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Review

Electrophysiological mechanisms underlying the initiation of atrial arrhythmia in genetically modified murine hearts

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ABSTRACT

Atrial fibrillation (AF) is an important and poorly understood condition whose incidence increases with age and certain ion channel mutations. We compare evidence concerning mechanisms for its initiation, from genetically modified murine hearts. *Arrhythmic substrate* has been attributed to abnormal action potential (AP) recovery altering AP duration (APD) and effective refractory period (AERP), and compromised AP propagation reducing conduction velocity (θ). These abnormalities increase the APD/AERP ratio and decrease AP wavelength (λ). *Triggering* is attributed to early and/or delayed after-depolarisations produced by abnormal Ca^{2+} homeostasis. *Scn5a*$^{+/-}$ atria with loss of cardiac Na^{+} channel (Nav1.5) function show reduced θ. Young but not aged *Scn5a*$^{+/-}$ atria additionally show atrial arrhythmias accompanied by prolonged APDs despite normal AERPs. In contrast, aged but not young atria from gain of Nav1.5 function, *Scn5a*$^{+/\Delta KPQ}$, hearts show atrial arrhythmias accompanied by prolonged APDs, shortened AERPs, reduced θ, and reduced Nav1.5 expression. Young *Scn5a*$^{+/-}$ and aged *Scn5a*$^{+/\Delta KPQ}$ thus shared arrhythmic phenotypes resulting from substrate produced by shortened λ and increased APD/AERP ratio. Finally, both heterozygotic and homozygotic ryanodine receptor type 2 (*RyR2)-P2328S* atria with gain of Ca^{2+}-release channel-RyR2 function show normal APD and AERP, but reduced θ and therefore reduced λ but unchanged APD/AERP ratio, and arrhythmic substrate following extrasystolic and isoproterenol challenge. Homozygotic but not heterozygotic *RyR2-P2328S* show spontaneous DADs triggering arrhythmia. These comparisons lead to a simple scheme for the initiation and perpetuation of AF. Once established, chronic AF leads to the subsequent, previously established, electrophysiological and anatomical remodelling further exacerbating arrhythmic tendency.

KEYWORDS: atrial fibrillation, sodium channels, ryanodine receptors, action potential, conduction velocity, wavelength

INTRODUCTION

Atrial arrhythmias most frequently manifest as atrial fibrillation (AF), the most clinically common sustained cardiac arrhythmia with a 2% prevalence in the general population [1]. AF is assuming increasing clinical importance [2, 3]. It is a costly public health problem in ageing western populations, with prevalence rising from 0.2% at age 45-54 y to 8% at age 75 y and over [1, 4]. It cost the National Health Service £243.9 million in 1995 accounting for ~0.62% expenditure. Other hospitalisations associated with a secondary coding of AF cost a further £241 million.

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Furthermore, one third to one half of all AF patients may be undiagnosed. Finally, AF predisposes to both cardiac and cerebrovascular morbidity and mortality [5, 6], increasing risks of stroke fivefold [7, 8]. With aging populations and improved healthcare extending human longevity, its prevalence is likely to increase dramatically over the next few decades.

AF shows variable patterns of clinical presentation often associated with advanced age and/or structural heart disease [9]. Genetically normal AF patients show a median age of onset of 75 y with ~70% of such patients falling between 65 and 85 y [10]. Furthermore, age and genetic factors may together influence the onset of AF. Genetic conditions, particularly those involving ion channel abnormalities have been implicated in early onset, particularly lone AF [11, 12, 13, 14, 15, 16, 17]. Available therapeutic approaches are only moderately effective often entailing significant risks of side-effects that may themselves be pro-arrhythmic. All these factors make understanding of atrial arrhythmias and the development of novel therapeutic approaches based on their mechanisms a matter of critical importance [18, 19].

Physiological mechanisms underlying AF are complex and poorly understood [20]. This review relates atrial arrhythmogenesis to electrophysiological properties bearing on action potential (AP) initiation and recovery, and to abnormal, triggered activity. It first summarizes the major ion channel processes involved in atrial activation, recovery, conduction and excitation-contraction coupling. It next relates alterations in AP initiation, AP propagation, ion channel expression, and changes in some of these effects with age, to initiation of atrial arrhythmia that would in turn lead to the electrophysiological remodelling of chronic AF. It then reviews experimental evidence from genetic murine exemplars of ion channel dysfunction used to model the initiation of atrial arrhythmias. These include models showing altered gap junction (Cx40 and Cx43) function compromising spread of excitation, loss and gain of Na^+ channel (Nav1.5) or subunit (Navβ3) function altering cell excitability, and abnormal ryanodine receptor (RyR2) characteristics modifying Ca^{2+} homeostasis. These alter not only cell excitability but have also recently been shown to modify AP propagation. This comparative approach of several genotypes implicated a number of common strategic features that could be associated with atrial arrhythmic predisposition.

Normal atrial activity

The periodic cycles of atrial and ventricular activity required for cardiac contraction are initiated by the sino-atrial node (SAN). Each cycle depends upon a wave of propagation of AP excitation followed by its recovery. The waveform of the ventricular AP has classically been divided into 4 phases (Figure 1). The AP begins with a rapid phase 0 depolarization that depends on a rapidly developing Na^+ current (I_{Na}) attributable to Nav1.5 activation. The initial phase 1 repolarization that follows results from Nav1.5 inactivation and transient outward (I_{to}) K^+ current activation. The phase 2 plateau reflects activation of voltage-gated, dihydropyridine-sensitive L-type Ca^{2+} currents (I_{Ca}). Phase 3 repolarization reflects opening of delayed rectifier I_{Kr} and I_{Ks}, and inwardly rectifying I_{K1}, K^+ channels which restore the membrane potential to the resting potential (phase 4). Nav1.5 channels then recover from refractoriness thereby permitting a fresh excitation cycle. Atrial resting potentials are more depolarized than ventricular resting potentials, mainly reflecting a smaller $I_{K1.}$ In contrast to ventricular APs, atrial APs are triangular with more prominent phase 1 owing to a larger I_{to}, and specifically expressed ultra-rapid delayed rectifier K^+ currents, I_{Kur}, and acetylcholine-activated K^+ currents, $I_{K–Ach}$. Together these result in a less prominent plateau phase 2. Phase 3 repolarization is more prolonged reflecting smaller I_{Kr}, I_{Ks} and I_{K1} currents [21, 22].

Extracellular Ca^{2+} entry into the cytosol mediated by I_{Ca} during phase 2 initiates the release of sarcoplasmic reticular (SR) Ca^{2+} through intracellular RyR2-Ca^{2+} channels by Ca^{2+}-induced Ca^{2+} release (CICR). In ventricular cells APs propagate into transverse tubules containing the L-type Ca^{2+} channels (Figure 2). The smaller atrial cells show much less developed transverse tubules, particularly in small mammals such as the mouse. Nevertheless, L-type Ca^{2+} channels are

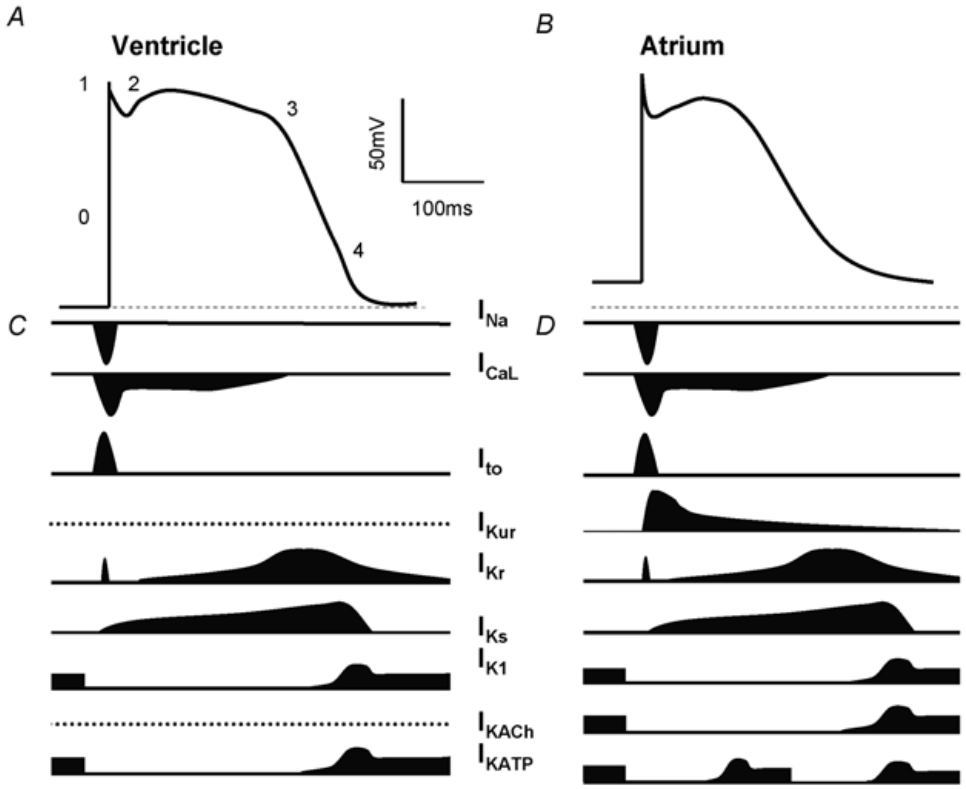

Figure 1. The ventricular and atrial action potential waveforms and the underlying ion currents. Comparison of typical action potential shapes (A, B) in human ventricular (A) and atrial tissue (B) and of the timecourses of their underlying ionic currents (C, D) plotted against time. Currents labeled with dashed lines are not present in the ventricles and specific to atrial myocardium.

