

Review article

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NEW ASPECTS OF PATHOGENESIS OF ATRIAL FIBRILLATION: REMODELING OF INTERCALATED DISCS

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This review deals with the understanding of the role of key factors of ageing, oxidative stress and inflammation, in relation to development of age-related cardiovascular disease, atrial fibrillation. Increased production of reactive oxygen species and systemic inflammation promote cardiac structural and electrophysiologic remodeling that is crucial with respect to development and sustaining of atrial fibrillation. Data suggest that alterations in atrial connexin-43 and/or connexin-40 expression, phosphorylation and distribution affect cell-to-cell electrical coupling and molecular signaling that is proarrhythmogenic. However, studies showing causal relationship in the context of pathogenesis of atrial fibrillation are still scarce. Nevertheless, gap junctional connexin channels are considered as targets for arrhythmia prevention and therapeutic interventions aimed at mitochondria-related reactive oxygen species appear to be challenging. In addition, ageing is accompanied by abnormalities in adhesive junctions that most likely promote asynchronous contraction and arrhythmias. It is consistent with recent data that highlight a new perspective in regulation of intercalated disc function *via* adhesive junctions, fascia adherens and desmosomes. The crosstalk between adhesive and gap junctions is suggested to be implicated in pathogenesis of arrhythmias. On the other hand, modulation of adhesive proteins, N-cadherin and catenin may be promising tool aimed to synchronize heart function. Despite the progress in this field many questions remain to be answered by further research.

Key words: heart, ageing, atrial fibrillation, intercalated disc, oxidative stress, reactive oxygen species, connexin

AGEING AND OXIDATIVE STRESS AS KEY PLAYERS IN THE DEVELOPMENT OF ATRIAL FIBRILLATION

Atrial fibrillation (AF) is characterized by rapid, fragmented, asynchronous electrical activation of the atria resulting in re-entrant waves, atrial contractility rates of 350 to 600 beats per minute and cardiac output reductions of up to 30% in otherwise normal individuals. Generally AF is categorized as lone (i.e., in the absence of cardiopulmonary disease), paroxysmal (i.e., recurrent but terminating spontaneously), persistent (i.e., sustained up to 7 days and requiring cardioversion for termination), or permanent (i.e., long-lasting where cardioversion has failed or not been attempted). In most cases, AF is self-perpetuating and progressive ('AF begets AF'). AF may be triggered by ectopic pacemaker activity arising from increased vagal or sympathetic tone, calcium overload, or sinoatrial dysfunction. In particular, the cardiomyocyte sleeves that extend into the pulmonary veins are a prime source of ectopic electrical activity, and radiofrequency catheter ablation of the pulmonary veins and surgical techniques have been shown to result in apparent elimination or improvement of AF in over 75% of patients (1, 2). Electrophysiological basis of AF initiation, maintenance and progression is discussed in details in recent review (3).

AF is the most common arrhythmia in clinical practice and is becoming an increasing problem worldwide as its prevalence increases. Recently it has been reported that in response to high

atrial rates there are changes in metabolism of bioactive compounds such as sphingolipids in heart ventricles (4). Systemic diseases like diabetes, hypertension, dyslipidemia and ageing are risk factors for AF. Importantly, each of these risk factors is accompanied by an increase in the systemic markers of oxidation. The association of oxidative stress and AF is robust and suggests that AF is possibly a manifestation of a systemic disease (5). Moreover, atrial sources of ROS vary with the duration and substrate of AF and this fact may explain why statins are effective in the primary prevention of AF but not in its management (6).

Serious issue is the prevalence of asymptomatic AF, particularly in elder population. These individuals have a significant risk of embolic stroke and other AF-related complications, including heart failure. Despite the burden of morbidity and mortality burden incurred with AF, there are limited therapeutic options that may improve the outcomes of AF patients. Moreover, these options are associated with significant cases of AF recurrence. Nevertheless, catheter ablation is superior to drug therapy in suppressing AF and improving arrhythmia-based symptoms, quality of life and exercise capacity (1, 2). One possible explanation for the limitations of current therapies is that they do not address effectively or completely the underlying causes of AF (5). Thus, further studies to reveal novel approaches in prevention of AF and its occurrence or recurrence after ablations are warranted.

The human life span has been extended and is expected to increase further with a supplementary prolongation of life expectancy. The exponential increase in mortality rate related to cardiovascular diseases in the geriatric population implies that cardiac ageing itself may be a major risk factor of cardiovascular pathology (7). Age is one of the key risk factors for AF, while genetic factors play a major role when AF occurs at young ages (8). AF is emerging as 'a new epidemic' in cardiovascular disease since its incidence rapidly increases. The lifetime risk of developing AF is about 1 out of 4 for men and women over 50 years of age.

Recently, a novel marker of biological ageing has been emerged - a telomere length. Exposure to chronic stressors is associated with accelerated biological ageing as indicated by reduced leukocyte telomere length (LTL). Importantly, the latter is linked with higher inflammatory biomarkers and excessive production of reactive oxygen species (ROS). Accordingly, shorter LTL was significantly associated with higher C-reactive protein and interleukin-6 (IL-6) (9). Gene-targeting 'knockout' technology has revolutionized the study of oxygen free radicals in aging showing that telomere DNA may be particularly sensitive to oxidative damage (10). Thus ageing likewise systemic diseases is accompanied and accelerated by oxidative stress (11). ROS are constantly generated in cells as a by-product of oxygen metabolism and they are important cell signaling molecules at physiological levels. However, excessive ROS induce an imbalance between the oxidant and antioxidant responses since antioxidant gene expression and activity are affected during ageing as well. Individual genetic variations in the antioxidant defense system may affect oxidative stress and subsequent development of injury (12). ROS also favor accumulation of misfolded proteins that, in turn, further enhances oxidative stress. Recently, misfolded or unfolded proteins have been found to play a role in arrhythmogenesis during human heart failure, and blocking of unfolded proteins has an antiarrhythmic effect (13).

