Europace (2010) **12**, 160–172
doi:10.1093/europace/eup328

REVIEW

Animal models for atrial fibrillation: clinical insights and scientific opportunities

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Received 24 September 2009; accepted after revision 27 September 2009; online publish-ahead-of-print 29 October 2009

Atrial fibrillation (AF) is the most common arrhythmia in clinical practice. A variety of animal models have been used to study the pathophysiology of AF, including molecular basis, ion-current determinants, anatomical features, and macroscopic mechanisms. In addition, animal models play a key role in the development of new therapeutic approaches, whether drug-based, molecular therapeutics, or device-related. This article discusses the various types of animal models that have been used for AF research, reviews the principle mechanisms governing atrial arrhythmias in each model, and provides some guidelines for model selection for various purposes.

Keywords Arrhythmias • Anti-arrhythmia therapy • Anti-arrhythmic drugs • Ablation • Re-entry • Triggered activity • Transgenic • Knockout

Introduction

Atrial fibrillation (AF) contributes to significant cardiovascular morbidity and mortality.[1] Despite the availability of numerous therapeutic agents, the available treatments have significant limitations,[2,3] and AF continues to be a clinical challenge. Several experimental models have been developed in which AF pathophysiology may be studied; however, many important questions concerning mechanisms underlying AF remain unanswered.[4] The advantages of using animal models over clinical samples are manifold: full access to tissue and cells from large regions of the heart and the ability to perform high-density epicardial or even optical mapping experiments, to name but a few. Knowledge gained from experimental models complements clinical studies and may lead to therapeutic advances. This article reviews animal models available for studying AF, presents their contribution to the body of knowledge regarding underlying mechanisms, and discusses how these models may be used.

Insights into mechanisms underlying atrial fibrillation

Moe *et al.*'s[5] multiple wavelet hypothesis and Allessie *et al.*'s[6] leading circle concept have long been dominant theories of mechanisms underlying AF, in which multiple transient wavelets perpetuate AF, with a balance between new wavelet formation and wavelet extinction allowing the arrhythmia to be maintained. The number of coexisting wavelets depends on the balance between atrial size and wavelength, i.e. the product of the refractory period and the conduction velocity.[6] However, many recent studies support the concept that driver regions, maintained by single or multiple spiral waves or rapidly discharging ectopic foci, perpetuate AF, with fibrillatory conduction contributing to activation irregularity.[7] Recent observations suggest that sources in the thoracic veins, particularly the pulmonary veins (PVs), play important roles in AF initiation and maintenance.[8]

The basic mechanisms underlying AF are presented in *Figure 1*. Rapid ectopic foci arise by abnormal automaticity originating in regions other than the sinus node, or as a result of early (EADs) or delayed after-depolarizations (DADs). Early after-depolarizations involve the reactivation of L-type Ca^{2+} channels during prolonged repolarization, whereas DADs appear when Ca^{2+} is released from the sarcoplasmic reticulum (SR) during diastole. Diastolic Ca^{2+} rises activate the Na^{+}–Ca^{2+} exchanger (NCX), which carries three Na^{+} ions into the cell in exchange for each Ca^{2+} transported out, causing net inward movement of one positively charged ion per cycle and depolarizing the cell. Oscillations in membrane potential that surpass the threshold potential trigger ectopic beats, and ectopic firing provides the critical premature activation that initiates re-entrant activity, in the form of either a single rotor or multiple rotors or wavelets that sustain fibrillation. Alternatively, repeated rapid firing from a focal

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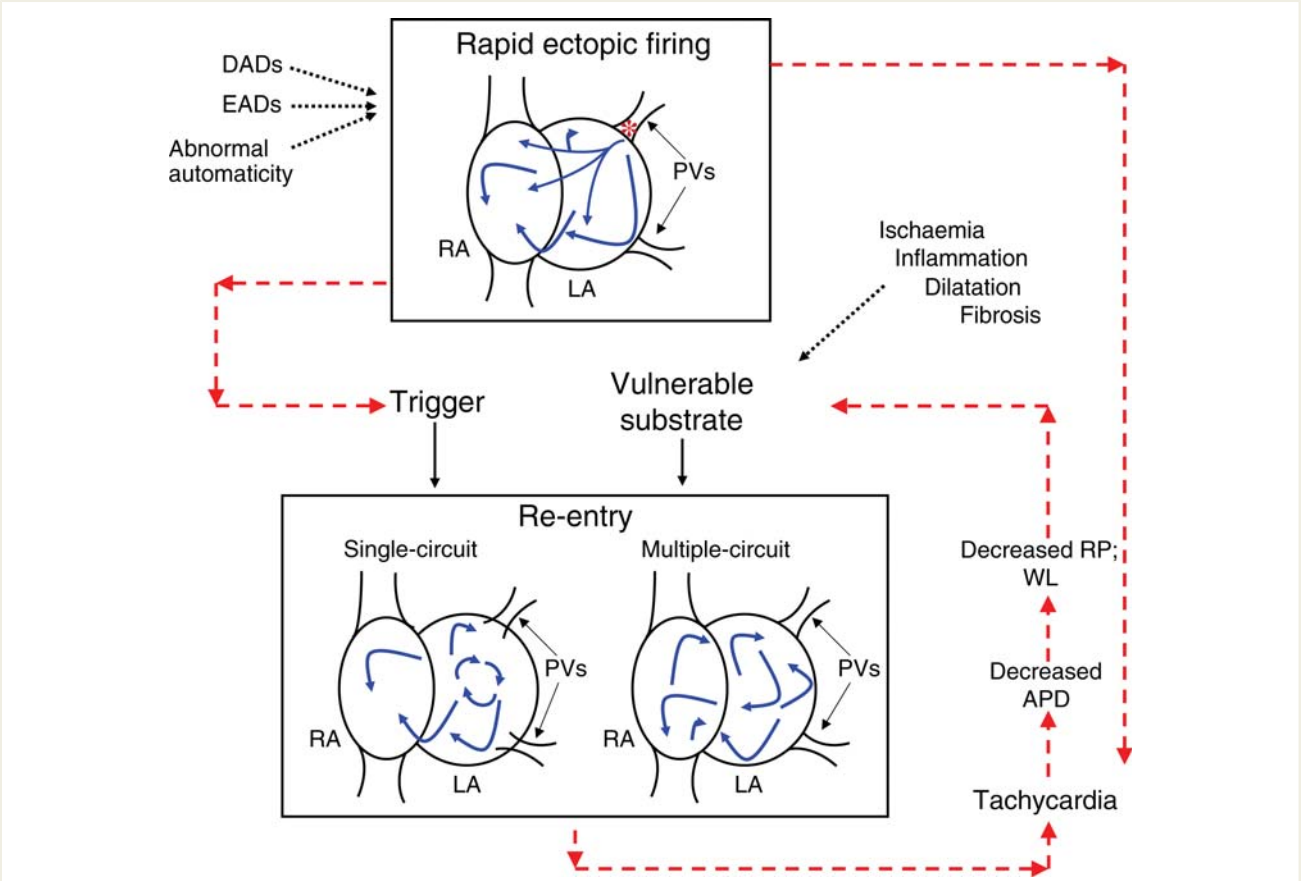