expressed on the cell surface and come into close proximity with SR junctional elements close to the surface membrane that contain RyR2s. Their Ca^{2+} release then initiates an inward, centripetal, propagation of a wave of CICR by cytoplasmic SR that also contains RyR2s [23, 24, 25]. This in turn activates troponin leading to contractile activation [26]. Finally AP recovery is associated with Ca^{2+} transport from cytosol to SR by sarcoplasmic reticular Ca^{2+}-ATPase (SERCA) and to the extracellular space by Na^{+}-Ca^{2+} exchange (NCX).

Propagation of these events between cells depends upon electrical, gap junction, conduction of local circuit currents generated by the Na^{+} channel activation [27, 28, 29, 30]. Gap junctions are transmembrane channels made up of contacting hemichannels (connexons) each comprising 6 connexin (Cx) subunits surrounding an aqueous pore. The principal atrial and ventricular connexin isoforms are respectively Cx40 and Cx43; Cx43 is also found in the atria [31, 32, 33, 34, 35].

Initiation of atrial arrhythmia

Disruption of this sequence of physiological events results in the breakdown of the normal atrial rhythm, which may in turn lead to arrhythmia. This disruption classically has been attributed to substrate and/or triggering events [36, 37]. Triggering has been attributed to extrasystolic beats resulting from early (EADs) and/or delayed after depolarisations (DADs). EADs result from membrane potential oscillations late in the AP timecourse produced by Ca^{2+} current reactivation that in turn initiate subsequent, premature, APs, in the event that Nav1.5 channels have then recovered from refractoriness. The resulting further depolarizing wavefront then results in a triggered beat. DADs occur following full repolarization, and can result

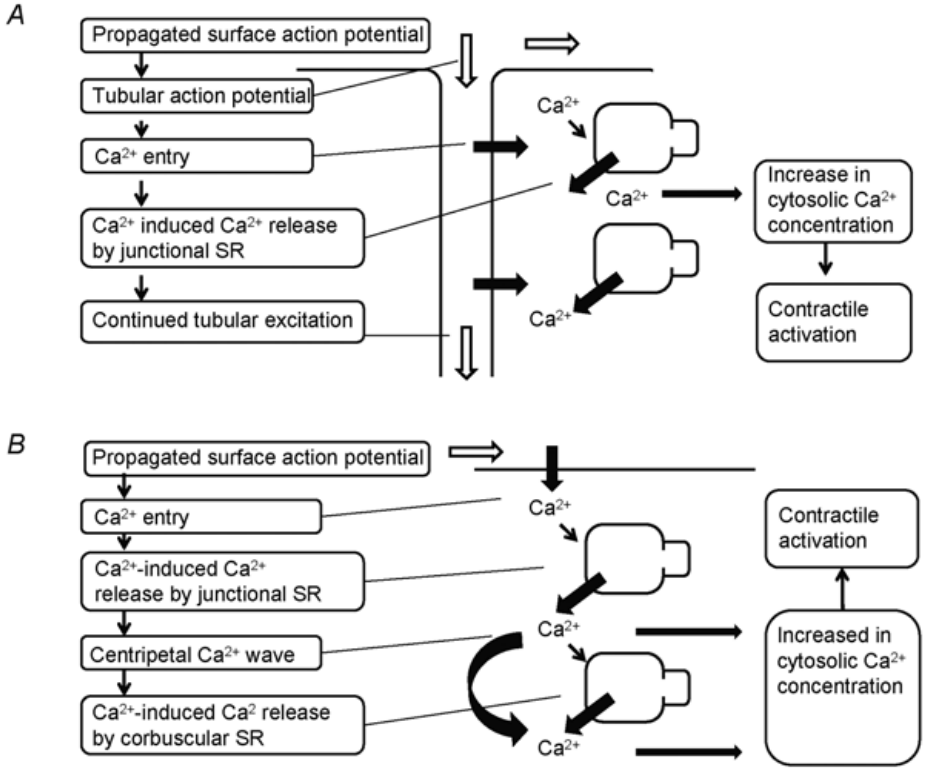

Figure 2. Ca^{2+} signalling mechanisms in ventricular and atrial myocytes leading to myofilament contraction. Mechanism of Ca^{2+} induced Ca^{2+} release (CICR) in ventricular (A) and atrial (B) myocytes. An action potential activates voltage-dependent L-type Ca^{2+} channels, leading to a small Ca^{2+} influx. This opens sarcoplasmic reticular RyR2 channels causing SR Ca^{2+} release through CICR and muscle contraction. Whereas ventricular myocytes (A) show a tubular action potential propagation in which action potential-mediated depolarisation results in CICR close to the tubular membrane, (B) atrial myocytes show less developed T-tubules and so surface action potentials activates a CICR in SR close to the membrane surface that then triggers a centripetal wave of CICR into the fibre interior [23].

from increased release of intracellularly stored SR Ca^{2+} in turn increasing electrogenic NCX activity. In the atria, triggering also can originate from the pulmonary or the superior caval veins [38].

Arrhythmic substrate can arise from abnormalities in AP generation with consequent alterations in conduction velocity (θ). It can also result from abnormalities resulting in spatial heterogeneities in AP recovery which alters action potential duration (APD) and/or shortens atrial effective refractory period (AERP). Together these make up the AP wavelength (λ) given by the product of the excitation parameter, θ, and the recovery parameter, either APD or AERP. This in turn defines the spatial extent of excitation by the travelling wave. Furthermore, the longer the λ, the lower the likelihood that the travelling wave will break up, particularly upon encountering tissue heterogeneities. Thus, reductions in λ would permit additional AP waves to occur within the atrial myocardium. In small mammals, this would likely take place primarily within the plane of the atrial wall, and between atria. This is in contrast to the transmural effects suggested for the ventricular wall which is considerably thicker and differentiated into epicardial and endocardial tissues. Marked increases in heart rates can decrease APD, AERP and θ and thereby exert arrhythmic effects [39, 40].

Remodelling following prolonged AF

Chronic atrial arrhythmias have also been attributed to re-entrant substrate associated with conduction slowing [41, 42, 43]. Following