Mitochondrial dynamics and autophagy are progressively impaired over time, contributing to the ageing process. It seems that mitochondrial dysfunction represents a common feature of the ageing and plays a fundamental pathologic role during the ageing process in the heart. This fact is not surprising based on the evidence that the heart relies on extensive energy production to ensure cardiac cycle (7). There has been found a growing mechanistic link between mitochondria dysfunction and arrhythmogenesis (14). The process of ageing is associated with accumulation of damaged proteins and organelles, partially due to the defects in protein quality control systems. Thus, accumulation of dysfunctional and abnormal mitochondria is most likely an important pathophysiological feature of the ageing process associated with overproduction of ROS (7). Moreover, excessive or uncontrolled free radicals production can induce an inflammatory response that is progressing with age and causing low-grade chronic systemic proinflammatory state (15). Similarity of the patterns in ageing and disease suggested a kind of „early ageing“ of diseased subjects or alternatively a „disease-like“ ageing process with respect to particular parameters. The heart is highly sensitive to ageing, which also affects the cardiac response to stress. Taken together, aged heart is prone to develop AF, whereby oxidative stress associated with mitochondria dysfunction is one of the crucial factors. In this context it should be noted that mitochondria-targeted antioxidants rather than general antioxidants or inhibitors of other sources of oxidative stress could prevent AF and its sustaining (14, 16), and most likely to hamper ageing process as well. For details see review Wolke *et al.* (17) on redox-sensitive signaling pathways implicated in the pathogenesis of AF. However, further studies are needed focusing on ROS/RNS as one of the factors implicated in occurrence of AF *via* modulation

of intercalated disc (ID) proteins and particularly Cx channels as suggested in this review.

There are numerous excellent reviews discussing mostly myocardial alterations induced by AF that may be implicated in perpetuation of AF and/or its recurrence after invasive treatment. However, prevention is the best treatment, therefore, in this review we focus on the possible factors and mechanisms facilitating initiation of AF. In this context we consider ageing heart as the most relevant model. Comprehensive understanding of AF pathogenesis is expected to foster the development of improved pharmacological and non-pharmacological therapeutic approaches. It appears that one of the missing puzzles in the mosaic is the elucidation of the role of intercalated disc in the development of AF.

AGEING-RELATED MYOCARDIAL ALTERATIONS FACILITATE THE DEVELOPMENT OF ATRIAL FIBRILLATION

Abnormal calcium handling

Oxidative stress injury, systemic inflammatory state, autonomic dysbalance, neurohormonal activation, including thyroid hormones and renin-angiotensin cascade, were discerned as important modifiers that affect AF susceptibility (2), whereby alterations in myocardial calcium homeostasis might be implicated. ROS associated with glycolytic inhibition and atrial energy deficit that accompanies ageing alters Ca²⁺ handling, causing elevation of [Ca²⁺]_i (18) and consequently facilitates incidence of AF (19, 20) or ventricular fibrillation (VF) in aged heart (21). Furthermore, there is a strong evidence showing that oxidative stress can lead to AF due to oxidized Ca²⁺ and calmodulin-dependent protein kinase II (CAMKII), which promotes sarcoplasmic reticulum Ca²⁺ leak from RyR2 (22). Thus, an important aspect of AF pathophysiology is altered intracellular Ca²⁺ handling mainly due to ROS-induced alterations. This aspect is discussed in recent review (23) focused on central role of Ca²⁺-handling abnormalities and ionic determinants. Taken these facts into consideration, new antiarrhythmic targets to control intracellular calcium handling, such as CAMKII, RyR2 and its associated protein FKBP12.6 (Calstabin), Na/Ca exchanger (NCX) and the late component of the sodium current (I_{Na-Late}) have been proposed (24). In addition, a mechanistic link between the renin angiotensin system activation-induced oxidative stress, intracellular Ca²⁺ handling, and the inducibility of AF helps to explain the antiarrhythmic effects of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (25). Consistent with the concept that AF begets AF, i.e. perpetuation of this arrhythmia, it is likely that aggravation of ROS induced myocardial injury, including abnormal Ca²⁺ handling, is involved (17, 23). Of note, ageing heart is prone to develop Ca²⁺ overload (18), and in addition to Ca²⁺-induced triggered activity, high diastolic Ca²⁺ may inhibit electrical coupling and promote cardiac cell-cell uncoupling as described in the next chapter. Moreover, Ca²⁺ disorders may affect cell-cell adhesion *via* structural alterations of adhesive junctions proteins (19, 21). Taken together, these defects most likely impair synchronized propagation of myocardial excitation-contraction waves. As myocardial disorders are distributed heterogeneously (dispersed) throughout cardiac muscle, they can facilitate electrical instability and disturbances in contraction, hence promoting reentry and AF occurrence.

Structural remodeling

Ageing-related increase of ROS and inflammation promote cardiac structural remodeling (7). Indeed, the heart tends to

become hypertrophic and fibrotic in the elderly human population likewise in animals (26). Hypertrophy of cardiomyocytes is induced in response to mechanical stress (stretch) and activation of signal transduction pathways involving cAMP and/or angiotensin II. Fibrosis is facilitated by the activation of fibroblasts and increased production of extracellular matrix proteins (27, 28). Both procollagen type II and transforming growth factor β (TGF- β) are implicated in fibrogenic effects in the setting of ROS and inflammation (29). Structural remodeling was identified as the main mechanism of AF persistence confirming dominant role of atrial fibrosis (1, 2). Noteworthy, age-related myocardial fibrosis may account for the heterogeneity of electrical conduction promoting AF which itself aggravates electrical remodeling (30). One mechanism of abnormal cardiac conduction is failure of local circuits generated by a propagating action potential to flow adequately through the gap junctions. This may be affected by fibrosis in the extracellular space as this may impair adequate local circuit flow. Fibrosis can promote AF by interrupting fiber bundle continuity and causing local conduction disturbances. Changes in extracellular matrix may include alterations of collagen IV, which is a structural component of cardiac cell membrane (31). Thus, it may affect properties of ion channels and their conductivity. This aspect should be studied more intensively. It has been shown that myocardial conductance is strongly dependent on the topology of gap junctions. In healthy heart, there is a dominant end-to-end distribution of gap junctions at the intercalated disc and less frequent at lateral borders. However, when the side-to-side gap junctions are reduced or not functional (uncoupling), the effective transverse propagation of conduction velocity decreases. Consequently, conduction is changed from uniform anisotropy to non-uniform (32). High-resolution optical mapping in atria revealed that preferential direction of conduction block changes from longitudinal to transverse with increasing age. A zigzag propagation based on lateral uncoupling would predispose the elderly to multiple reentry and a higher incidence of AF. (33).