Figure 1 Mechanisms underlying atrial fibrillation. APD, action potential duration; DAD, delayed after-depolarization; EAD, early after-depolarization; LA, left atrium; PV, pulmonary vein; RA, right atrium; RP, refractory period; WL, wavelength.

source can be conducted irregularly through the atrial substrate, producing fibrillatory activity. The very rapid atrial rate resulting from re-entry or triggered activity in turn abbreviates the effective refractory period (ERP), which perpetuates re-entrant activity and promotes AF.[6] Ischaemia, inflammation, fibrosis, and atrial dilatation also contribute to the AF substrate, and spatial variability of refractoriness is another important determinant of sustained episodes of AF.[9,10]

Large animal models

Atrial fibrillation has been studied in large animal models with rate-related electrical remodelling or with atrial-structural remodelling, following acute atrial insults, and in the presence of autonomic nervous system modulation. *Table 1* summarizes these models and the corresponding clinical paradigms.

Rate-related remodelling

Wijffels *et al.*[11] first demonstrated experimentally that maintained AF alters atrial electrophysiology to enhance AF vulnerability and persistence, hypothesizing that AF begets AF. In their goat model, the authors demonstrated that atrial burst pacing led to atrial ERP shortening, enhanced perpetuation of AF, and lack of ERP adaptation to rate changes.[11] The authors coined the term 'atrial remodelling' to describe AF-promoting changes caused by AF itself. Remodelling induced by AF is virtually indistinguishable from that produced by any rapid atrial tachyarrhythmia, which has come to be known as 'atrial tachycardia (AT)-induced remodelling'. Atrial tachycardia remodelling has since been demonstrated in dogs,[10,12] sheep,[13] and pigs.[14,15] Rapid atrial rates cause heterogeneous remodelling of refractoriness, increased vulnerability to AF induction, and increased arrhythmia persistence.[10–12] Only ATs with rates $\geq$300 bpm promote AF vulnerability and maintenance in dogs, suggesting that the clinical association between paroxysmal ATs and AF is not due to AT remodelling alone.[16] Atrial tachycardia models are particularly useful for evaluating drugs that may prevent electrical remodelling.

Atrial tachycardia remodelling alters ionic currents and gene expression of ion channels in a manner that promotes the occurrence of AF (*Figure 2*).[17,18] Reduced I_{CaL}, due to $Ca_v1.2$ mRNA and protein expression downregulation, results in action potential duration (APD) shortening and reduced rate adaptation.[17,18] This is mediated by rate-induced intracellular Ca^{2+} overload,[19,20] activating the Ca^{2+}-dependent calmodulin–calcineurin–nuclear factor of activated T cell system causing transcriptional downregulation of

Table 1 Large animal models of atrial fibrillation

Large animal model	Clinical counterpart	Species
Rate-related remodelling	AT or fibrillation remodelling	Dog, goat, pig, sheep
Atrial structural remodelling		
CHF	CHF	Dog, sheep, rabbit
MR	Mitral valve disease	Dog
Sterile pericarditis	Post-cardiac surgery	Dog
Atrioventricular block	Severe bradycardia	Goat
Chronic volume overload	Cardiac shunt disease, arteriovenous shunt	Sheep, goat, rabbit
Hypertension	Hypertension	Sheep, rat
Acute atrial insults		
Atrial stretch	Acute volume overload	Dog, rabbit, sheep
Aconitine	Focal AF	Dog
Ischaemia	Acute myocardial infarction, coronary disease	Dog
Autonomic models		
Vagal nerve stimulation	Cholinergic AF	Dog, sheep
Acetylcholine perfusion	Cholinergic AF	Sheep
Sympathetic nerve stimulation and hyperinnervation	Autonomic nervous system hyperactivity	Dog

CHF, congestive heart failure; MR, mitral regurgitation.