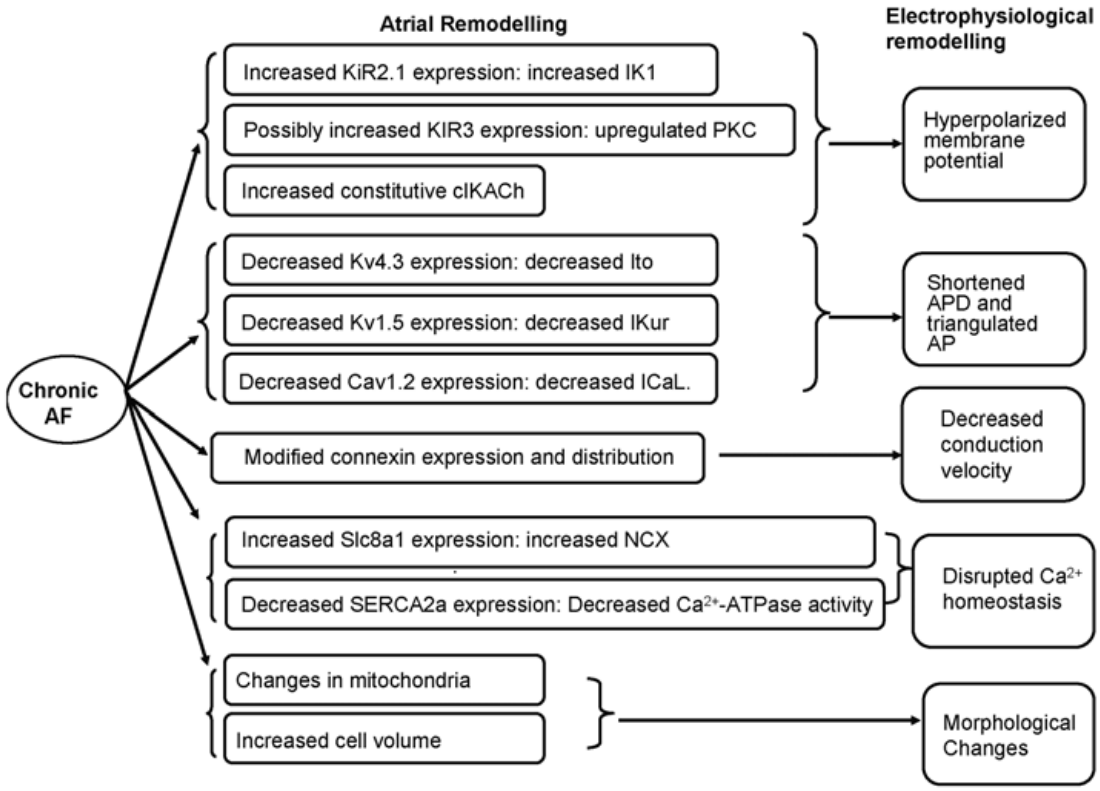

Figure 3. Atrial remodelling in chronic AF. Remodelling in the expression of particular molecules leads to associated changes of hyperpolarised resting membrane potential, the shortened and triangulated AP timecourses, with reduced APD, reduced conduction velocity, alterations in Ca^{2+} homeostasis and morphological changes.

chronic AF, atrial tissue undergoes reversible and subsequently irreversible alterations in electrophysiological properties, and irreversible anatomical changes (Figure 3). This remodelling process further exacerbates arrhythmic tendency and is attributable to changes in membrane protein expression with significant consequences for myocyte function. The extent of these changes depends on the progression of such remodelling.

First, resting membrane potentials become more negative [44, 45, 46]. This may reflect alterations in K^+ channel function with increases in I_{K1}, reflecting increased Kir2, particularly Kir2.1 subunit expression [44, 47]. Stimulated $I_{K\text{-}ACh}$ is reduced but constitutive, agonist-independent $I_{K\text{-}ACh}$ activity is enhanced [48, 46, 49, 50]. This may relate to upregulated phosphokinase C (PKC) activity [51], rather than altered Kir3 subunit expression [49, 50]. PKC inhibition reduces constitutive $I_{K\text{-}ACh}$ activity. Nevertheless, clinical AF is frequently initiated with the activation of $I_{K\text{-}ACh}$ that occurs under vagotonic conditions.

Secondly, AP timecourse becomes triangulated, reducing APD, AERP and consequently reducing both λ and their rate adaptation. These together in turn exacerbate arrhythmic substrate [52, 53]. Such changes may be attributed to K^+ current alterations. There are the increased I_{K1} and $I_{K\text{-}ACh}$ indicated above. In addition, reduced mRNA and protein expression of the pore-forming Kv4.3 subunit might reduce I_{to} [54]. There is also a reduced Kv1.5 expression expected to reduce I_{Kur} [55] whose action on the AP may vary with its initial waveform. Before atrial remodelling, I_{Kur} block shortens APs by accentuating plateau voltages thereby enhancing I_{KR} and I_{Ks} activation [56, 57]. In contrast, during chronic AF, I_{Kur} block prolongs the triangulated APs [58].

Thirdly, experimental systems show remodelled connexin expression and distribution dependent

upon the species and the associated pathology [59, 60]. Goat hearts showing AF have increased spatial heterogeneity, leading to reduced Cx43 but not Cx40 expression [61], Canine heart failure models showed a reduced expression and an increased extent of phosphorylation of Cx43 in both the left and right atria, and reduced Cx40 in the left atria [62]. Patients with persistent AF similarly display a heterogeneous distribution of Cx40 and to a lesser extent Cx43 [63, 64]. AF patients thus show slowed θ during regular pacing prior to the onset of arrhythmia [65].

Fourth, atrial remodelling also involves alterations in Ca^{2+} homeostasis. Paroxysmal AF patients show normal APDs, L-type Ca^{2+} and NCX currents, unchanged RyR2 expression [47, 66, 67, 68] yet increased SR Ca^{2+} leak [69] and increased incidences of DADs [70]. Chronic AF patients also show DADs now attributable not only to enhanced SR Ca^{2+} leak but also to increased NCX action [71]. Both a reduced I_{CaL}-mediated Ca^{2+} entry and this NCX upregulation [72, 73, 74] would accelerate cytoplasmic Ca^{2+} extrusion, and increase depolarizing inward NCX current, potentially increasing the likelihood of triggering APs. In long term AF, SR Ca^{2+} thus ultimately returns to normal [18, 74, 75, 76] or even becomes elevated [69]. Thus, atrial samples showed normal intracellular diastolic Ca^{2+} concentrations and SR Ca^{2+} content despite the increased SR Ca^{2+} leak and higher RyR2 open probabilities [71]. There is also a decreased SERCA expression. Together with the decreased I_{CaL}, this may account for observed decreases in SR Ca^{2+} [73], as well as eventual decrease in SR Ca^{2+} release, amplitudes of evoked intracellular Ca^{2+} transients and prolongations of their decays [77].

Fifth, RyR2 hyperphoshorylation may contribute to the alterations in Ca^{2+} leak. Atrial samples from patients in chronic AF showed increased calmodulin expression and autophosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) at Thr-287 resulting in its persistent activity. They also showed a RyR2 phosphorylation at the phosphokinase A (PKA) and CaMKII-sensitive Serine (Ser) 2808, and the CaMKII-sensitive Ser 2814 sites. Furthermore the CaMKII blocker KN-93, though not the PKA inhibitor H-89, conversely decreased SR Ca^{2+} leak, frequencies of spontaneous Ca^{2+} release events, and RyR2 open probabilities [71]. There may also be altered kinetics of FKBP12.6 binding to RyR2. SERCA changes have been related to phospholamban (PLB) hyperphosphorylation [73] and down regulation of the atrial selective PLB inhibitor sarcolipin [78]. Both these changes reduce their inhibition of SERCA Ca^{2+} uptake [73].

Finally, atrial contractility is impaired, initially due to the electrophysiological alterations above and then due to longer-term structural changes [79, 57]. Cell volumes increase with glycogen accumulation, sarcomere depletion, chromatin redistribution and areas of myolysis in which the SR loses its organization, and many mitochondria are visible. These mitochondria show altered sizes and shapes, but not signs of degeneration in that their cristae remain normal [80]. These cell morphological abnormalities correlate with the extent of atrial dilatation [81, 39] and the latter in turn correlates with AF inducibility likely through promoting re-entry [82].

Experimental studies of murine models

Mouse hearts are useful in studies of human myocardial electrophysiological disorders [83, 84]. Mice share significant genetic homology with humans [85]. Both human and murine atrial and ventricular excitation propagate by electrotonic spread driven by $Na_v1.5$ and $Ca_v1.2$-mediated depolarization followed by outward K^+ current-mediated repolarization [86]. Genetically modified murine hearts permit disease genotypes to be replicated, and pathological effects over time, including ageing, to be followed without complex pharmacological manipulations [87]. Significant differences do exist between human and murine electrophysiology, especially in the form of differing contributions of I_{KR} and I_{Ks} to ventricular repolarization [88]. Nevertheless, genetically modified murine hearts have replicated a wide range of ventricular arrhythmias and have also been used as experimental models for atrial arrhythmia [89, 90, 91, 92, 93].

Such studies permit assessment for atrial arrhythmic tendency thereby confirming the clinical arrhythmic phenotype in experimental systems.