In this context, it should be noted that excess of extracellular matrix proteins in aged or diseased heart form electrically insulating laminae, which force impulse conduction to follow tortuous pathways resulting in slow overall conduction, discontinuous conduction, and conduction blocks (28, 29). The severity of these conduction disturbances is dependent on the amount and specific spatial distribution of excessive extracellular matrix deposition within the myocardium, which is categorized as interstitial, compact, diffuse, and patchy fibrosis (28, 34). In particular, diffuse or patchy fibrosis has been related to increased propensity of the diseased or aged heart to AF (19, 20). An important issue with respect to fibrotic remodeling of the myocardium is related to cell types responsible for excess of extracellular matrix proteins production. It has been demonstrated that activation of fibroblasts is associated with appearance of myofibroblasts, and these substantially contribute to fibrogenesis, which is mediated mostly by TGF- β and angiotensin II (35).

Interestingly, *in vitro* studies suggest that paracrine fibroblast-cardiomyocyte interactions can cause alterations in cardiomyocyte electrophysiology and hence, arrhythmogenic implication of fibroblast-myocyte interactions has been discussed recently (28). The crosstalk between fibroblasts and cardiomyocytes, contributing to myocardial structural and electrical remodeling appears to be involved in the pathogenesis of various heart diseases as well as rhythm disorders (36). However, further studies are urgently needed to be addressed to this issue because myocardial fibrosis is a key factor contributing to both malignant arrhythmias and heart failure.

Taken together it can be concluded that changes in cardiac conduction due to myocardial remodeling are attributed to changes in three morphologic parameters: (1) cardiomyocyte geometry (size and shape), (2) interstitial space (size and localization) and (3) gap junctions (GJ distribution and conductivity). Based on the above mentioned arrhythmogenic potential of the fibrotic myocardium, therapeutic efforts to counteract this condition by either suppressing of fibroblast activation or by interfering with collagen degradation are substantial. In this regard it would be interesting to pay attention to microRNAs which are implicated in the process of myocardial fibrosis, such as miR-29b (37) and miR-21 (38).

Impairment of electrical coupling

The coordinated contraction of the heart is dependent on the proper mechanical and electrical coupling of cardiomyocytes. To achieve this goal, cardiomyocytes are connected end-to-end by specialized structure called intercalated disc (ID). Cardiomyocytes are electrically coupled through GJ that are made up of proteins named connexin (Cx) family and they are organized into intercellular Cx channels. Cx43 and Cx40 are primary components of atrial myocytes GJ that are dominantly localized at the ID, while lateral contacts between cardiomyocytes (*Fig. 1B*) are less frequent. Different to atria the cardiomyocytes in the healthy heart ventricles express Cx43 only that is localized mainly at the ID-related GJs, and lateral junctions are scarce. The major role of GJ in the myocardium is to enable the rapid and coordinated electrical excitation, a prerequisite for normal rhythmic function (39, 40). Conduction velocity is known to be determined by cell-to-cell coupling *via* GJ Cx40/Cx43 channels and voltage-gated Nav 1.5 channel (41). In the condition of oxidative stress, alterations of Nav 1.5 and Cx40/Cx43 channels can contribute to electric remodeling and form the AF substrate. As already mentioned, alterations in extracellular matrix and fibrosis deteriorate atrial side-to-side electrical coupling (*Fig. 1A*) resulting in conduction disturbances (32). Such disorders are enhanced by abnormal expression of Cx40 and/or Cx43 at the ID (42). Interestingly, reduced Cx43 expression and coupling can even contribute to excessive collagen deposition due to enhanced fibroblast activity, leading to increased conduction inhomogeneity and proarrhythmia demonstrated in aged mice ventricles (43). These changes should be explored in atria as well.

Advanced age in humans is associated with an increase in atrial Cx40 expression and a corresponding increase in GJ resistivity suggesting mechanistic explanation for changing of atrial conduction and age-related pro-arrhythmic tendency (42). Moreover, a germ-line mutation in the *GJA5* gene (which encodes Cx40) resulting in a truncated Cx40 (Q49X) is capable to impair the gap junction distribution and the function of key atrial Cx predisposing the heart to AF (44).

Animal rodent models have shown down-regulation of atrial Cx43 on both transcriptional and post-transcriptional levels (45, 46). It may account for observed increased propensity of aged guinea pig hearts to AF (19, 47). Of note, c-Jun N-terminal kinase activation has been implicated in Cx43 reduction and development of AF in aged rabbits (46). In addition, stress, ageing or diseases (which all increase the risk of AF) are accompanied by an excessive ROS production (48) that can accelerate Cx43 degradation (49). This process includes internalization of GJ that can be revealed in electron microscope by the presence of intracellular annular GJ (41) as they are found in aged heart prone to AF (19) or VF (50). In addition, oxidative stress causes limited microtubule interaction with adherens junctions at ID and reduces connexon delivery to the membrane and GJ coupling and thereby slows the spatial spread of