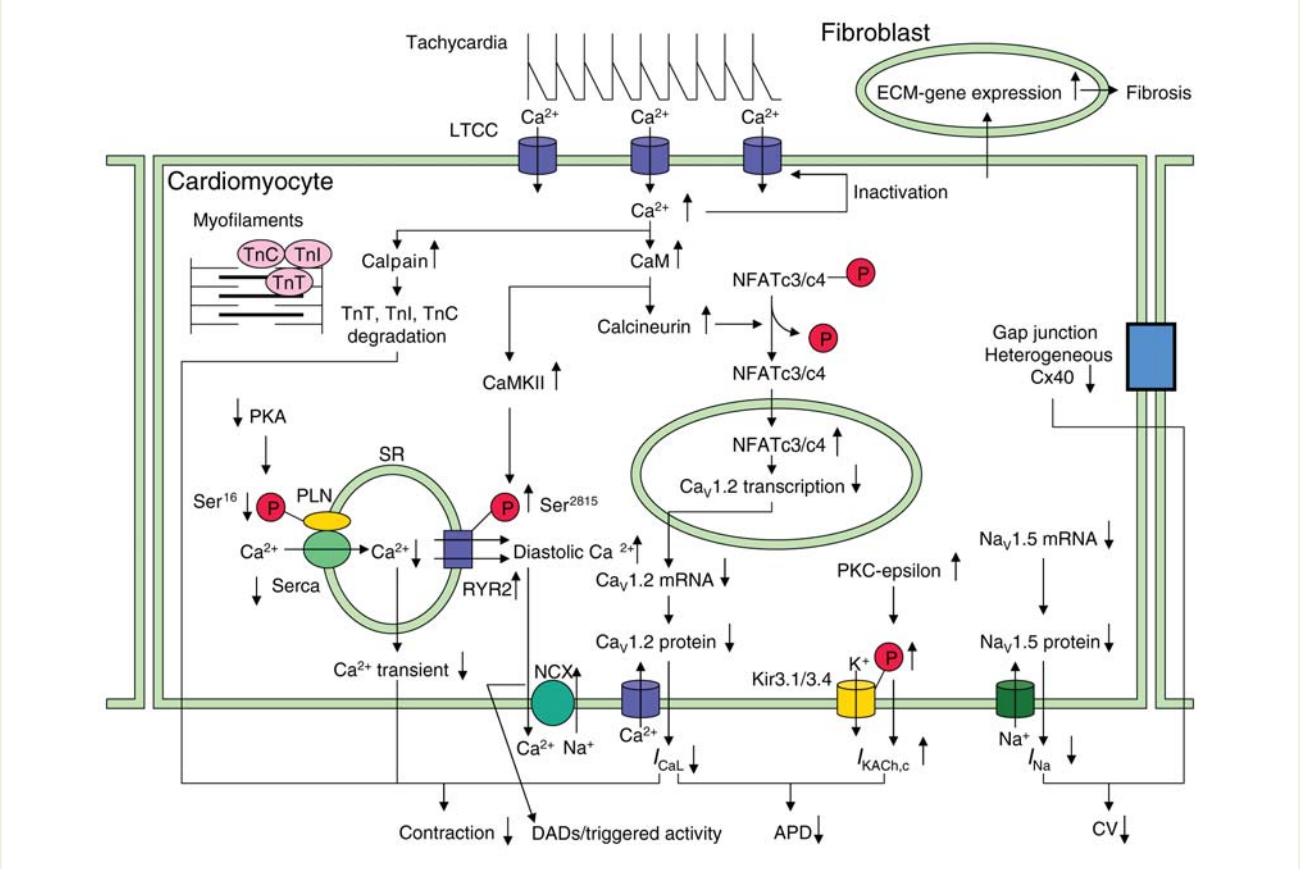

Figure 2 Schematic representation of the pathogenesis of atrial tachycardia remodelling. APD, action potential duration; CV, conduction velocity; CaM, calmodulin; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; Cx40, connexin40; DAD, delayed after-depolarization; ECM, extracellular matrix; I_{CaL}, L-type Ca^{2+} current; $I_{KACh,c}$, constitutive acetylcholine-regulated K^+ current; I_{Na}, Na^+ current; LTCC, L-type Ca^{2+} channel; NCX, Na^+/Ca^{2+} exchanger; NFAT, nuclear factor of activated T cells; PKA, protein kinase A; PKC, protein kinase C; PLN, phospholamban; RYR2, ryanodine receptor; SR, sarcoplasmic reticulum; TnC, troponin C; TnI, troponin I; TnT, troponin T. For a detailed discussion, see text.

I_{CaL} in an attempt to restore intracellular Ca^{2+} at the expense of arrhythmogenic APD changes.[20] Further APD abbreviation is mediated by changes in the constitutive, agonist-independent, acetylcholine-regulated K^+ current ($I_{KACh,c}$).[21,22] Atrial tachycardia causes spontaneous $I_{KACh,c}$ channel opening,[23] but does not affect mRNA or protein expression of Kir3.1/3.4 subunits underlying I_{KACh}.[23] Chronic AF is associated with increased expression of protein kinase C (PKC)-ϵ, suggesting that abnormal channel phosphorylation by PKC leads to increased $I_{KACh,c}$ activity.[24] A recent work suggests that PKC-isoform switching is responsible for $I_{KACh,c}$ activation with AT remodelling.[25] Furthermore, the selective Kir3 antagonist, tertiapin-Q, terminates AF without affecting ventricular electrophysiology,[21] indicating that $I_{KACh,c}$ may be a potentially interesting anti-arrhythmic target.

Long-term rate-related remodelling may also lead to conduction velocity slowing.[26] Decreased Na^+ current (I_{Na}), due to the down-regulation of the underlying Na^+ channel α-subunit expression,[17,26] and changes in the content and distribution of gap junction connexins are thought to contribute to this finding.[27] Additionally, maintained rapid atrial activation reduces transient outward current (I_{to}) and increases background inward-rectifier K^+ current (I_{K1} and $I_{KACh,c}$), which can modulate conduction changes by, respectively, opposing early depolarizing currents and removing I_{Na} inactivation by hyperpolarizing atrial cells.[18,23,28]

Contractile dysfunction, responsible for intra-atrial thrombus formation and increased risk of embolic stroke in AF patients, occurs after several days of AF,[29,30] and is associated with reduced I_{CaL}.[31,32] In canine atrial cardiomyocytes, sustained AT reduces systolic Ca^{2+} transients, impairs cellular Ca^{2+} handling, and reduces cellular contractility.[32] Preventing Ca^{2+} overload during tachycardia prevents Ca^{2+} handling abnormalities.[33] Moreover, altered SR Ca^{2+} release, intracellular [Ca^{2+}] changes, and I_{CaL} inactivation contribute to AP abbreviation and the loss of rate adaptation commonly observed in this model.[34] Phospholamban dephosphorylation and ryanodine receptor (RYR2) hyperphosphorylation underlie reduced SR Ca^{2+} load in goats with AF.[35] Degradation of the myofibril structure also contributes to contractile dysfunction following AT remodelling.[36] Ca^{2+}-overload-induced calpain activation plays a central role in the degradation of troponin T.[36]

Atrial structural changes develop after long-term pacing-induced sustained AF[37,38]: loss of myofibrils, accumulation of glycogen, altered mitochondrial shape and size, fragmentation of SR, and dispersion of nuclear chromatin are observed.[39] Remodelling of the cellular ultrastructure develops progressively over 4 months of AF, and recovery remains incomplete 4 months post-AF.[37,38] Oxidative and inflammatory stresses are also involved in AT remodelling.[40–42] Simvastatin, with antioxidant properties, attenuates AF promotion in dogs.[41] Prednisone prevents electrophysiological and AF-promoting effects of AT remodelling, possibly by an anti-inflammatory action.[42] The signalling pathways involved are not fully understood; however, these mechanisms remain attractive potential therapeutic targets.

Several animal studies implicate the PVs in the promotion of AF.[43,44] In dogs with rapid atrial pacing, PV cardiomyocytes with pacemaker activity have been found to have a higher incidence of DADs or EADs.[43] Non-re-entrant focal activations have also been reported in the PVs of a canine model of pacing-induced sustained AF.[44] However, AT in dogs produces qualitatively similar ionic remodelling in left atrium (LA) and PVs and reduces PV–LA AP differences.[45] Furthermore, resection of all PVs fails to alter atrial tachyarrhythmia inducibility in AT-remodelled LA preparations.[45] These findings suggest that PVs are not essential for AT-induced atrial tachyarrhythmia promotion in this model.[45]

AV block is often created in AT models to avoid rapid ventricular response and tachycardia-induced ventricular dysfunction. In the absence of AV block, concomitant rapid ventricular response accelerates atrial fibrosis and the development of AF.[13,46] In sheep without His bundle ablation (non-HBA), persistent AF develops significantly earlier than in those that undergo ablation (HBA). Non-HBA sheep have diminished atrial matrix metalloproteinase (MMP)-2, increased tissue inhibitor of metalloproteinase (TIMP)-2 expression, and more extensive atrial fibrosis.[13] Inhibition of the angiotensin pathway has been shown to suppress fibrosis and the development of persistent AF in non-HBA sheep.[13]

Indeed, rapid atrial activation alone can promote atrial fibrosis.[47,48] In a chronic canine model of rapid pacing-induced AF with AV node ablation, the arrhythmia occurs in the presence of atrial fibrosis but in the absence of any ventricular dysfunction.[47] In this model, AT induces extracellular matrix (ECM) remodelling that promotes fibrosis. Canine atrial fibroblasts cultured in the medium from rapidly paced atrial cardiomyocytes adopt an activated myofibroblast phenotype, as indicated by increased α-smooth muscle actin (SMA) protein expression.[48] Furthermore, increased secretion of collagen and fibronectin by fibroblasts may explain increased atrial fibrosis with AT.[48]

Atrial structural remodelling

The importance of structural remodelling of the atrial architecture, particularly involving enhanced fibrosis, was first emphasized by studies in a dog model of congestive heart failure (CHF).[49] Atrial fibrosis is a common motif seen in many conditions associated with clinical AF, including ageing, valvular heart disease, hypertension, and cardiomyopathy.