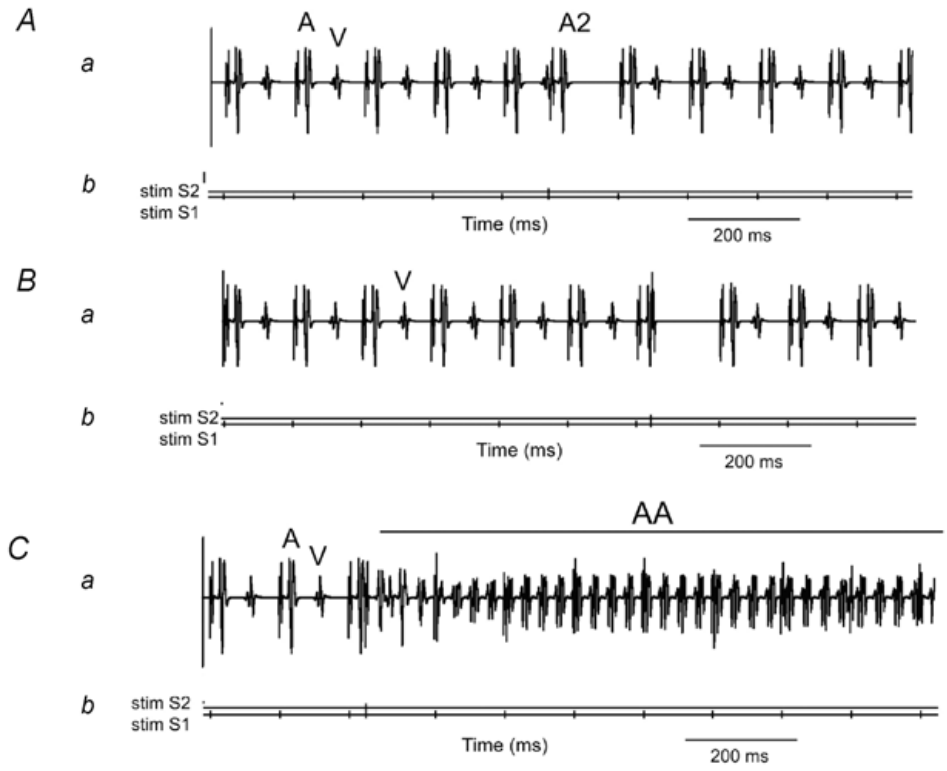

Figure 4. Assessment of atrial arrhythmias. Recorded atrial bipolar electrogram (BEG) traces obtained from a typical *Scn5a*$^{+/-}$ atrium subjected to programmed electrical stimulation (PES), prior to refractoriness (Aa). Vertical markers indicate the timing of the S1 stimuli (single marker) and S2 extrastimuli (double marker). S2 extrastimuli were delivered following a train of eight S1 stimuli at progressively shorter S1S2 coupling intervals (Ab). S1 stimulation artefacts and their respective evoked atrial ('A') and ventricular ('V') electrograms are succeeded by the extrastimulus (S2) artefact and its resulting atrial electrogram ('A2'), which is then followed by the first of eight S1 stimulation artefacts and its corresponding 'A' and 'V' electrograms. At refractoriness (Ba) the S2 no longer elicits an action potential at a given S1S2 coupling interval (Bb). (Ca) Initiation of an atrial arrhythmia (AA) in response to a premature S2 stimulus. The S1 stimulation artefact is closely followed by an S1 evoked 'A' electrogram and S2 stimulation artefact (Cb) in turn succeeded by an AA (Reproduced from Dautova, Y., Zhang, Y., Grace, A. A. and Huang, C. L. H. 2010, Exp. Physiol., 95, 994 with permission from John Wiley and Sons).

This may involve (a) *in vivo* electrocardiographic (ECG) records of both ambulatory and intact anaesthetized animals [94] that might show spontaneous disruptions of electrical rhythm. This can be followed by *ex vivo* experiments with isolated Langendorff-perfused preparations, which provide well-established experimental systems and permit the investigation of pathophysiological and pharmacological processes [83, 95]. They additionally permit observation of (b) spontaneous arrhythmic events taking place during intrinsic and regular activity from epicardial stimulation delivering regular S1 pacing beats and (c) provoked arrhythmic phenomena during programmed electrical stimulation (PES) typically comprising stimulus cycles of 8 regular S1 pacing beats followed by an S2 extra-stimulus delivered at progressively shorter S1S2 coupling intervals (Figure 4) [90, 96]. Some studies also include burst pacing protocols involving delivery of successive high frequency stimulus trains to the right atrium [93].

Further studies of the corresponding excitation properties have examined (a) features of AP initiation, propagation and recovery, including refractoriness. Monophasic AP (MAP) recordings provide AP durations (APDs) and AERPs. Bipolar electrogram (BEG) recordings yield electrogram durations (EGDs) and EGD ratios from EGDs

observed with S2 and S1 stimulation [96]. These also provide APD_{90}/AERP ratios, increases in which are associated with increased arrhythmogenicity [90]. Intracellular atrial AP recordings provide APDs, and maximal rates of rise $(dV/dt)_{max}$ of intracellular APs. (b) Isochronal mapping of patterns and timings of excitation wavefront propagation using multiple electrode arrays (MEAs). (c) Studies concerned with altered Ca^{2+} homeostasis and arrhythmic tendency explore Ca^{2+} signalling properties in single enzymically isolated fluo-3 loaded atrial cells [97]. Further effects of age on electrophysiological properties have also been studied in young (3 months) and aged (12 months) mice.

Assessments of longer-term factors contributing to arrhythmia have involved examination of gene expression characteristics, by quantitative polymerase chain reaction (qPCR) and western blotting, of a wide range of ion channels and regulatory genes. These included genes for Na^+ channel subunits, Ca^{2+} channel subunits and Ca^{2+}-handling proteins, K^+ channel subunits, hyperpolarisation-activated cyclic nucleotide-gated K^+ channels, gap junction proteins, transcription factors and other selected genes, including TGF-β_1 and vimentin gene expression.

Finally, explorations of morphological and fibrotic changes have assessed associated age and/or genotypically related changes. This included gross tissue architecture, as well as picrosirius red staining and vimentin fibroblast immunostaining.

Such physiological and structural studies have been applied to a range of genetic paradigms in order to demonstrate the existence of atrial arrhythmia and clarify their underlying mechanisms, additionally examining the consequences upon these of the ageing process where such data is available. These examined experimental systems with loss of cell-cell coupling, loss and gain of function in Nav1.5, altering atrial excitation and recovery, and increased RyR2-mediated release of SR Ca^{2+}.

Murine connexin cardiac models

Altered expression in cardiac connexin proteins appears to lead to abnormal conduction and arrhythmia [98]. Polymorphisms in the regulatory genes for connexin-40 (*Cx40*), specifically located within the atria as opposed to the ventricles appear linked to familial atrial standstill and increased vulnerability to AF [99, 100]. In the mouse, immunohistochemical studies indicate that of the three connexins (Cx40, Cx43, and Cx45) known to be expressed in myocytes, Cx40 occurs mainly in the atrial myocardium and His-Purkinje system [101, 102, 103]. Strands of Cx43- and Cx40-positive atrial cells protrude into the Cx45-positive sinus nodal area [104].

Homozygous Cx40 knockout ($Cx40^{-/-}$) mice correspondingly showed impaired sino-atrial (SA) propagation, with development of atrial ectopic pacemakers then maintaining overall cardiac rhythm. In $Cx40^{-/-}$ mice, ECG P-wave durations [105], PQ intervals, and QRS durations were prolonged [106, 107, 108]. It has been speculated that P-wave prolongation may result from local block with consequent prolongation of activation path length and therefore of intra-atrial conduction time. These changes were not associated with significant differences in resting membrane potential, $(dV/dt)_{max}$, AP amplitude, or APD compared to wild type $Cx40^{+/+}$ [105]; AERPs were shortened in the homozygous but not heterozygous *Cx40-A96S* mutants [109].

Murine $Scn5a^{+/-}$ cardiac models

Murine $Scn5a^{+/-}$ cardiac models have previously been used to model Brugada syndrome (BrS), a clinical ventricular arrhythmic disorder *associated with* slowed AP propagation and potentially fatal ventricular arrhythmic episodes [110, 111]. However, 10-30% of BrS patients additionally show increased AF tendency [112, 113, 114, 115]. The AF often precedes the onset of ventricular manifestations [116, 117] and reflects a severe BrS phenotype [118]. BrS patients thus demonstrated an occurrence of prolonged ECG P waves that correlated with the presence of the coved, type I, chest lead QT alterations [119] associated with severe disease [120, 121]. There were also late atrial potentials not shown by normal patients. Extrasystolic stimuli consistently induced sustained AF lasting >5 min in 11 patients with BrS-type ECGs [122]. Another study

demonstrated provoked AF in all 18 BrS but in none of the age-matched (age~50 y) control patients. The BrS patients showed atrial repolarisation abnormalities in the form of prolonged APDs at the S1S2 interval before the AERP was reached [123].