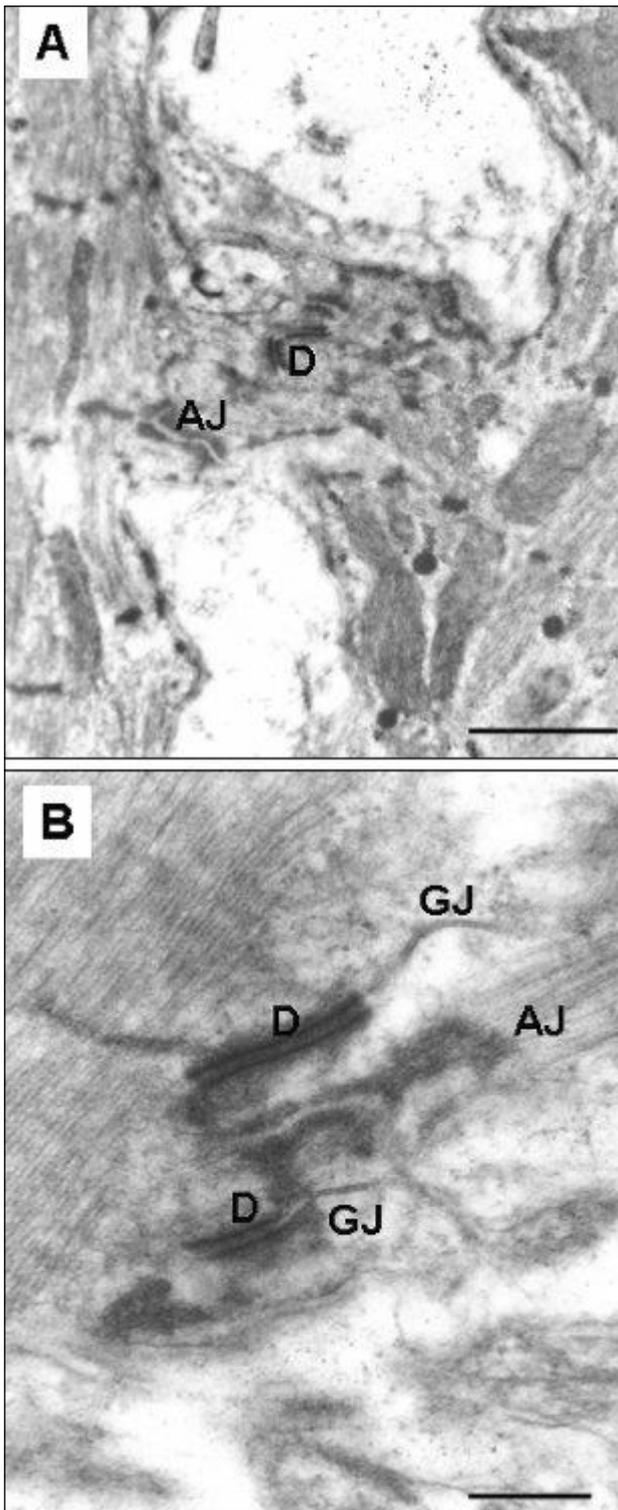


Fig. 1. Electron microscopic images demonstrate lateral, side-to-side type of intercellular connections in atria of old (A) and young (B) guinea pig hearts. Note lost of gap junctions (GJ) as well as degradation of adherent junctions (AJ) and desmosomes (D) in old comparing to well organized junctions in young animals. Bar - 1 μ m.

excitation (49). Moreover, remodeling of microtubule-associated mechanical junction proteins promotes cardiac Cx43 lateralization (51-53), which affects anisotropy of conduction that may facilitate reentry arrhythmias (32).

Furthermore, circulating cytokines during inflammation can modulate Cx43 expression in the heart (54). Interestingly, inflammation due to viral myocarditis has been shown to repress ventricular Cx43 expression *via* microRNA-1 (55). In turn, down-regulation of this microRNA in response to chest irradiation resulted in up-regulation of myocardial Cx43 (56) indicating modulation of Cx43 expression by microRNA-1. The latter can regulate cardiac arrhythmogenic potential by targeting of both genes GJA1 (which encodes Cx43) and KCNJ2 (which encodes the K⁺ channel subunit Kir2.1 (57). However, whether and how microRNA-1 affects Cx43 or Cx40 and Kir2.1 in the atria is not known.

Number of studies indicate that ageing-related changes in spatial organization of GJ and/or myocardial level of cardiac Cx43 or Cx40 are intimately associated with arrhythmogenesis promoting AF (19, 32, 33, 42, 44, 47, 50, 58-60). AF itself enhances these alterations that may contribute to its persistence (61-64). The involvement of changes in myocardial gap junctions mediated communication as a possible cause of AF is discussed in details in a comprehensive review (60).

An important issue that should be elucidated more in the context of development as well as persistence of AF, is Cx protein phosphorylation because it is a prerequisite for Cx channels function. Different to the wide spectrum of protein kinases implicated in Cx43 phosphorylation in ventricles, little is known about their actions in atria. Moreover, there are no data about Cx40 phosphorylation, although it has been found that it exist in phosphorylated form (65).

It should be also noted that unitary conductance of both Cx40 and Cx43 is voltage- and pH-dependent. Thus, under conditions of atrial hypoxia and subsequent intracellular acidification, loss of coupling may occur, which is a form of functional remodeling. Moreover, as already mentioned ageing is accompanied by alterations in Ca²⁺ handling causing elevation of [Ca²⁺]_i. The latter is known to inhibit Cx43 channels and induce cardiac cell-to-cell uncoupling (66). In addition, it should be taken into consideration that there are numerous compounds that can acutely modulate electrical and metabolic signal propagation as reviewed previously (67). However, most studies on regulators have been focused on Cx43 in heart ventricles. Therefore, to explore modulation of atrial Cx40 and/or Cx43-mediated electrical coupling and communication by endogenous or exogenous compounds is desirable. This approach is supported by recent clinical and experimental studies, including ours, indicating that the so called "upstream" therapy is efficient to prevent the occurrence malignant ventricular arrhythmias by targeting arrhythmogenic factors and substrate (68). Accordingly, additional (pleiotropic) effects of currently used drugs to treat hypertension, dyslipidemia or diabetes, in particular, antioxidant, anti-inflammatory and anti-fibrotic, most likely underlie their antiarrhythmic efficacy. Consistent with it, cardioprotective n-3 polyunsaturated fatty acids have been reported to alter expression of fibrotic and hypertrophic genes in dog model of atrial cardiomyopathy (69).