Congestive heart failure

Congestive heart failure, one of the most common clinical causes of AF, has been investigated in experimental dog and sheep models involving ventricular tachypacing (VTP).[49–51] Experimentally, CHF does not alter atrial ERP or global conduction velocity, in contrast to AT remodelling. Indeed, CHF may even prolong atrial ERP, leaving wavelength unchanged or even increased.[49] Furthermore, refractory period heterogeneity is unchanged.[49] At the ionic current level, CHF decreases atrial I_{to}, I_{Ca}, and I_{Ks} and increases I_{NCX}: the balance between net outward and inward currents results in no overall change or an increase in atrial ERP.[52] Atrial cells isolated from CHF hearts display prolonged APDs, more positive resting membrane potentials, and DADs,[53,54] implicating focal activity and DAD-induced triggered activity as potential mechanisms of CHF-mediated AF.[53–56] High-resolution mapping of experimental animals with CHF suggests that focal activations and complex wavefronts originate in the PV region during AF,

although the precise role of PV-derived activity in maintaining AF in this model is unclear.[56]

Interstitial fibrosis interferes with conduction (causing areas of slowed conduction and spatial heterogeneity) and stabilizes re-entrant circuits.[49] In sheep with CHF, AF frequency and dynamics are affected by the quantity, type (patchy vs. diffuse), and spatial distribution of fibrosis.[50,51] Furthermore, patchy rather than diffuse fibrosis contributes to wavebreak and intramural rotor formation.[51] The cessation of tachypacing is associated with full recovery of ventricular function, followed by normalization of atrial function and reversal of ionic remodelling. However, changes in fibrosis and conduction are not reversed, and a substrate that supports prolonged AF remains,[57,58] implicating structural, rather than ionic, remodelling as an important contributor to the maintenance of AF in the presence of experimental CHF.[57–60]

The canine model has been fundamental in enhancing our knowledge of the signal transduction pathways involved in atrial structural remodelling during experimental CHF.[60,61] Tissue angiotensin II concentration is elevated as early as 6 h following the onset of tachypacing.[61] The effects of angiotensin II are mediated by three important mitogen-activated protein (MAP) kinases: ERK, p38, and JNK. Activation and increased expression of the phosphorylated forms were observed in this model prior to changes in the pro-apoptotic factors, Bax and caspase-3.[61] Treatment with an angiotensin-converting enzyme (ACE)-inhibitor prevented angiotensin II concentration increases and ERK hyperphosphorylation, but did not affect p38 or JNK. Angiotensin-converting enzyme-inhibition failed to prevent necrosis and only attenuated fibrosis, suggesting that both angiotensin II-dependent and -independent pathways are involved in atrial structural remodelling.[61] Omega-3 polyunsaturated fatty acids (PUFAs) also reduce atrial structural remodelling and AF promotion in VTP-induced CHF.[62,63] Interestingly, PUFAs suppress the phosphorylation of both ERK and p38, in contrast to ACE-inhibition.[62]

Atrial fibrosis is more prevalent in the LA than in the LV in CHF dogs,[64,65] and is reduced by ACE-inhibitors and the anti-fibrotic drug pirfenidone,[66] which acts on the important profibrotic mediator, transforming growth factor (TGF)-β1. Simvastatin reduces atrial structural remodelling by attenuating TGF-β1-stimulated atrial myofibroblast differentiation.[41] Growth factors such as foetal bovine serum, platelet-derived growth factor, angiotensin-II, and TGF-β1 cause atrial fibroblasts to proliferate to a greater degree than ventricular fibroblasts.[65] Platelet-derived growth factor signalling may be particularly important for atrial-selective fibroblast responses and fibrosis.[65]

Figure 3 shows atrial molecular expression changes in CHF-associated remodelling observed in animal experiments.[67,68] Connective-tissue growth factor gene expression is enhanced by 24 h VTP.[67] Connective-tissue growth factor is upregulated by angiotensin II, TGF-β1, or alterations in the cytoskeleton and promotes fibrosis in pathological conditions by blocking the negative TGF-β1 feedback loop and allowing continued TGF-β1-related activation.[67] Upstream activators of MAP kinase signalling are upregulated early in VTP remodelling, notably the small G-protein-signalling element Rac1, which is a target for statins.[68] Extracellular matrix remodelling appears to be important in CHF-induced AF since collagen, fibronectin, and TIMP-1 gene expression increase after 24 h VTP.[68] Apoptotic signalling is also altered in experimental CHF.[68]

Mitral valve regurgitation

Atrial fibrillation is maintained by multiple wavefronts, non-uniform conduction, bidirectional block, and macro-re-entrant circuits in dogs with experimentally induced mitral valve regurgitation (MR).[69] Effective refractory period is increased homogeneously throughout the LA and RA,[70] whereas interstitial fibrosis and chronic inflammation are observed only in the dilated LA.[71] No change in the spatial distribution of connexins is seen,[71] but LA conduction slowing, likely due to fibrosis, accounts for increased AF inducibility.[72]

Sterile pericarditis

Atrial fibrillation associated with open-heart surgery results from multiple factors, with sterile pericarditis being an important contributor. A canine model of sterile pericarditis developed by the Waldo laboratory[73] provides an experimental counterpart to the clinically observed phenomenon (*Table 1*). This model exhibits a high incidence of sustained AF,[73] maintained by unstable re-entrant circuits around the atrial septum, or a stable LA driver causing fibrillatory activation, particularly in the RA.[74] Sterile pericarditis also causes atrial flutter, and changes in the length of the line of functional block in the RA free wall are critical for the conversion of AF into flutter and back to AF.[73] Altered gap junction connexin distribution and an inflammatory response contribute to abnormal atrial conduction and AF vulnerability, and agents with anti-inflammatory properties like atorvastatin and prednisone prevent AF in this model.[75,76]