Nav1.5-haploinsufficient (*Scn5a*$^{+/-}$) murine hearts have experimentally modelled loss-of-function BrS and its associated ventricular arrhythmia [124], as well as alterations in SA pacemaker function [125, 126]. PES experiments demonstrated that Langendorff-perfused *Scn5a*$^{+/-}$ hearts showed greater atrial arrhythmogenicity than wild-type (WT) hearts during BEG recording [126, 90]. Furthermore, young *Scn5a*$^{+/-}$ showed the greatest arrhythmic tendency in comparison to either aged *Scn5a*$^{+/-}$ or young WT hearts. The latter findings correlated with the following electrophysiological differences between WT and *Scn5a*$^{+/-}$, and between young and aged *Scn5a*$^{+/-}$. First, aged *Scn5a*$^{+/-}$ showed lower heart rates than either aged WT or young *Scn5a*$^{+/-}$ hearts. The differences were attributable to independent effects of both age and the *Scn5a*$^{+/-}$ condition [127]. Secondly, the *Scn5a*$^{+/-}$ condition was associated with longer P wave durations [128], particularly in young rather than aged *Scn5a*$^{+/-}$ [129]. Young *Scn5a*$^{+/-}$ hearts similarly showed the most prolonged APDs [90]. However, it was the aged *Scn5a*$^{+/-}$ atria that showed increased AERPs and consequently the smallest APD_{90}/AERP ratios. Thirdly, *Scn5a*$^{+/-}$ atria, particularly those from young *Scn5a*$^{+/-}$ hearts showed the most prolonged EGD ratios prior to refractoriness during BEG recordings [127]. Finally, *Scn5a*$^{+/-}$ atria, again, particularly those of aged as opposed to young hearts, showed slower AP conduction as indicated by isochronal MEA maps of intervals required for activation of 50% of MEA recording sites [129, 127] and by ECG PR intervals [128, 129] (Figure 5). These findings thus implicate slowed conduction and prolonged APD relative to AERP in the AF tendency particularly shown by young *Scn5a*$^{+/-}$ hearts.

These physiological findings were accompanied by significant molecular and morphological changes. Nav1.5 mRNA expression was downregulated with both aging and the *Scn5a*$^{+/-}$ genotype giving the following sequence of Nav1.5

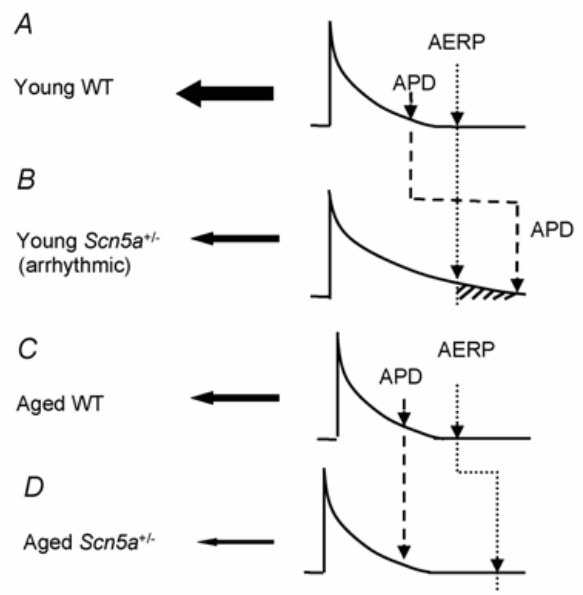

Figure 5. Electrophysiological features of young and aged wild-type (WT) and *Scn5a*$^{+/-}$ atria. The differing relationships between atrial AP recovery properties between groups are displayed with dotted lines. The conduction properties are displayed with arrows to the left, with higher velocities represented with thicker arrows. The slowed conduction in both young and aged *Scn5a*$^{+/-}$ atria (B, D) compared to respective WT (A, C) potentially exert arrhythmic effects. The prolongation in APD relative to the AERP in the young *Scn5a*$^{+/-}$ (B) would exert further arrhythmic effects, whereas the prolongation in AERP relative to the APD in the aged *Scn5a*$^{+/-}$ atria (D) would potentially reduce such arrhythmic effects [127].

expression: young WT > young *Scn5a*$^{+/-}$ > aged WT > aged *Scn5a*$^{+/-}$. Thus, Nav1.5 transcription was markedly reduced in both young and aged *Scn5a*$^{+/-}$ compared to young WT. In contrast, the greatest and smallest levels of expression of all ion channel types were observed in the young and aged *Scn5a*$^{+/-}$, respectively. There were accompanying, corresponding, increases in collagen density, determined by picosirius red immunostaining, and fibroblast density as measured by vimentin immunostaining in atrial regions surrounding the SAN. The *Scn5a*$^{+/-}$ genotype also correlated with greater mRNA levels of the fibroblast marker transforming growth factor-β_1 (TGF-β_1). All these effects were most marked in aged *Scn5a*$^{+/-}$ hearts. Furthermore, in all the animals studied, levels of

vimentin immunostaining and TGF-β_1 expression were linearly correlated; both these variables increased in hearts showing marked reductions in Nav1.5 expression. Finally, acute reductions in Nav1.5 channel expression or activity brought about by Nav1.5-E3 antibody appeared to upregulate TGF-β_1 and vimentin transcripts in either cardiac myocytes or cardiac fibroblasts, both known to express Nav1.5 [128].

These observations together implicate *Scn5a*$^{+/-}$ in a comprised SAN pacemaker function and atrial conduction, propagation and recovery in the initiation of arrhythmia in *Scn5a*$^{+/-}$ hearts. Thus, young *Scn5a*$^{+/-}$ show a combination of a prolonged APD yet normal AERP thereby increasing the APD/AERP ratio compared to aged *Scn5a*$^{+/-}$. In addition, there is reduced AP wavelength due to the reduced atrial θ combined with a normal AERP, changes taking place under conditions of a normal heart rate. Both would increase arrhythmic tendency. In contrast, aged *Scn5a*$^{+/-}$ hearts show a further reduction in Nav1.5 expression and increased fibrosis due to the presence of fibroblasts and increased TGF-β1. This lowers heart rates, prolonging the cycle length available for atrial AP recovery. This effect accompanies increased refractory periods. Together with the normal APD this reduces APD/AERP ratio. It also partially corrects the reduction in AP wavelength brought about by slowed conduction. These factors together would be expected to at least partially correct the AF arrhythmic tendency.

Murine *Scn5a*$^{+/\Delta KPQ}$ cardiac models

Long QT syndromes (LQTS) form a group of inherited cardiac disorders classically associated with prolonged ECG QT intervals [130]. They are associated with sudden death from ventricular arrhythmias, particularly through torsade de pointes. However, LQTS patients also show typical ages of AF onset that are substantially younger than in normals, typically at age 50 ± 14 years [131, 132]. Of the numerous characterized LQTS, long QT syndrome type 3 (LQT3) is associated with a gain of function Nav1.5 mutation involving a deletion of the three conserved amino acids, KPQ1505-1507, resulting in incomplete Nav1.5 inactivation [133] and prolonged ventricular APD. It is associated with an increased risk of sudden cardiac death typically within the first four decades of life [134, 135]. Early onset AF has also been described in a family with LQT3 and the *Y1795C* mutation in *SCN5A* [136]. A further report related early onset AF to a novel gain-of-function, *M1875T, SCN5A* mutation in a Japanese family [137].

The physiological relationships between the *SCN5A*$^{+/\Delta KPQ}$ mutation and its related ventricular arrhythmic phenotypes have been described in studies in a murine *Scn5a*$^{+/\Delta KPQ}$ model [138]. However, subsequent studies have further examined atrial arrhythmogenicity and have correlated these with corresponding electrophysiological properties and the dependence of both of these upon age [96, 139, 140, 141]. Thus, in parallel with the clinical findings, Langendorff-perfused, murine *Scn5a*$^{+/\Delta KPQ}$ cardiac preparations showed a progressive development of atrial arrhythmic tendency with age. Thus, whilst young *Scn5a*$^{+/\Delta KPQ}$ showed either indistinguishable or even reduced arrhythmogenesis compared to young WT [96, 139], aged *Scn5a*$^{+/\Delta KPQ}$ hearts showed higher incidences of arrhythmia than aged WT. These observations correlated with differences in electrophysiological characteristics. First, both young and aged *Scn5a*$^{+/\Delta KPQ}$ mice showed slowed heart rates relative to those of their WT counterparts, suggesting inhibited SAN function, in common with findings in the *Scn5a*$^{+/-}$ hearts.

Secondly, *Scn5a*$^{+/\Delta KPQ}$ hearts showed distinct electrophysiological features, in the form of generally longer atrial recovery characteristics as reflected in greater P wave durations and APDs during regular pacing, particularly in aged *Scn5a*$^{+/\Delta KPQ}$ [96, 139]. Similarly, at low (<2 Hz) pacing rates, whole cell patch-clamped left atrial *Scn5a*$^{+/\Delta KPQ}$ cardiomyocytes showed prolonged APDs and frequent EADs [142], which might reflect rate-dependent I_{Na} enhancements at low frequencies [143]. Thus, ranolazine, known to block slow, I_{NaL}, Na^+ currents, in preference to peak, I_{Nap} current reversed this APD prolongation and inhibited the EADs [142]. Young *Scn5a*$^{+/\Delta KPQ}$ showed similar AERPs as young WT. However, whilst WT showed increased AERPs with age, *Scn5a*$^{+/\Delta KPQ}$ did not. As a result, aged *Scn5a*$^{+/\Delta KPQ}$ showed shorter AERPs than the corresponding WT.