Taken together, age- or disease-related changes of GJ connexin channels are expected to be linked with functional alterations since the structure-function relationship is generally accepted. Data suggest that alterations in atrial Cx43 and/or Cx40 expression and/or distribution, indeed, affect cell-to-cell electrical coupling and molecular signals propagation. However, due to methodological limitations when evaluating functional impact of Cx43/Cx40 alterations, the causal relationship is still insufficient (60). Nevertheless, myocardial connexins are considered to play an important role in arrhythmias development. It challenges for further research aimed to reveal targeted approaches to modulate cell-to-cell communication and fight AF.

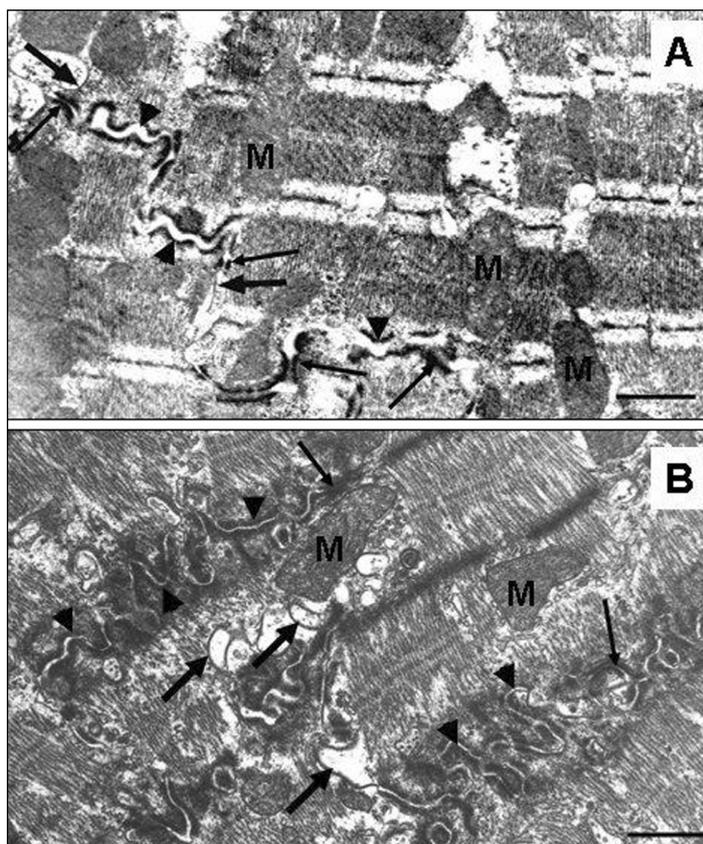


Fig. 2. Electron microscopic images of intercalated disc related, end-to-end type of intercellular connections in atria of old (A) and young (B) guinea pig heart. Note short or internalized (annular profile) gap junctions (thick arrows) as well as widened extracellular space at the “flattened” adherens junctions (arrowheads) in old comparing to numerous GJ and narrow extracellular space at the “plicate” AJ in young animals. Desmosomes - thin arrows, M - mitochondria. Bar - 1 μ m.

Impairment of mechanical coupling

With respect to myocardial remodeling of intercellular coupling in relation to arrhythmogenesis, more attention is paid to GJ compared to adhesive junctions. However, it is generally accepted that three distinct junctional complexes of ID (*Fig. 2A* and *2B*), i.e. fascia adherens junctions (AJ), desmosomes (D) and GJ are required for mechano-electrical coupling and synchronized contraction in the heart. Of note, occurrence of GJ precedes development of AJ and D, as indicate postnatal studies of human heart (70) and animal studies dealing with age- or disease-related myocardial structural and GJ remodeling (43, 48). Importantly, as revealed by electron microscopy (*Fig. 2A* and *2B*), there is a difference in ID features between old and young hearts. Atrial myocytes of old heart exhibit less GJ and widened extracellular space at the AJ compared to those in young heart. AJ and D provide mechanical attachment between cardiomyocytes by anchoring the actin filaments of myofibrils and intermediate cytoskeleton filaments at the ID respectively. The AJ consists of N-cadherins that mediate Ca-dependent cell-cell adhesion. According to the classical description, the ID, (i.e. AJ, D and GJ) mediate mechanical and electrical coupling of cardiomyocytes.

The organization of ID is, however, more complex as shown in *Fig. 3* (see in details 52, 53). Accordingly, AJ adhesive activity is dependent on cytosolic proteins called catenins that link the cadherin cytoplasmic domain to the actin myofilaments. Catenins can be divided into two families, the armandillo domain containing catenins: cadherin β -catenin, λ -catenin and p120ctn, that directly bind to the cadherin, and the vinculin-homology domain containing catenins: α E-catenin and α T-catenin. From adhering proteins, β -catenin, λ -catenin, are also known as plakoglobin. Desmosomes consist of three families of proteins,

desmosomal cadherins, armadillo proteins and plakins. Desmosomal cadherins, desmogelins and desmocollins form the extracellular connections. The cytoplasmic tails of desmosomal cadherins bind to the armandillo protein plakoglobin and plakophilin, which bind to the plakin proteins, i.e. plakophilin-2. Moreover, as recently reviewed (53), the morphological and molecular studies indicate that AJ and D components are capable to mix together resulting in ‘hybrid adhering junctions’ or ‘area composita’ (*Fig. 3*) that may regulate ID function. Accordingly, desmosomal plakophilin-2 directly interacts with AJ protein α T-catenin, providing a new molecular link between the cadherin-catenin complex and desmosome.

