Review

Animal models for arrhythmias

David J. Milan, Calum A. MacRae*

Cardiovascular Research Center, Massachusetts General Hospital, 149 13th Street, Charlestown, MA, USA
Harvard Medical School, Boston, MA, USA

Received 15 February 2005; received in revised form 31 May 2005; accepted 6 June 2005

Time for primary review 14 days

Abstract

The complex pathophysiology of human arrhythmias has proven difficult to model. Direct correlations between the traditional arrhythmia mechanisms, including abnormal excitability, conduction, or repolarization and underlying molecular or cellular biology are poorly defined, as the primary etiologies of many human arrhythmias remain unknown. Since the causes of several arrhythmic syndromes have been identified, genetic models reproducing the mechanisms of these arrhythmias have become feasible. Initial murine modeling has revealed that in many cases the pathophysiology of the respective human disease is more complex than had been suspected. Insights from human genetic studies and animal models strongly suggest that the primary molecular defects may contribute at many stages in the causal chain leading to arrhythmia. The comprehensive analysis of each arrhythmia will require knowledge not only of the membrane effects of the primary defects, but also downstream intracellular signals, the developmental results of these perturbations, and the integration of compensatory responses and environmental factors. Precise modeling will require not only the mutation of specific residues in known disease genes, but also the systematic study of each of the many steps in arrhythmogenesis. Ultimately, such models will enable unbiased screens for disease mechanisms and novel therapies.

© 2005 European Society of Cardiology. Published by Elsevier B.V.

Keywords: Arrhythmias; Modeling; Genetics; Drugs

1. Introduction

Arrhythmias remain among the most challenging human disorders to diagnose and to treat [1]. A substantial number of clinical arrhythmias are fatal at presentation or result in a catastrophic event such as resuscitated ‘sudden death’ or stroke. The paroxysmal nature of many arrhythmias also has made the efficacy of antiarrhythmic therapies difficult to assess. Decades of human observation and animal experimentation have led to the proposal of a broadly accepted, mechanistic framework for most arrhythmias (see Fig. 1). Empiric therapies directed at individual steps in this arrhythmogenic framework have in some instances reduced both the morbidity and mortality associated with clinical arrhythmias, but even the most successful of these interventions target events several steps downstream of the primary defects. It is likely that many of the primary defects in human arrhythmic syndromes act through several of the traditional mechanisms at multiple time points. The identification of the primary causes of many human arrhythmias has opened the possibility of genetic models capable of reproducing many or all of the events in the pathophysiology of these disorders.

1.1. Arrhythmogenesis

The concept that membrane function may be perturbed by primary abnormalities of individual channel proteins or dysregulated channel expression is well established from seminal human genetic studies [2]. Increasing evidence now implicates abnormalities not only of the ionic currents

* Corresponding author. Cardiovascular Research Center, Massachusetts General Hospital, 149 13th Street, Charlestown, MA, USA. Tel.: +1 617 726 4343; fax: +1 617 726 5086.
E-mail address: cmaacrae@partners.org (C.A. MacRae).
carried by the mutated channels, but also disruption of signaling pathways and adaptor proteins in cardiac arrhythmias [2–4]. Similarly, metabolic abnormalities and mitochondrial dysfunction also may affect the electrical stability of the cardiomyocyte [5,6].

At a cellular level, specialized populations of pacemaker, nodal and conduction system cells long have been recognized, but their roles in arrhythmogenesis are only now beginning to be explored [7,8]. Perhaps the best-characterized source of myocardial heterogeneity is infarct-related scar [9], but elegant ex vivo models have demonstrated that there are physiologic heterogeneities in myocyte electrophysiology between endocardium, mid-myocardium, and epicardium [8]. The normal and pathological roles for these and other myocardial cell subpopulations have yet to be fully understood.

Superimposed on any arrhythmogenic substrate are a host of transient physiologic stimuli including autonomic activity, immune and environmental triggers each of which may contribute to the initiation of a clinical event [5,10,11]. Perhaps the most important modifiable environmental contributors to clinical arrhythmias are drugs. Many cardioactive drugs, particularly antiarrhythmic agents themselves, are in some situations proarrhythmic [12]. To generate a framework for the treatment and prevention of rhythm disorders we must aim to describe and model the relationships between the multiple etiologic factors leading to the specific arrhythmia.

