slowing and block. In addition to voltage regulation, many of the connexin isoforms are found as phosphoproteins. While the sites or physiological relevance of Cx40 phosphorylation are not known, Cx40 has been shown to exist in a phosphorylated form [68] and has been shown to be phosphorylated by PKC and MAP kinase pathways [69]. One study has shown that cAMP increases dye coupling in cells transfected with Cx40 [70] but to date studies of whether phosphorylation of Cx40 in atrial tissue is altered under pathological conditions have not been done. Cx43 phosphorylation, on the other hand, has been widely studied. It is known that Cx43 protein within a functional Cx43 gap junction channel exists in a highly Serine/Threonine phosphorylated state [71]. Under pathological conditions Cx43 may be dephosphorylated or the phosphorylation may change from Serine/Threonine to Tyrosine phosphorylation, rather than a complete dephosphorylation of the protein [71]. For example, under conditions in which the non-receptor tyrosine kinase cSrc is activated, Cx43 loses Serine/Threonine phosphorylations but gains a Tyrosine phosphorylation [72]. Depending on the phospho-residues in question, both loss of Serine Threonine phosphorylation as well as a gain of Tyrosine phosphorylation are associated with loss of cell-cell coupling [71,72]. It has been shown that phosphatases are activated in atrial fibrillation [73,74] but direct studies on the relationship between phosphatases and connexins in the fibrillating atrium have yet to be published. Activation of tyrosine kinase pathways in atrial fibrillation has also been described [75] although again, not in connection with the role they might play in regulation of connexins. Most studies quantifying connexins in AF have not evaluated changes in the phosphorylated state.

Inflammation of atrial tissue has been documented in patients with atrial fibrillation [76]. Inflammatory cells migrate into the atria and cytokines increase within the tissue. Both Tumor Necrosis Factor alpha (TNFa) and Interleukin-6 are increased [76]. Both TNFa and members of the Interleukin family have been reported to close gap junction channels, although IL-6 in particular has not been studied [77,78]. While the mechanism by which these cytokines close gap junctions is not clear, TNFa may be acting via activation of Src [79].

Since the atria have at least two and likely three types of connexins that are involved in gap junction formation and these connexins can interact in a number of different configurations, it is difficult to formulate a unified hypothesis concerning the relationship of connexin remodeling to the occurrence of atrial fibrillation from the available data on quantitative changes. The problem is further enhanced by methodological considerations some of which have been mentioned but are worth reiterating. In human and some animal studies, quantification of connexins have mostly been confined to small pieces of right atrial appendage or trabeculated right atrial wall, regions that are probably not important in the genesis of atrial fibrillation. Although it has often been assumed that these regions are representative of the rest of the atria, in our opinion it is likely that differences in quantity and relationships of the different connexins and the response to AF are regionally dependent. Changes in right atria

may not be representative of changes in left atria where fibrillation often originates. In an animal study in which connexins in both right and left atrial appendage were measured, a different response to atrial fibrillation occurred in each chamber [80]. Another difficulty in the interpretation of the data is methodologies for quantification of connexins. Both Western blotting and immunofluorescence confocal analysis have been used, and both have their shortcomings. Quantification of connexin by immunofluorescent confocal analysis has numerous methodological difficulties including the potential for epitope masking, an event known to occur with Cx43 under hypoxic conditions [81]. Also, appropriate pixel counts in confocal microscopy can be difficult to assess across samples due to differences in antibody affinity or high background fluorescence. Whole-cell Western blot methods, the most commonly used immunoblotting methods, do not show whether the connexins measured are confined to cell membranes. Neither method (Western blotting or immunofluorescence confocal microscopy) shows that the connexins being quantified form functional gap junctions. A third difficulty is due to the mixing of connexin isoforms. If gap junctions are heteromeric/heterotypic, how do changes in the quantity of a specific connexin affect the makeup of individual gap junctions. Finally different pathophysiological causes of AF, mitral valve disease or ischemic heart disease for example, may result in different patterns of gap junction remodeling. Animal models of AF do not clearly replicate pathophysiological events in humans.