Importantly, plakoglobin (λ -catenin), the only junctional component found in both AJ and D, seems to play a critical role in regulation of cell-cell adhesion. Mutations in the human gene encoding desmosomal protein, plakophilin-2, have been identified to cause inherited heart disease, arrhythmogenic right ventricular cardiomyopathy (71). In addition, it is associated with reduction of Cx43 content, a redistribution of Cx43 from the ID to intracellular pools and remodeling of desmin and desmoplakin (72). Furthermore, defects in desmoplakin (Carvajal syndrome) and plakoglobin (Naxos disease) result in substantial abnormalities in the ultrastructure of ID and a reduction in the size and abundance of GJ plaques (73, 74). Destabilization of AJ resulting in disruption of cell-cell mechanical contacts and the disassembly of GJ has been detected in cultured adult rat cardiomyocytes with dominant negative suppression of N-cadherin (75). Furthermore, N-cadherin CKO hearts exhibit an increase in dephosphorylated Cx43, consistent with less functional GJ at the sarcolemma associated with slowing of conduction velocity (76). Of note, reduction in epicardial conduction velocity is similar to that observed in the Cx43 CKO mice, which also die from

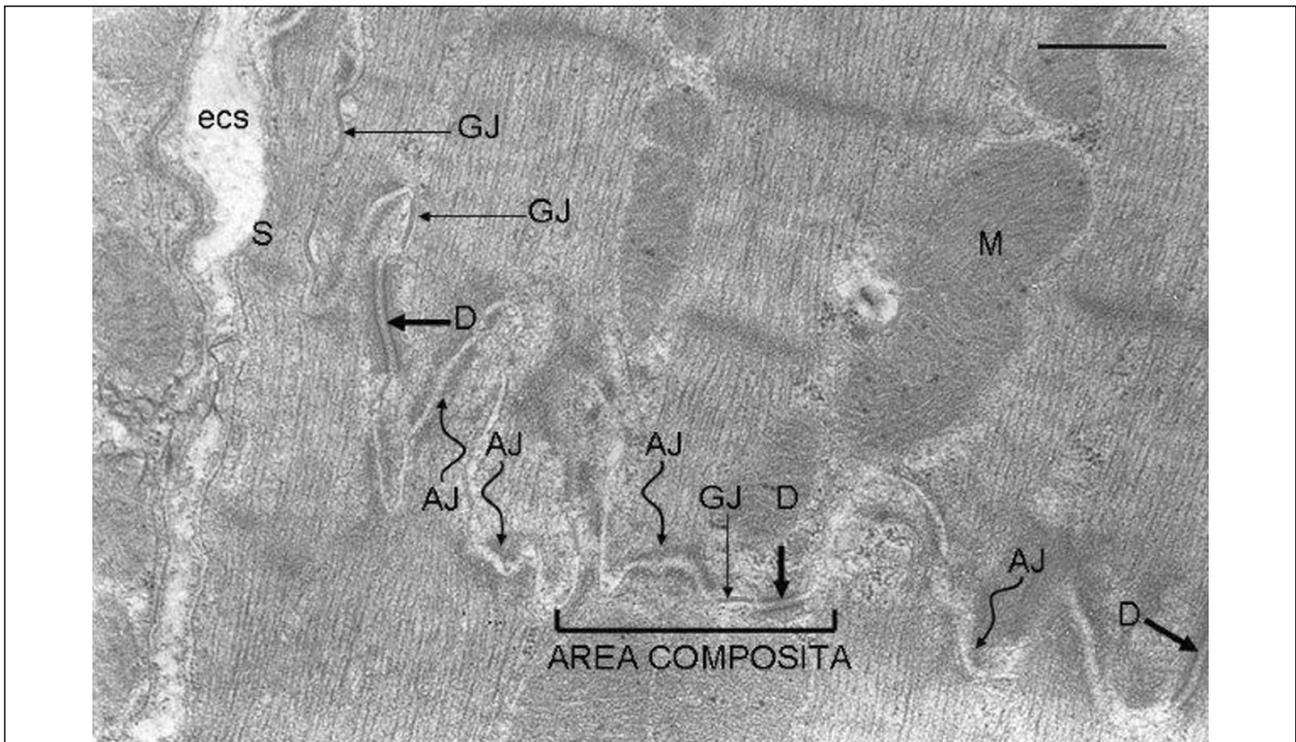


Fig. 3. Representative electron microscopic image demonstrates typical ID-related intercellular junctions and mixed type of junction in myocardium of adult guinea pig heart. Intercalated disc (ID) contains three different intercellular junctions: fascia adherentes junctions (AJ), desmosome (D) and gap junctions (GJ) that are composed of specific proteins and associated proteins (in italics) to fulfill proper ID function. Area composita is mixed type junction that may play role in arrhythmogenesis as discussed recently.

Adherens junction: N-cadherin anchors actin myofilaments, *α T-catenin*, *α E-catenin*, *β -catenin*, *λ -catenin* (*plakoglobin*).

Desmosome: desmosomal cadherins, desmoglein-2 and desmocollin-2 anchor desmin filaments, *desmoplakin*, *plakoglobin*, *plakophilin-2*.

Gap junction: Cx43 or Cx40 form channels for electrical and molecular signals propagation.

Bar - 1 μ m.

spontaneous lethal arrhythmias (77). Loss of α T-catenin alters the hybrid adhering junctions that is associated with reduced ID-related Cx43 (including co-localization with N-cadherin) and its lateralization that increased propensity of the heart to ventricular arrhythmias (78).

In addition to electrical and mechanical junctions, the ID contains a variety of ion channels. One of them, the voltage-gated cardiac sodium channel Nav1.5, is responsible for the rapid upstroke of the cardiac action potential and in that respect, together with gap junctions, is critically important for maintenance of impulse propagation. Postnatal human studies revealed that Nav1.5 channels are localized in ID much earlier than gap junction channels (70). Moreover, studies using desmoglein-2 mutant mice indicate that ID abnormalities are linked with reduced Na^+ current density and conduction slowing prior to cardiomyopathic changes (79). Noteworthy, ankyrin-G-dependent protein platform links Nav 1.5 channels with broader intercalated disc signaling/structural nodes, as *in vivo* ankyrin-G loss results in remodeling of plakophilin-2. Ankyrin-G associates with Nav1.5 and recruits the channel to the ID while loss of ankyrin-G results in defects in cardiac excitability and arrhythmia (80). Based on the *in vivo* link between ankyrin-G and plakophilin-2, it will be important to further investigate the mechanistic roles of ankyrin-G in regulation of intermediate filaments at the ID. Remodeling of the actin cytoskeleton including downregulation of the actin-binding scaffold protein cortactin is likely responsible for decreased voltage-gated potassium channel Kv1.5 in the N-cadherin CKO model

facilitating arrhythmias (81). Further studies will be necessary to determine whether trafficking of Kv1.5 to the ID is dependent on N-cadherin-mediated cytoskeletal organization.