Atrioventricular block

Chronic (4-week) AV block in goats leads to progressive atrial dilatation and prolonged AF.[77] Atrial ERP and AF cycle length remain constant,[77] whereas local conduction delays are observed during rapid pacing. Hypertrophy is present, but no atrial fibrosis is observed.[77] Gap junction connexin expression is not altered,[77] and structurally based spatial differences in atrial wall stress may explain the conduction heterogeneities.[77] Sarcoplasmic reticulum Ca^{2+} load decreases due to phospholamban dephosphorylation and RYR2-hyperphosphorylation, along with reduced myofilament phosphorylation, further compromise contractility.[35]

Chronic volume overload

Chronic atrial dilatation and persistent AF can be produced in volume overload models.[78–80] In a goat aortic-to-LA shunt model, progressive LA dilatation, ERP prolongation, and increased AF duration occur, without changes in conduction or tissue collagen.[78] An aorto-pulmonary artery shunt in sheep induces moderate, isolated LA dilation, rendering the atria vulnerable to AF.[79] Atrial myocytes from these hearts show enlargement and myolysis, and many are inexcitable.[79] Effective refractory period is unchanged, I_{Ca} is reduced by ~45%, and APs have a characteristically small amplitude and triangular morphology.[79] Heterogeneous APD shortening and loss of excitability may be pro-arrhythmic factors.[79] Conduction slowing is also observed in a rabbit model

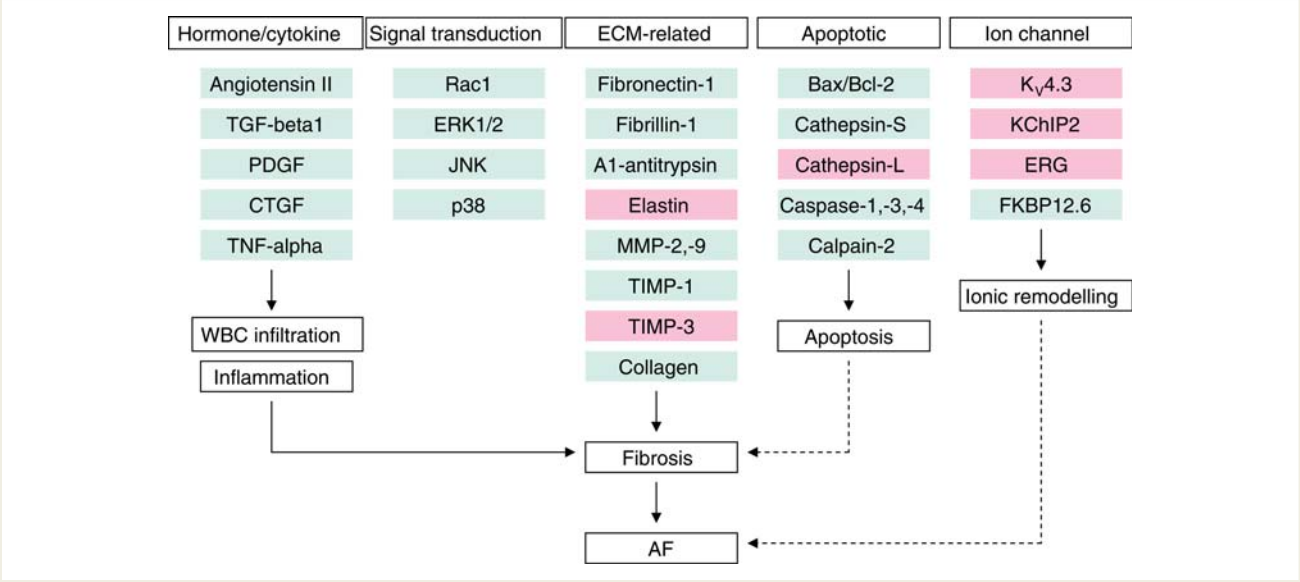

Figure 3 Potential molecular mechanisms of congestive heart failure-associated atrial remodelling. Blue boxes indicate increased or activated molecules, and pink boxes indicate decreased molecules. TGF, transforming growth factor; PDGF, platelet-derived growth factor; CTGF, connective-tissue growth factor; ERK, extracellular signal-related kinase; JNK, c-Jun N-terminal kinase; p38, p38 kinase; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; KChIP2, K_v channel-interacting protein 2; FKBP12.6, FK506-binding protein; ECM, extracellular matrix; ERG, ether-à-go-go-related gene; TNF, tumour necrosis factor; WBC, white blood cell.

with an arteriovenous shunt, and atrial tachyarrhythmias are associated with re-entrant and focal excitation originating in the posterior LA.[80]

Hypertension

Sheep with pre-natal corticosteroid-induced blood pressure increases exhibit enlarged LAs and increased AF durations. Widespread conduction abnormalities and atrial wavelength shortening are observed; however, refractoriness does not change significantly.[81] Atrial tissue undergoes significant structural remodelling: central myofibrillolysis, myocyte hypertrophy, mitochondrial and nuclear enlargement, and fibrosis, along with evidence of apoptosis.[81]

Acute atrial insults

Some acute atrial insults can promote AF without chronic alterations in atrial structure and function. Since such models do not involve long preparation periods, they may be useful in screening drugs for anti-AF activity.

Atrial stretch (volume overload)

Increased atrial pressure leads to ERP shortening and increased AF vulnerability in isolated rabbit hearts, changes that reverse completely within 3 min of stretch release.[82] Sustained AF is reliably induced in preparations lacking the pericardium, whereas in pericardially intact preparations, atrial stretch is limited by passive constraint despite elevated atrial pressures,[83] suggesting that AF promotion with acute atrial volume loading relies on atrial stretch rather than increased atrial pressure.[83]

Stretch-induced AF is suppressed by the stretch-activated channel (SAC) blocking actions of gadolinium (Gd^{3+}) and a specific tarantula venom toxin, GsMTx-4, in the absence of ERP changes.[84,85] *Figure 4* illustrates putative mechanisms of AF promotion and ERP shortening in response to acute atrial stretch. Non-selective cationic SACs are permeant to Ca^{2+}, Na^+, and K^+, whereas other SACs are selective for K^+ and, possibly, Cl^-.[85] It is possible that K^+-selective SACs, which are resistant to Gd^{3+} and GsMtx-4, shorten the AP under stretch.[85] The direct mechanisms by which atrial stretch promotes AF are still poorly understood. However, non-selective cation SACs may promote AF by causing Ca^{2+} overload.[86,87] Activation of SACs elevates intracellular Ca^{2+} via increased Ca^{2+} influx.[86] Since SACs are permeable to Na^+, increased intracellular Na^+ leads to the activation of the NCX and the accumulation of further intracellular Ca^{2+}.[86]