Keeping these methodological difficulties in mind, studies on connexins in atrial fibrillation have focused on quantification of Cx43 and Cx40 messenger RNA (using Northern blot analysis) and protein (using Western blot and immunofluorescent analysis) of right atrial tissue in humans and animal models of AF, and the distribution of these connexins. Connexin quantification in left atrium has rarely been done [82,83]. Increased Cx40 [47,49,82,84], decreased Cx40 [49,54,85] and no change in Cx40 [54,83] have been reported. A common finding irrespective of quantitative changes in humans or animal models has been an increased heterogeneity in Cx40 distribution manifested as increased regions in which Cx40 containing myocytes are located adjacent to myocytes mostly lacking Cx40 [47,49,54,83,85,86]. Lateralization of Cx40 where increased Cx40 protein is located on lateral sarcolemmal membranes that are usually devoid of Cx40 has been noted [49,84] along with lateralization of adhesion proteins of the intercalated disk [49]. In one study Cx40 was increased in one region sampled and decreased in another showing that there can be heterogeneity in quantitative remodeling of connexins [49]. In the goat model of AF the appearance of increased heterogeneity without a change in Cx40 quantity paralleled the progression from unsustained to sustained AF [86]. An increase in Cx43 has also been measured [82,87,88], as well as a decrease [49] but the most common finding is no change [47,54,84,85]. Unlike Cx40, Cx43 is uniformly distributed rather than inhomogeneously distributed but like Cx40, lateralization of Cx43 has been observed [49,84]. Quantification of Cx45 has not been included in any of the studies on chronic atrial fibrillation although it was measured in a study on patients who had postoperative AF and did not change [47].

It is difficult to reconcile the disparity in some of the results as well as relate them to electrophysiological changes associated with AF. Since the measurements of connexin quantity and distribution have been confined to small pieces of right atrium in most studies (left atrium in one human and animal study) and since there may be heterogeneous remodeling [49] it is possible that different regions may show quantitative differences in connexin remodeling. If this occurred it could contribute to heterogeneities of electrophysiological properties that might be instrumental in causing atrial fibrillation or fibrillatory conduction. In addition, it doesn't appear that connexin measurements have been made in any of the regions previously indicated to be a possible source of AF, for example where rotors are located in the left atrium. As to functional significance, a number of issues should be considered. The most important is that connexin quantity cannot be equated with structural and/or functional gap junctions. In general, antibodies that have been used have measured both phosphorylated and dephosphorylated

isoforms except in one study [85] when only the phosphorylated isoform may be functional. Connexin proteins may also locate in the membrane without forming structural gap junctions [62]. Cx40 or Cx43 quantity cannot be equated with quantity, makeup or function of heterotypic/heteromeric gap junctions. Each of the changes in Cx quantity individually or in combination might lead to different changes in conduction properties and contribute to the heterogeneities in conduction in fibrillating atria.

An increase in quantity of Cx40 as found in several studies does not necessarily mean increased coupling or conduction and in fact, this would not be predicted to occur in AF, whereas a decrease in Cx40 should lead to conduction slowing as demonstrated in the atrial of a transgenic mouse model [89] (however, see reference 48 for an hypothesis of how decreased Cx40 might improve conduction). Similarly, in our opinion, lateralization of Cx40 is unlikely to increase lateral coupling and improve transverse conduction since lateralized connexin protein probably does not form structural or functional gap junctions [62]. The most common finding in human and experimental studies is heterogeneity of Cx40 distribution which might lead to conduction block and reentry. However, measurements of conduction in such regions have not shown this, perhaps because the spatial resolution of the mapping electrode array has not been sufficiently small (54,83). Similarly we do not think it is likely that an increase in Cx43 or lateralization of Cx43 leads to improved coupling and increased conduction although one study on rat atrium did show a decrease in anisotropy ratio [84]. A decrease in Cx43 in the atrium did not slow conduction in a transgenic mouse model [90].

Conclusions

Alterations of intercellular communication through gap junctional connections are likely contributing factors to the occurrence of atrial fibrillation. Such alterations may be as simple as microfibrosis interrupting gap junctional communication without a change in connexin quantity or distribution or as complex as remodeling of the 3 different connexin isoforms to change the makeup of heteromeric/heterotypic gap junctions. We propose that in future studies designed to elucidate the role of remodeling of intercellular communication, the following issues need to be addressed; 1) regional differences in connexin quantity and location with particular attention to atrial regions that are the source of AF, 2) quantification of structural gap junctions in intercalated disc and lateral sarcolemmal membranes in addition to connexin protein alone, 3) quantification of the phosphorylated state of remodeled connexins to provide an indication as to whether gap junctions are functional, 4) quantification of heterotypic/heteromeric gap junctions and their make up 5) measurements of coupling conductance in atrial cell pairs isolated from fibrillating atria, and 6) correlation of quantitative changes and distribution with measurements of local conduction properties. This appears to be an enormously complex undertaking.

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Figure 1.

Topology of the three atrial connexin proteins. All have four transmembrane domains and an intracellular amino, loop, and carboxyl domain. Areas of red indicate alpha helices while green regions are the beta sheets within each molecule. Conserved prolines are seen in black. Areas of highest divergence are within the cytoplasmic domains of the connexins [43].

	Cx43	Cx40	Cx45
Cx43	Homotypic	Functional Heterotypic	Functional Heterotypic
Cx40	Functional Heterotypic	Homotypic	Functional Heterotypic
Cx45	Functional Heterotypic	Functional Heterotypic	Homotypic

Figure 2.

Table showing the possible combinations which form functional channels. All combinations of Cx40,Cx43, and Cx45 potentially form functional channels within the atrium.