Altogether, data suggest the crosstalk among different junctions and their implication in the pathogenesis of arrhythmogenic diseases. However, exact consequences of such alterations on cardiac electrophysiology are not elucidated. N-cadherin/catenin complex is believed to be a master regulator of ID function, whereby disruption of α T-catenin/plakophilin-2 complex with actin destabilizes ID (53). Disruption of AJ due to higher serum levels of p-cresol (in chronic kidney disease) was linked with dissociation of p120 catenin from N-cadherin, which might be related to asynchronous contraction (82).

In the context of arrhythmogenesis, the role of zonula occludens proteins (localized at cytoplasmic junctional sites and interacting with α -catenin and p120 catenin) providing the structural basis for the assembly of multiprotein complexes (including Cx) and a link with filamentous cytoskeleton (83, 84) should be further elucidated. Recent data indicate that vinculin, another actin-binding scaffold protein, and ZO-1 together regulate GJs in the heart (85).

Taken into consideration all the mentioned facts it appears that both mechanical and electrical coupling is important for maintenance of synchronized contraction in the heart. Moreover, Ca^{2+} handling disorders resulting in alterations in $[\text{Ca}^{2+}]_i$ may be implicated in ID remodeling, however, this needs to be explored. Consistent with evidence pointing to the role of AJ in arrhythmogenesis our previous ultrastructural studies (19, 50, 86)

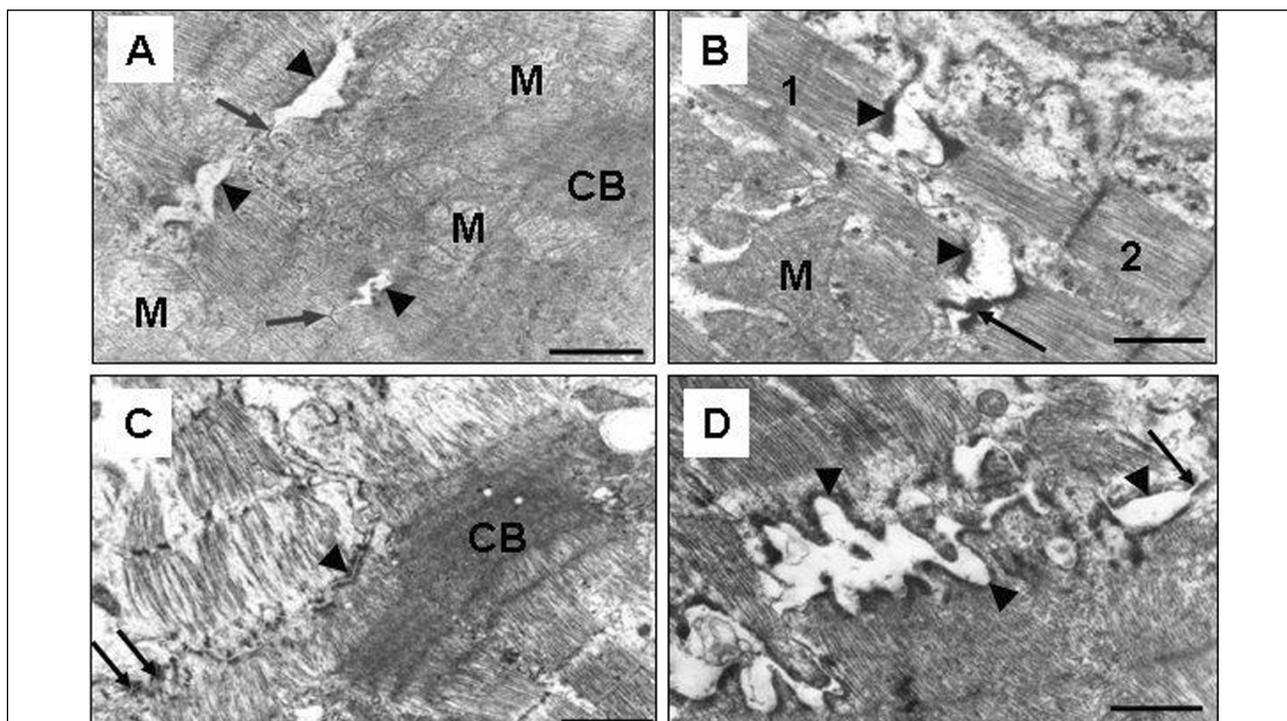


Fig. 4. Electron microscopic images showing intercellular junctions in atria of old (A, B) and young (C) guinea pig heart. Note short gap junctions (thick arrows) and dramatic widening of extracellular space at the adherens junctions (arrowheads) that is present already after 5 – 10 minutes of burst stimulation to induce AF in isolated heart model (A, B). In contrast, cell-to cell adhesion is still preserved in young animals (C) despite much prolonged pacing (> 1 hour). Moreover, fast pacing induced Ca^{2+} overload, as indicated by hyper-contraction of myofibrils (C, B), occurred earlier in old comparing to young guinea pig atria. Interestingly, that similar feature of dehiscence of adhesive junctions is seen in the heart subjected to Ca^{2+} deficient perfusion (D). However, such changes are uniform throughout myocardium unlike burst pacing-induced widening of AJ that is non-uniform. M - mitochondria. Bar - 1 μ m.