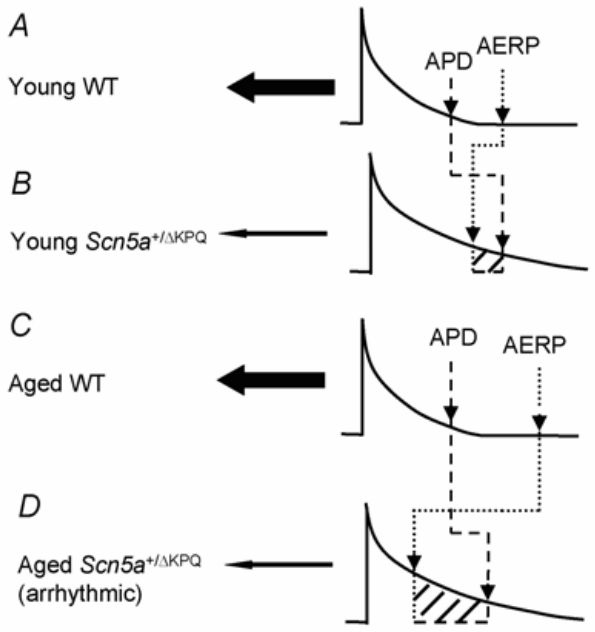

Figure 6. Electrophysiological features of young and aged wild-type (WT) and *Scn5a*$^{+/\Delta KPQ}$ atria. The relationships between atrial AP recovery properties among groups are displayed with dotted lines and the conduction properties are displayed with arrows to the left, with higher velocities represented with thicker arrows. The slowed conduction both in the young and the aged *Scn5a*$^{+/\Delta KPQ}$ atria (B, D) compared to their WT counterparts (A, C) potentially exert arrhythmic effects. The prolonged APD [141] together with the shortened AERP values in the *Scn5a*$^{+/\Delta KPQ}$ would potentially exert arrhythmic effects. However, the aged *Scn5a*$^{+/\Delta KPQ}$ additionally shows a marked reduction in AERP relative to the corresponding WT that would be expected to exert additional arrhythmic effects [96].

Consequently, young *Scn5a*$^{+/\Delta KPQ}$ showed greater APD/AERP ratios than young WT. Similarly, aged *Scn5a*$^{+/\Delta KPQ}$ showed greater APD/AERP ratios than aged WT. Thirdly, the prolonged PR intervals suggested that both young and aged *Scn5a*$^{+/\Delta KPQ}$ had slower atrial-ventricular conduction than WT [96]. MEA recording studies similarly suggested that *Scn5a*$^{+/\Delta KPQ}$ showed a slowed intra-atrial conduction [140] (Figure 6). Studies of related molecular and morphological changes demonstrated that young *Scn5a*$^{+/\Delta KPQ}$ atria actually showed higher Nav1.5 expression levels than did young WT. However, Nav1.5 expression then increased with age in WT but not *Scn5a*$^{+/\Delta KPQ}$. Finally, with ageing, *Scn5a*$^{+/\Delta KPQ}$ atria showed morphological changes in the form of increased atrial diameter, but without fibrotic change [141] in contrast to findings in *Scn5a*$^{+/-}$.

Together these findings clarify the development of an atrial arrhythmic tendency in aged *Scn5a*$^{+/\Delta KPQ}$. Although, both young and aged *Scn5a*$^{+/\Delta KPQ}$ showed high APD_{90}/AERP ratios relative to corresponding WT, aged *Scn5a*$^{+/\Delta KPQ}$ showed deficient Nav1.5 expression, an increased atrial extent relative to AP wavelength, and increased tendency to AF. These phenotypic characteristics overlap those of the *Scn5a*$^{+/-}$.

Murine cardiac models containing mutations in *RyR2*

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is one of the most malignant cardiac channelopathies. It mainly presents in young patients (age ~<30 y) with otherwise structurally normal hearts [144, 145]. Despite typically normal resting 12-lead ECGs, physical and emotional stress results in potentially life-threatening ventricular arrhythmic episodes, characteristically bidirectional VT, during exercise stress testing [146, 147]. CPVT is associated with more than 150 pathological allelic variants involving *RyR2* [144, 148]. A number of *RyR2* abnormalities are also associated with atrial arrhythmias [149, 150, 151, 152]. Variants associated with atrial arrhythmic disorders include the *RyR2-P2328S*, *RyR2-G3946A* [153], *RyR2-S4153R* [152], *RyR2-A7420G* [154], *RyR2-M4109R*, *RyR2-I406T* [155], and *RyR2-W4645R* [156] mutations.

A number of murine *RyR2* CPVT models correspondingly exhibit atrial arrhythmic abnormalities. *RyR2*$^{R176Q/+}$ mice did not show spontaneous AF during ambulatory ECG recordings but did show AF episodes during rapid oesophageal pacing under anaesthesia [94] *RyR2*$^{R2474S/+}$, *RyR2*$^{N2386I/+}$ and *RyR2*$^{L433P/+}$ mice also showed atrial tachyarrhthymias following rapid oesophageal pacing [93]. More detailed comparisons of heterozygotic (*RyR2*$^{+/S}$) and homozygotic (*RyR2*$^{S/S}$) *RyR2-P2328S* mice demonstrated that (a) neither anaesthetized *RyR2*$^{+/S}$ nor *RyR2*$^{S/S}$ mice showed spontaneous atrial arrhythmias even following isoproterenol challenge, even with rapid oesophageal pacing.

Similarly, (b) intrinsically beating isolated, Langendorff-perfused *RyR2*$^{+/S}$ and *RyR2*$^{S/S}$ hearts did not show AF whether before or after isoproterenol challenge. However, (c) regularly paced, *RyR2*$^{S/S}$ but not *RyR2*$^{+/S}$ hearts showed increased AF incidences than WT before but not following isoproterenol challenge. Neither showed increased AF incidences following isoproterenol challenge [91, 157]. Finally, (d) during PES, *RyR2*$^{S/S}$ but not *RyR2*$^{+/S}$ hearts showed increased incidences of provoked AF before isoproterenol challenge. In *RyR2*$^{S/S}$, AF occurred at higher S1S2 intervals when compared to WT [157]. However, both *RyR2*$^{S/S}$ and *RyR2*$^{+/S}$ then showed increased incidences of AF following isoproterenol challenge. These results together suggest that *RyR2*$^{+/S}$ possesses arrrhythmic substrate but not triggered activity, but that *RyR2*$^{S/S}$ shows both trigger and substrate potentially leading to AF [91].

Electrophysiological studies suggested normal ECG features. Thus, *RyR2*$^{R176Q/+}$, *RyR2*$^{+/S}$ and *RyR2*$^{S/S}$ mice demonstrated similar heart rates, RR and PR intervals, QRS durations and QTc intervals as WT [91, 94]. Optical mapping correspondingly demonstrated that atrial recovery was unaltered; *RyR2*$^{R176Q/+}$ and WT atrial *myocytes* showed indistinguishable APD_{80} values. Similarly, in *RyR2*$^{R176Q/+}$ mice there were no significant differences in AERP from WT [94]. MAPs from Langendorff-perfused *RyR2*$^{S/S}$, *RyR2*$^{+/S}$ and WT hearts also showed indistinguishable APD_{90} and AERP values whether before or following isoproterenol treatment [91, 157] (Figure 7). Thus, *RyR2*$^{R176Q/+}$, *RyR2*$^{+/S}$, *RyR2*$^{S/S}$ showed APD_{90}/ AERP ratios similar to those of WT hearts.

However both *RyR2*$^{R176Q/+}$ and *RyR2*$^{S/S}$ atria also showed triggering phenomena. *RyR2*$^{R176Q/+}$ atria showed more frequent ectopic beats that occurred over a wider range of pacing intervals than WT. Mapping studies revealed that such ectopic beats produced re-entrant waves in 3 of 5 *RyR2*$^{R167Q/+}$ atria [94]. Intracellular, sharp electrode recordings in *RyR2*$^{S/S}$ atria in isolated perfused hearts similarly revealed local DADs and propagated ectopic APs [157] in common with previous findings in *RyR2*$^{S/S}$ ventricles [158].

The occurrence of triggered events acutely leading to atrial arrhythmia correlated with alterations in cellular Ca^{2+} homeostasis in the form of diastolic Ca^{2+} events even in WT atrial myocytes. Thus diastolic Ca^{2+} waves were observed in regularly stimulated isolated atrial myocytes <5 min following caffeine application. These were abolished by previous SR Ca^{2+}-ATPase inhibition or L-type Ca^{2+} channel block by cyclopiazonic acid or nifedipine, respectively. These findings directly correlated with the presence or absence of atrial arrhythmia in intact Langendorff-perfused hearts [25, 159].