Acute atrial stretch also promotes AF *in vivo*; however, changes in ERP depend on the method of stretch.[88,89] Balloon catheter-induced LA dilatation in dogs shortens atrial ERP and lengthens atrial conduction time.[88] Saline infusion-induced RA volume overload in anaesthetized dogs lengthens atrial ERPs (greater effects in the thin free wall than in the thick-walled crista terminalis), producing ERP dispersion.[89] Ectopic drivers originating at the PV region may underlie stretch-induced AF.[90]

Aconitine-induced atrial fibrillation

Aconitine is a neurotoxin that opens tetrodotoxin-sensitive cardiac Na^+ channels, causing triggered activity and AF. Acontinine-induced AF can be used for drug screening: e.g. the I_{KACh} blockers tertiapin and NIP-151 have been shown to dose-dependently convert AF to sinus rhythm in the aconitine model.[91,92]

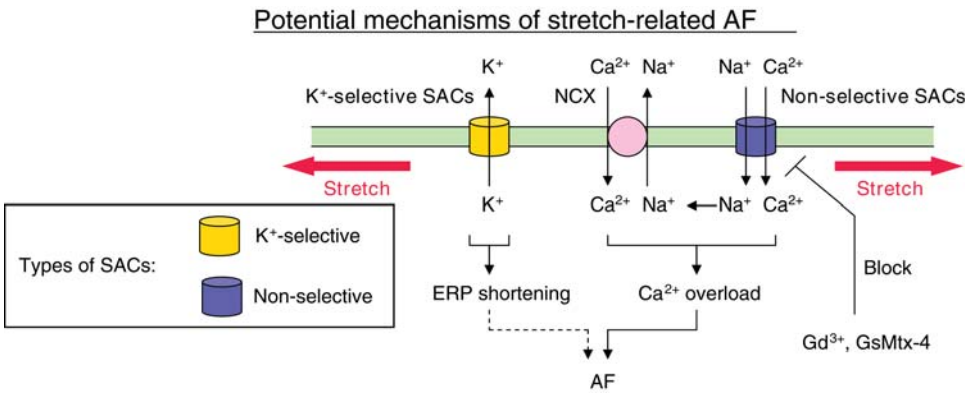

Figure 4 Potential ionic mechanisms of acute stretch-induced atrial fibrillation. SAC, stretch-activated ion channel; ERP, effective refractory period; GsMtx-4, Grammostola spatulata mechanotoxin 4; AF, atrial fibrillation; NCX, Na^+-Ca^{2+} exchanger.

Acute ischaemia

Coronary artery disease is a significant risk factor for AF; however, the underlying mechanisms remain to be elucidated.[93] Isolated acute atrial ischaemia, produced in dogs by occluding an atrial arterial branch that does not provide blood flow to the ventricles, increases AF duration.[93] Severe conduction slowing occurs in the ischaemic zone, and histological examination reveals ischaemia-induced necrosis at sites of conduction delay.[93] Ischaemia-induced atrial conduction slowing and AF promotion are suppressed by β-adrenoceptor blockade, Ca^{2+} current inhibition, and induction of heat shock protein by geranylgeranylacetone,[94] whereas Na^+ or K^+ channel blockers appear ineffective, indicating that cardioprotective manoeuvres are more effective in ischaemic AF than more traditional AF-suppressing drugs.[94,95] Rotigaptide, a peptide that enhances gap junction conduction, improves conduction in several AF models but only suppresses ischaemia-induced AF promotion.[96] Together, these results suggest that acute atrial ischaemia may be an important underlying mechanism of AF in the context of coronary artery disease and may have a specific therapeutic profile.

Autonomic models

Vagus nerve stimulation readily promotes AF induction and maintenance. This method induces stable AF at a relatively low cost, and it has been useful for *in vivo* screening of potential anti-arrhythmics.[97,98] Dogs are typically used but other species, like sheep, also show clear vagal AF promotion.[99] Vagally mediated AF results from acetylcholine activation of the K^+ current I_{KACh}, which abbreviates APD and ERP.[97,98] Vagal stimulation also increases ERP heterogeneity, which correlates well with the duration of inducible AF.[9] Acetylcholine perfusion has also been used to induce AF *ex vivo*,[100,101] and optical mapping experiments in Langendorff-perfused sheep hearts reveal a decrease in the dominant frequency gradient of AF from LA to RA.[100] Rotors are more often observed in the LA; dose-dependently increasing in frequency and stability with increased acetylcholine concentration.[101] This may be explained by a greater abundance of the inward rectifier channel subunits underlying I_{KACh}, Kir3.1 and 3.4, and larger I_{KACh} current density in LA cardiomyocytes.[101] Computer simulations show that either a single dominant rotor or multiple re-entrant spiral generators can initiate fibrillatory activity in this model.[102,103] At lower acetylcholine concentrations, extensive spiral wave meander prevents the emergence of single stable rotors. Therefore, prolonged fibrillatory activity only occurs when atrial size is large enough to permit a sufficiently large number of rotors that simultaneous extinction of all is unlikely.[103] At higher acetylcholine concentrations, single primary rotors anchored in low acetylcholine concentration zones maintain activity, and substrate dimensions are not critical.[103]

Sympathetic nerve stimulation is less effective than vagal activation in promoting AF for intensities that produce similar effects on ERP and wavelength.[104] The discrepancy may be due to the lack of sympathetic effect on the hetereogeneity of repolarization.[104] In a canine model with MI and complete AV block, sympathetic hyperinnervation induced by either infusion of nerve growth factor or subthreshold electrical stimulation of the left stellate ganglion produces paroxysmal AF.[105] A recent study revealed a relationship between autonomic activation and paroxysmal AF for arrhythmias observed after several weeks of rapid LA pacing.[106] Immunohistochemistry indicates nerve sprouting and sympathetic hyperinnervation, and continuous autonomic nerve activity monitoring reveals simultaneous sympathovagal discharges preceding the onset of atrial arrhythmias.[106] Cryoablation of extrinsic sympathovagal nerves eliminates paroxysmal AF and AT, suggesting a causal relation between simultaneous sympathovagal discharges and arrhythmias.[106]

The importance of the PVs in the substrate for AF has been highlighted by several studies utilizing autonomic stimulation.[107,108] Optical mapping experiments showed that acetylcholine-induced sustained tachycardias in an *ex vivo* canine PV preparation are due to re-entrant activity.[107] In anaesthetized dogs, stimulation of autonomic ganglia at the base of the right superior PV converts PV firing into AF.[107] Autonomic ganglion stimulation reduces the number of premature stimuli required for AF induction.[108]