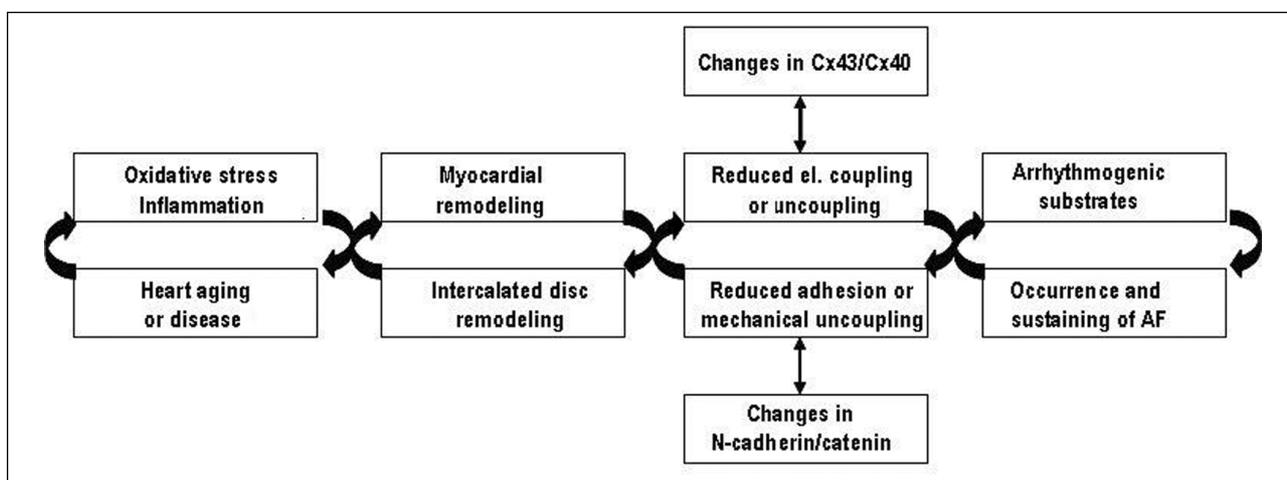


Fig. 5. Scheme depicts possible factors and alterations associated with age-related development and persistence of atrial fibrillation. Note, that both electrical and mechanical coupling are suggested to be implicated in this process according to available experimental studies.

suggest that age- or disease-related remodeling of ID is associated with widening of intercellular space at the AJ (Fig. 2A). These junctions anchor actin myofilaments, hence they are important for contractile force propagation between neighboring cardiomyocytes. AJ and desmosomes ensure mechanical coupling and integrity of myocardial tissue. However, when mechanical coupling is impaired together with Cx43 alterations, it may affect myocardial electro-mechanical synchronization and contribute to the development of arrhythmogenic substrate

facilitating AF (19, 45, 47, 50). Of note, age-related weakened adhesion at AJ has been shown to aggravate by burst stimulation of the heart to induce AF, while young heart is more resistant to this injury (Fig. 4A-4C). Dehiscence of AJ was accompanied by asynchronous contraction as indicated by non-uniform sarcomere shortening between neighboring cardiomyocytes. Accordingly, we hypothesize (as depicted on Fig. 5) that both mechanical and electrical uncoupling is highly arrhythmogenic. It is supported by the findings that ID abnormalities, including intercellular space

widening (at the level of desmosome/adherens junctions), and concomitant reduction in Na^+ current density increases arrhythmia susceptibility that precedes the onset of fibrosis or necrosis during early stage of arrhythmogenic right ventricular cardiomyopathy (79). Noteworthy, burst pacing-induced dehiscence of AJ is not uniform throughout myocardium. Most likely such heterogeneity in mechanical uncoupling promotes asynchronous contraction since there are no arrhythmias when widening of intercellular space is uniform (Fig. 4D) as it is in the case of Ca^{2+} -paradox injury model (86).

The question arises how does this age-related widening of AJ occur? Cell-cell adhesion by N-cadherins is Ca^{2+} -dependent, thus it can be expected that deficiency of external Ca^{2+} in the heart with altered Ca^{2+} -handling might be involved in this process. Likewise, it has been seen in the conditions of Ca^{2+} -depletion (86). It is also likely that the N-cad/catenin complex and its associated filaments will be altered during cardiac remodeling due to ageing. The paramount importance of N-cadherin in the regulation of ID structure and function in the normal heart is well established (53). Another factor implicated in adhesive junctions' dysfunction might be matrix metalloproteinases (MMPs). Advancing age itself is associated with significant extracellular matrix remodeling, whereby MMPs and their inhibitors (TIMPs) constitute one of the important pathways of proteolysis, which degrade extracellular and basement membrane proteins, such as collagen IV. Plasma MMP-2 levels have been shown to be increased in humans with aging (27) and its atrial tissue expression was up-regulated in old guinea pig heart (47).

New approaches will be necessary to investigate the changes in N-cadherin function during ageing or disease progression, especially the effects of ROS and inflammation. The N-cad haploinsufficiency model may be useful in this regard, since mice are healthy and show only signs of arrhythmia after programmed stimulation (53). It appears that the topic is more complicated as the recent discovery of 'hybrid adhering junction' or 'area composita' in the mammalian heart suggests. Nevertheless, it would have important implications for us to understand the molecular mechanisms underlying arrhythmias and perhaps heart diseases. The exact mechanisms by which adhesion proteins influence connexon trafficking, channel assembly and/or stability at the ID cannot be fully understood until the functional interdependence of the junctional proteins is known. Recent data indicate that oxidative stress can disrupt Cx43 interactions critical for connexon transport to the ID (49). Most proteins such as Cx43 are capable of interacting with multiple proteins and thus have the potential to form large macromolecular complexes. In this context, it will be of interest to explore implication of various protein kinases, which are responsible for connexin phosphorylation in both atrial and ventricular myocardium.

Age-related atrial structural and electrophysiologic remodeling facilitated by oxidative stress is a time-dependent maladaptive process directed at preserving cardiomyocyte function in the presence of external stressors. Consequently, electrical uncoupling and slow conduction (dominantly attributed to Cx43, Cx40 channels alterations) predispose the heart to AF and are aggravated by persistence of AF. In addition, alterations in adherens junctions highlight the importance of understanding of the crosstalk between the junctional proteins of ID, i.e. adhesive junctions and gap junctions and their implication in asynchronous contraction and arrhythmogenesis.

Recent data highlight a new perspective in regulation of intercalated disc function via adhesive junctions and desmosomes. The crosstalk among adhesive and gap junctions is suggested to be implicated in pathogenesis of arrhythmias. Despite the progress in this field, there are many questions outlined in this review that

should be answered by further research. Mainly, how intercalated disc-related changes affect electrical properties of the heart muscle and its susceptibility to malignant arrhythmias.

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