Parallel findings emerged from studies in atria with modified *RyR2* [160]. Regularly paced *RyR2*$^{R2474S/+}$, *RyR2*$^{N2386I/+}$ and *RyR2*$^{L433P/+}$ atrial myocytes similarly showed increased diastolic SR Ca^{2+} leaks in the form of increased Ca^{2+} spark frequencies compared to WT. They also appeared to show correspondingly decreased SR Ca^{2+} store levels as estimated following a caffeine treatment protocol. The rycal drug S107, thought to stabilise the RyR2 closed state by inhibiting oxidation/ phosphorylation-induced dissociation of calstabin 2 (FKBP12.6) from RyR2, both reduced the cellular diastolic SR Ca^{2+} leak in atrial myocytes and decreased burst pacing induced AF in *RyR2*$^{R2474S/+}$ [93]. *RyR2*$^{R176Q/+}$ atrial myocytes, whilst showing simlar amplitudes and durations of evoked Ca^{2+} transients as WT, similarly showed greater SR Ca^{2+} leak [94]. Finally, Fluo-3 loaded atrial myocytes from *RyR2*$^{S/S}$ hearts showed increased incidences of diastolic Ca^{2+} release than WT and *RyR2*$^{+/s}$. This was accentuated by isoproterenol particularly in the *RyR2*$^{S/S}$. These findings directly correlated with electrophysiological evidence demonstrating higher incidences of atrial arrhythmia in isolated perfused *RyR2*$^{S/S}$ as opposed to *RyR2*$^{+/s}$ in comparison with WT hearts [91].

Atrial biopsies from mice with atrial enlargement and spontaneous AF, goats with lone AF, and patients with chronic AF showed increased CaMKII-mediated RyR2 phosphorylation. Situations of increased RyR2-mediated Ca^{2+} release secondary to increased CaMKII-induced RyR2 phosphorylation have similarly been implicated in some acute murine AF models including *RyR2*$^{R2474S/+}$, *RyR2*$^{N2386I/+}$, *RyR2*$^{L433P/+}$ [93] and the *RyR2*$^{R176Q/+}$ hearts following burst pacing [94] and hearts with

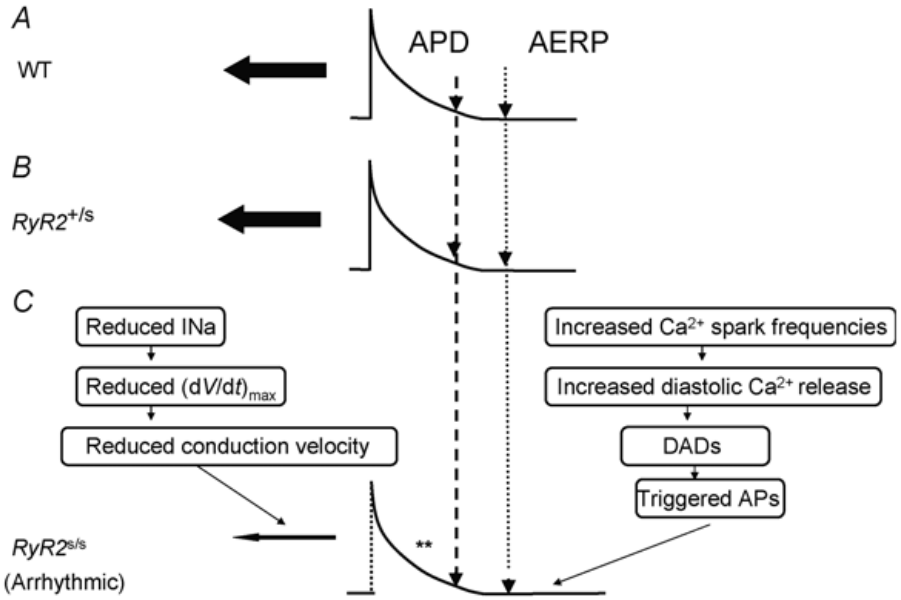

Figure 7. Electrophysiological features of wild-type (WT), *RyR2*$^{+/s}$ and *RyR2*$^{S/S}$ atria. Relationships between atrial AP recovery properties among groups are displayed with dotted lines. Conduction properties are displayed with arrows to the left, with higher velocities represented with thicker arrows. The reduced conduction velocity and presence of triggered APs exert arrhythmic effects in the *RyR2*$^{S/S}$ atria [162].

RyR2 phosphorylated at Serine (Ser)-2814 [71]. Thus, mapping studies revealed that ectopic beats produced re-entrant waves in 3 of 5 *RyR2*$^{R167Q/+}$ atria and these were sensitive to pharmacological or genetic CAMKII inhibition [94]. Knock-in mice with constitutively phosphorylated RyR2 at Ser-2814 showed higher incidences of atrial Ca^{2+} sparks and greater susceptibility to pacing-induced AF than controls. The CaMKII blocker KN-93, though not the PKA inhibitor H-89, conversely decreased the SR Ca^{2+} leak, frequencies of spontaneous Ca^{2+} release events, and RyR2 open probabilities [71].

These results directly parallel findings that similarly attribute ventricular arrhythmia in CPVT to altered myocyte Ca^{2+} homeostasis resulting from inappropriate RyR2-mediated release of intracellularly stored SR Ca^{2+}. These may also initiate spontaneous Ca^{2+} waves of Ca^{2+}-induced Ca^{2+} release (CICR), potentially responsible for the DADs that might trigger CPVT [144, 161, 162].

Finally, recent reports show reduced atrial epicardial AP conduction abnormalities following regular pacing in *RyR2*$^{S/S}$ compared to WT. These might potentially result in arrhythmic substrate. Thus, θ was reduced to extents comparable to those shown by either Nav1.5-haploinsufficient *Scn5a*$^{+/-}$ or flecainide-treated (20 μM) WT hearts. More detailed intracellular recordings demonstrated correspondingly reduced maximum rates of AP depolarization $(dV/dt)_{max}$ [157]. Furthermore, APs elicited by extrasystolic S2 stimuli that were imposed during PES showed inter-atrial θ and $(dV/dt)_{max}$ that fell with decreasing S1S2 interval in both *RyR2*$^{S/S}$ and WT. Nevertheless, θ was consistently (15-36%) lower in *RyR2*$^{S/S}$ than WT at any given S1S2 interval. However, appearance of sustained arrhythmia in *RyR2*$^{S/S}$ corresponded to the *same* value of θ and $(dV/dt)_{max}$ even when this took place at longer corresponding S1S2 intervals as WT. Thus, $(dV/dt)_{max}$ and θ emerge as major determinants for the induction of re-entrant arrhythmia. *RyR2*$^{S/S}$ atria thus showed a wider range of S1S2 intervals over which arrhythmias occurred than in WT.

These reductions in atrial θ in *RyR2*$^{S/S}$ did not appear to reflect fibrosis or other structural abnormalities detectable by either haematoxylin and eosin or Masson's trichrome staining in

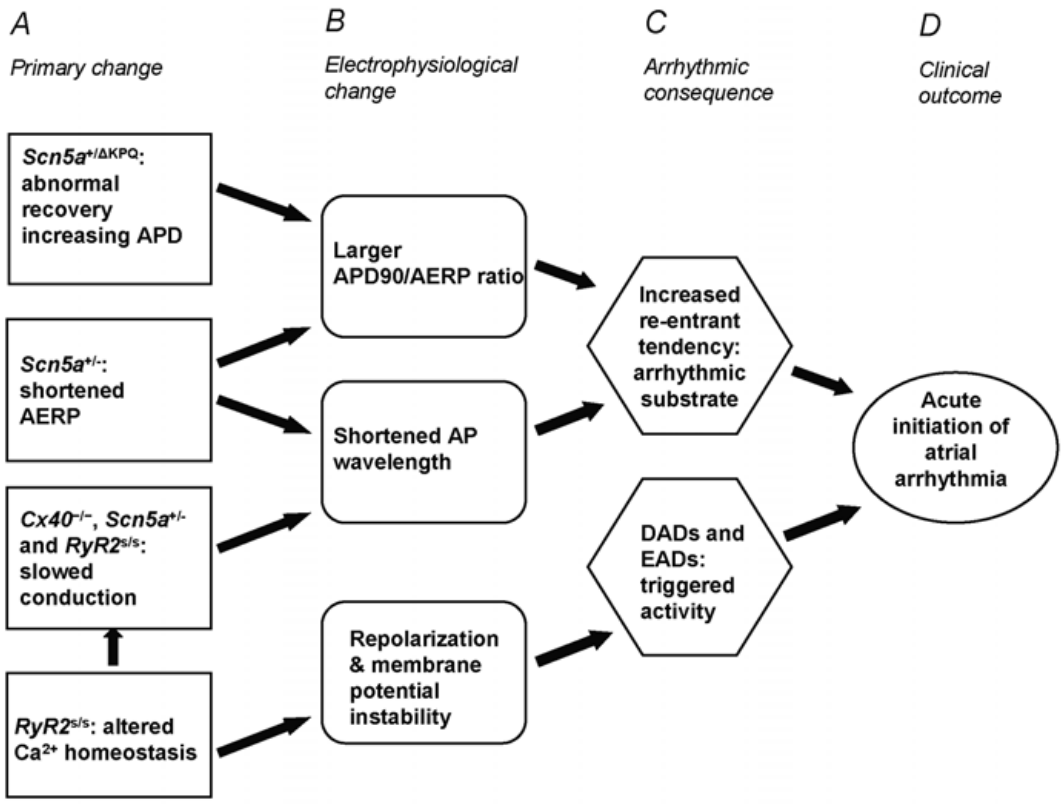

Figure 8. Overall scheme summarising the factors involved in initiation of atrial arrhythmias. Where genotypes are associated with primary changes in recovery and conduction properties (A) their electrophysiological change causes changes in APD/AERP ratios, wavelength and/or membrane instability (B). The resulting arrhythmic consequence in the form of arrhythmic substrate and/or triggered activity (C) would potentially result in the acute initiation of atrial arrhythmia (D).