Table 2 Genetic models of clinically relevant atrial fibrillation

Clinical counterpart	Predisposing cardiac factors	Genetically engineered models
Atrial pathology in CHF	Atrial fibrosis, atrial dilatation, connexin remodelling, AV block	Constitutive TGF-β1 activation,[127] overexpression of ACE[128] or JDP2[129]
Atrial electrical remodelling	Bradycardia, delayed conduction, AV block, accelerated repolarization, decreased I_{CaL}, reduced Ca^{2+} transients, impaired Ca^{2+} handling	Cx40,[130] $Ca_v1.3$,[132,133] KCNE1,[136] or NUP155[137] knockout; Kir2.1[131] or KCNE1–KCNQ1[135] overexpression; FKBP12.6 knockout,[139] R176Q mutation of RYR2; RYR2–S2814A knock-in[140]
Dilated cardiomyopathy	Dilated cardiomyopathy, atrial dilatation, atrial fibrosis, bradycardia, AV block, connexin remodelling	Rho-A,[117] MURC,[118] or TNF-α[119,120] overexpression
Hypertrophic cardiomyopathy	Ventricular hypertrophy, atrial dilatation, fibrosis, bradycardia, decreased connexin-40	Junctin,[121] junctate-1,[122] CRE modulator,[123] Rac1,[124] HopX,[125] or Gaq[126] overexpression

Pulmonary veins may be vulnerable sites for vagally induced arrhythmogenesis; however, intact PVs are not required for the maintenance of experimental cholinergic AF, since vagally stimulated sustained AF still occurs in dogs with electrically isolated PVs.[109]

Small animal models of atrial fibrillation

Rat models

Myocardial infarction

Left coronary artery ligation in rats leads to LV dysfunction, LA dilatation, and LA fibrosis. Mild-severe heart failure can be induced 3 months post-infarction. Elevated MMP activity and protein expression appear to be involved in ECM remodelling.[110,111] Fibroblasts and collagen accumulate between cardiomyocytes,[110,112] forming a potential substrate for AF; however, AF is not evident from ECG recordings in MI rats.[110–112] In addition, connexin-43 is redistributed to lateral cell borders, but there is little evidence to suggest that lateralized connexins form functional gap junctions.[112]

Spontaneously hypertensive rats

The spontaneously hypertensive rat, a genetic model of systemic hypertension, develops a substrate for AF that includes increased LA interstitial fibrosis in the absence of changes in ERP.[113,114] Hypertension-induced activation of the renin–angiotensin system may explain this observation, since angiotensin-II receptor blockade reduces fibrosis.[114]

Glycolytic inhibition

Sarcoplasmic reticulum uptake of intracellular Ca^{2+} is ATP-dependent, and the addition of sodium pyruvate to glucose-free perfusate selectively inhibits glycolysis in isolated rat hearts.[115] This model yields spontaneous AF in old (~28 months) but not young (~4 months) rats. Atrial fibrillation is most likely mediated by Ca^{2+}-handling abnormalities, since EAD-induced triggered activity occurs at the LA–PV junction. In old rats, EADs originating in this region are associated with prolonged APD and elevated diastolic $[Ca^{2+}]$.[115]

Asphyxia

An *in vivo* asphyxia rat model is associated with a high incidence of burst pacing-induced AF that may include elevated vagal and/or sympathetic nerve discharge as an underlying mechanism.[116] Atrial fibrillation inducibility and duration are reduced by sympathetic inhibition, e.g. by amiodarone or sotalol, although no change in plasma catecholamine values was reported.[116]

Genetic mouse models

Transgenic mouse models of AF (*Table 2*) are often associated with a substrate for atrial conduction abnormalities, with electrophysiological abnormalities that accelerate atrial repolarization, or with RYR2 dysfunction, which promotes triggered activity and focal discharges. Atrial fibrosis and AF are linked to genetic models of several clinical paradigms, e.g. dilated cardiomyopathy, hypertrophic cardiomyopathy, and AT remodelling. Dilated cardiomyopathy is induced by overexpressing the GTPase RhoA, muscle-restricted putative coiled-coil (MURC) protein, or tumour necrosis factor (TNF)-α.[117–120] RhoA is involved in the regulation of the *actin* cytoskeleton in actin stress fibre formation,[117] and is further activated by MURC overexpression.[118] Tumour necrosis factor-α overexpression also downregulates connexin40 (Cx40), which contributes to AF promotion.[119,120]

Hypertrophic cardiomyopathy can be mimicked by modulating SR Ca^{2+} handling.[121–126] Overexpression of junctin, a calsequestrin-binding protein which forms a Ca^{2+} release complex, or junctate, a Ca^{2+}-binding protein located on the SR membrane and closely associated with SR Ca^{2+} storage capacity,[122] produces atrial dilatation, fibrosis, and fibrillation.[122,123] Ventricular hypertrophy and atrial enlargement are produced by changes in the transcription factor cAMP-response element (CRE) modulator.[123] Overexpression of the GTPase, Rac1, which regulates NADPH oxidase activity, is implicated in the generation of oxidative stress and fibrosis.[124] Connexin-40 remodelling in the presence of cardiac hypertrophy is observed in a mouse model in which the transcriptional activity of serum response factor is inhibited by homeodomain-only protein X (HopX).[125] Overexpression of activated cardiac Gαq, an important mediator of α-adrenoceptor, angiotensin II, and endothelin