contrast with findings in *Scn5a*$^{+/-}$ [91, 163]. Nor were there alterations in gap junction expression that would have reduced intercellular coupling [107, 163, 164]. Instead, *RyR2*$^{S/S}$ atria showed reductions in both Nav1.5 expression and Na^+ currents measured by loose patch clamp, compared to WT, in common with findings in *Scn5a*$^{+/-}$ and findings in WT following acute increases in cytosolic Ca^{2+} produced by high extracellular Ca^{2+}, caffeine or cyclopiazonic acid [163]. These atrial findings thus extend previous reports in ventricular myocytes describing reductions in both $(dV/dt)_{max}$ and Na^+ current density following alterations in cytosolic Ca^{2+} concentrations [165]. They also reproduced observations in atrial myocytes of parallel increases in diastolic SR Ca^{2+} release and arrhythmic tendency produced by acute caffeine (1 mM) applications [97].

These reports implicate ectopic triggering attributable to altered Ca^{2+} release properties in the initiation of arrhythmia in CPVT murine models. However, they also demonstrate slowed conduction that could maintain arrthythmia in *RyR2-P2328S* atria, as AP wavelength would be reduced by a combination of slowed θ and unaltered AP recovery characteristics.

CONCLUSION

The incidence of atrial fibrillation (AF) increases with age; furthermore particular genetic mutations in ion cardiac channels are associated with early onset AF. This review surveys recent studies using genetically altered murine models and thereby identifies common electrophysiological, molecular and structural features associated with initiation of atrial arrhythmia. Thus, although the cardiac conditions of BrS, LQT3 and CPVT are

mostly associated with ventricular arrhythmia, they have been increasingly associated with the presence of atrial arrhythmia particularly accompanying severe ventricular phenotypes. These conditions are associated with cardiac ion channel gene mutations encoding currents essential for either normal AP generation or excitation-contraction coupling. The paradigms described result in loss or gain of function in Nav1.5, (*Scn5a*$^{+/-}$ and *Scn5a*$^{+/\Delta KPQ}$ respectively) and gain of function in RyR2 (heterozygous and homozygous *RyR2-P2328S*, *RyR2*$^{R176Q/+}$, *RyR2*$^{R2474S/+}$, *RyR2*$^{N2386I/+}$ and *RyR2*$^{L433P/+}$) in CPVT. Further paradigms include those affecting Cx-40, with consequences for gap junction conduction.

These variants were used to explore for alterations in atrial electrophysiology that might result in either arrhythmic substrate or trigger. The variants showed alterations in SA pacing rate, atrial AP recovery, refractoriness and conduction, thereby permitting derivation of AP wavelength which has been used as a predictor of propensity to arrhythmias. The findings led to investigations for molecular changes that might result from the genetic modifications. These investigations also explored for structural including fibrotic or gross anatomical changes, and the effect of ageing. Together, the findings could be assembled into a cohesive scheme for the acute initiation of arrhythmia (Figure 8).

Scn5a$^{+/-}$ atria showed an increased atrial arrhythmic tendency particularly in hearts of young as opposed to aged animals. As expected from an associated Nav1.5 haploinsufficiency associated with fibrotic change, this correlated with delayed conduction and prolonged APDs. These resulted in both larger APD_{90}/AERP ratios and shortened AP wavelength that would be expected to contribute to arrhythmic substrate. In contrast, aged but not young *Scn5a*$^{+/\Delta KPQ}$ atria showed an increased arrhythmic propensity. This correlated with prolonged APDs, reduced Nav1.5 expression but shortened AERP and consequently larger APD_{90}/AERP ratios. Finally, *RyR2*$^{S/S}$ atria also showed increased tendencies to AF. However, this was associated with normal AP recovery in the form of normal APDs and AERPs and therefore normal APD_{90}/AERP ratios. However, they showed slowed conduction resulting in shortened AP wavelength. This could be attributed to a surprising reduction in Nav1.5 expression in common with findings in *Scn5a*$^{+/-}$ and aged *Scn5a*$^{+/\Delta KPQ}$, in this case, attributed to actions of altered Ca^{2+} homeostasis upon Nav1.5 expression. However, these changes took place in an absence of fibrotic change, in contrast to the situation in *Scn5a*$^{+/-}$. *RyR2*$^{S/S}$ also showed an increased level of triggered activity resulting in ectopic action potential initiation associated with an increased frequency of DADs and abnormal Ca^{2+} homeostasis. Finally, the reduced θ expected from reduced intercellular conduction expected with a *Cx40*$^{-/-}$ modification would similarly result in arrhythmic substrate.

Taken together, this comparative approach implicates mechanistic relationships involving altered APD_{90}/AERP ratios and shortened wavelength, likely resulting in re-entrant substrate, and repolarization and membrane potential instability, likely related to triggered activity in the acute initiation of atrial arrhythmia. Furthermore, the approach clarifies previously reported overlapping clinical features [166] shared by contrasting genetic modifications, through the convergence of the examples discussed here onto common electrophysiological mechanisms all leading to arrhythmic tendency through interactions between aging, ion channel expression, and cardiac tissue excitability. This interplay would thus account for paradoxical alleviations in arrhythmic characteristics shown by aged as opposed to young *Scn5a*$^{+/-}$, and young as opposed to aged *Scn5a*$^{+/\Delta KPQ}$. Nevertheless in all these cases, the resulting initiation of acute arrhythmia, if persistent, would lead to the chronic remodelling changes outlined in Figure 3. These would result in the long term electrical and structural remodelling that could extend to atrial dilatation that would in turn increase atrial size relative to AP wavelength. Such remodelling could become the subject of future study in further genetic models that could similarly contribute to the development of future therapeutic manoeuvres.

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CONFLICT OF INTEREST STATEMENT

None declared.

ABBREVIATIONS

AF, atrial fibrillation; AP, action potential; BEG, bipolar electrograms; BrS, Brugada syndrome; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; Cav1.2, cardiac Ca^{2+} channel mediating I_{CaL}; CICR, Ca^{2+}-induced Ca^{2+} release; CPVT, Catecholaminergic polymorphic ventricular tachycardia; Cx40 and Cx43, connexin molecules types 40 and 43; DAD, delayed after-depolarization; $(dV/dt)_{max}$, maximal rate of rise of the action potential; EAD, early after-depolarization; ECG, electrocardiogram or electrocardiographic; FKBP12.6, calstabin 2; H89, a phosphokinase A inhibitor; I_{KR}, rapid K^+ current; I_{Ks}, slow K^+ current; Kir2.1, K^+ channel mediating I_{K1}; Kir3, K^+ channel mediating I_{KACh}; KN-93, a CaMKII blocker; Kv, voltage gated K^+ channel; Kv1.5, K^+ channel mediating I_{Kur}; Kv4.3, K^+ channel mediating I_{to}; LQT3, long QT syndrome type 3; LQTS, Long QT syndrome; λ, action potential wavelength; MEA, multiple electrode array; Nav1.5, cardiac-type Na^+ channel; Navβ3, β3 subunit of the Na^+ channel; NCX, Na^+/Ca^{2+} exchanger; PES, programmed electrical stimulation; PKA, phosphokinase A; PKC, phosphokinase C; PLB, phospholamban; qPCR, quantitative polymerase chain reaction; RyR2, ryanodine receptor type 2; SA, sino-atrial; S1, regular pacing stimulus in PES protocol; S2, extrasystolic stimulus in PES protocol; Ser, Serine; SR, sarcoplasmic reticular; θ, action potential conduction velocity; TGF-β1, transforming growth factor-β_1.

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