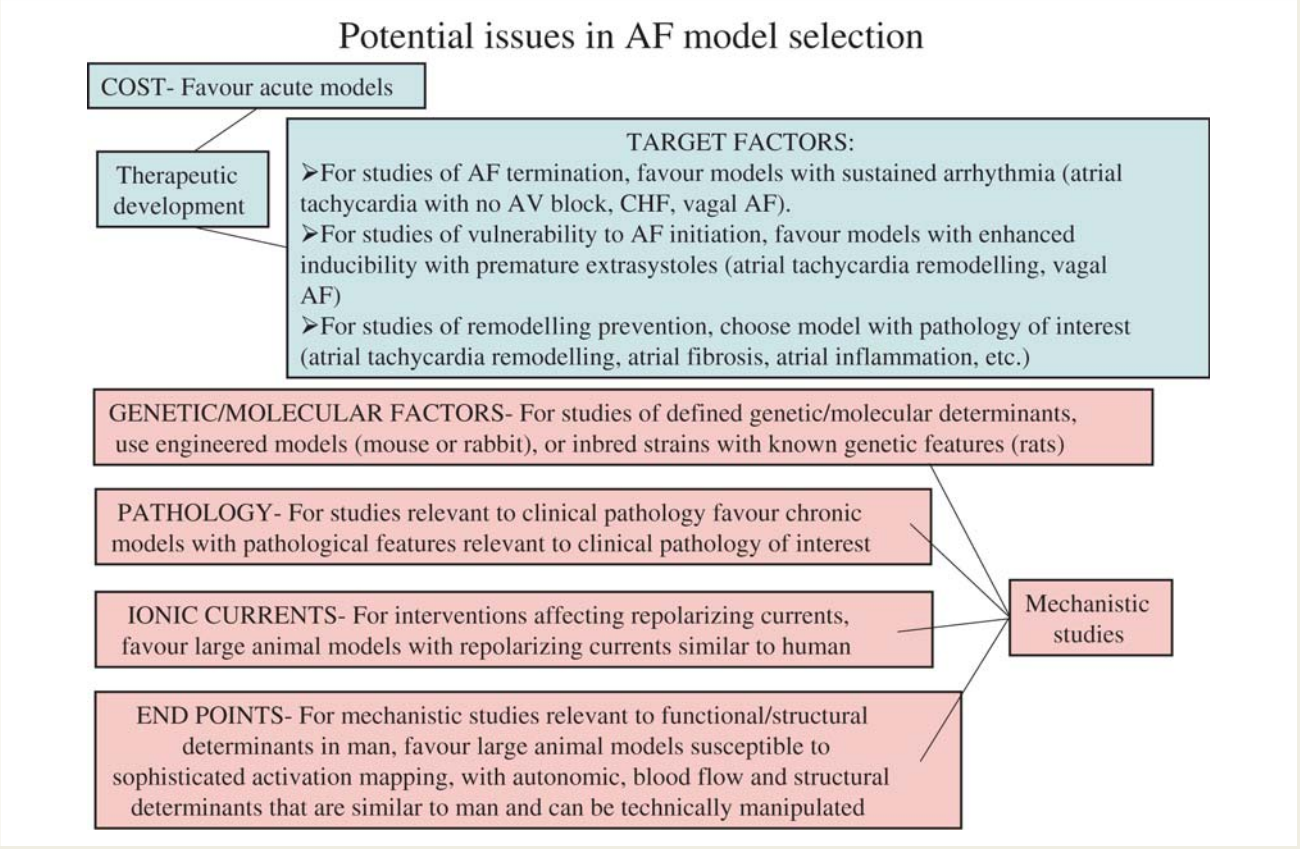

Figure 5 Considerations in selecting an animal model of atrial fibrillation (AF) for study. AV, atrioventricular; CHF, congestive heart failure.

effects, is also associated with ventricular hypertrophy, atrial dilatation, fibrosis, and prolonged atrial arrhythmias.[126]

Atrial structural remodelling-related genetic models of AF can be produced without accompanying ventricular dysfunction.[127–129] Transforming growth factor-β1,[127] ACE,[128] and JDP2[129] overexpression produce selective atrial changes. JDP2 is a transcription factor that represses transcription from promoters that contain certain elements, such as CRE. Indeed, JDP2 overexpression has a similar outcome to overexpression of CRE modulator.[129]

Models that involve cardiac electrical remodelling in the absence of cardiomyopathy include targeted deletion of Cx40[130] and modulation of proteins underlying cardiac ion currents such as I_{CaL}, I_{Ks}, and I_{K1}.[131–136] Deletion of Cx40 slows atrial conduction and increases vulnerability to AF.[130] Overexpression of Kir2.1, the principal protein underlying I_{K1}, markedly decreases APD and causes spontaneous AF.[131] Deletion of the $Ca_v1.3$ (α_{1d}) subunit underlying I_{CaL} has similar consequences and also leads to reduced intracellular Ca^{2+} transients and Ca^{2+}-handling abnormalities.[132,133] Congenital AF occurs with gain-of-function mutations in KCNQ1, and co-assembly of the KCNQ1-α and KCNE1-encoded β-subunit is required for the arrhythmogenic increase in I_{Ks} during repetitive stimulation.[134] Overexpression of KCNE1–KCNQ1 fusion protein produces prolonged atrial arrhythmias in response to β-adrenergic receptor stimulation.[135] Surprisingly, KCNE1 deletion leads to faster-activating I_K in atrial myocytes, abbreviated APD, and enhanced susceptibility to AF.[136] Genetic mapping of congenital AF led to the discovery that a mutation in the *NUP155* gene encoding a nucleoporin (member of nuclear pore complex) alters nucleocytoplasmic transport. Homozygous $NUP155^{-/-}$ knockout mice die during embryogenesis, but heterozygous $NUP155^{+/-}$ mice have abbreviated APs and spontaneous AF, although the mechanism linking nuclear pore complex abnormalities and AF are yet to be elucidated.[137]

Sarcoplasmic reticulum Ca^{2+} leak during diastole leads to DADs and triggered activity.[138] Atrial RYR2s are part of a macromolecular complex that includes protein kinase A, Ca^{2+}/calmodulin protein kinase II (CaMKII), protein-phosphatases, calmodulin, and FK-506-binding protein (FKBP12.6).[138] Mice lacking RYR2-stabilizing FKBP12.6 show larger SR Ca^{2+} leak and longer inducible AF episodes.[139] Genetic inhibition of CaMKII phosphorylation of RYR2 reduces AF inducibility.[140] Thus, mice models with RYR2 dysfunction are valuable for studying AF associated with triggered ectopic activity.

Use and abuse of animal models of atrial fibrillation

There is no such thing as a 'perfect' animal model of AF, any more than there is a single clinical mechanism of AF. The pathophysiology of AF in man is a complex function of the patient's cardiac status (including the presence or absence of organic pathology),

genetic predisposing factors, and environmental factors (potentially including diet, drug exposure, atmospheric pollutants, stress levels, toxic substances like tobacco or drugs of abuse, etc). Any animal model reproduces at best a very limited component of the pathophysiologic spectrum of clinical AF.[141] In choosing to work with an animal model, it is important to keep in mind the question(s) being asked. *Figure 5* presents some potential considerations and their application. Considerations that pertain particularly to therapeutic product development (drugs, ablation procedures, devices, etc.) are in grey boxes, whereas issues that pertain more typically to mechanistic studies are shown in stippled boxes. However, depending on the specific questions being asked, any or all of the considerations indicated may come into play.

Conclusions

Important insights into the mechanisms underlying AF have been made possible through the use of animal models. These have improved our understanding of the mechanisms of AF in the clinical setting and have allowed for the development of novel therapeutic approaches. It is reasonable to hope that further experimental studies, in combination with careful clinical investigation, will soon provide the means to control this challenging clinical problem.

Acknowledgements

The authors thank France Thériault for secretarial assistance.

Funding

This study was funded by The Canadian Institutes of Health Research (MOP 44365, MGP 6957), the Fondation Leducq (ENAFRA Network, 07/CVD/03), the German Federal Ministry of Education and Research (Atrial Fibrillation Competence Network grant 01Gi0204), the German Research Foundation (DFG, Do 769/1-3), and the MITACS Network. G.M. is a Heart and Stroke Foundation of Canada research fellow.

Conflict of interest: none declared.

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