1.2. Arrhythmia models

Spontaneous arrhythmias have proven difficult to model in intact animals, in part due to our incomplete understanding of the causal chain of events for most clinical rhythm disorders. Intrinsic to the multi-step model outlined above is the fact that, for most arrhythmias, individuals may exhibit several of the elements of an arrhythmogenic state, but rarely experience a clinical event. The ideal animal model would recapitulate not just individual components of the causal chain leading to an arrhythmia, but each step along the way. Thus, not only the final rhythm disturbance, but the antecedent interactions between substrate, modifiers and chance itself would be accessible. The study of genetic animal models will not only enable the development of a rigorous understanding of the pathophysiology of arrhythmias, but also allow systematic approaches to the discovery and testing of novel drugs, devices or interventions.

In this review we will categorize models by specific arrhythmia, although in reality several rhythm disturbances may coexist in a single model (as in human disease states). We will describe only genetic models in intact organisms; other in vivo and ex vivo models have been reviewed extensively elsewhere [13–16]. We have not attempted to be comprehensive, but rather have focused on the distinctions between empiric models, that capture one or more aspects of the underlying biology, and mechanistically faithful models that attempt to recapitulate the primary mechanism of disease in order to more completely model the entire chain of causality. We also will describe several spontaneously occurring animal arrhythmias, which may or may have the same pathophysiology as orthologous human disorders.

2. Sinoatrial disease

There has not been an extensive effort to model sinus node disorders in animals. Nevertheless, several genetically modified mice have shed light on the biology both of the normal and diseased sinus node. Classic interpretation of sinoatrial disease as a focal degenerative disorder of the anatomic sinus node does not explain the distinctive
associations of this syndrome with specific mutations causing structural human heart disease [17]. Murine models of these same human genetic disorders also exhibit sinoatrial arrhythmias. A ‘knock-in’ mutation of the mouse α-myosin heavy chain gene (the ortholog of a human hypertrophic cardiomyopathy mutation) results in sinus node failure [18]. These data suggest that either the hypertrophic process itself or a compensatory downstream response affects not only contractile myocytes but also specialized pacemaker and conduction tissue. Similarly, metabolic disruption through glycogen storage disorders or mitochondrial diseases often results in sinus bradycardia or sinus arrest, and here again related murine models reproduce these arrhythmias in the context of widespread myocardial dysfunction [19].

Involvement of the sinus node is highly dependent on the specific mutations introduced. An ankyrin B null mouse exhibits significant sinoatrial dysfunction as might be predicted from the clinical features seen with related human mutations in the long QT (LQT) 4 syndrome [20]. Several mice modeling other forms of the LQT syndrome exhibit sinoatrial disease that is not observed in the human disorders [21]. Importantly, when ‘knockin’ models of specific mutant alleles in these same genes are made, the mice recapitulate the repolarization phenotypes without any associated pleiotropy, suggesting that the human disorders are the result of very specific perturbations of the pathway(s) in which the mutant proteins participate [22,23].

The widespread availability of mouse telemetry has allowed heart rate to become part of the phenotyping repertoire used for most genetically engineered mouse lines. As a result many genes, some expected and some unexpected, have been found to play roles in the normal regulation of heart rate in the mouse. While the role of the pacemaker channels (hyperpolarization activated cation channels) in the regulation of heart rate integrates readily into models of pacemaker biology [24,25], the effects of several other genes are more difficult to explain. Sinus rhythm abnormalities in many of these models may reflect non-specific effects of the mutant protein rather than perturbations of pathways peculiar to pacemaker or conduction tissue.

Finally, spontaneous genetic conditions do occur in animals and may model human syndromes. Several dog breeds exhibit inherited forms of sinoatrial disease with important parallels for human disease. Middle-aged miniature Schnauzers often present with syncope due to sinus pauses in the context of structural heart disease similar to the myxomatous degeneration of the mitral valve seen in humans [26]. In some Spaniel kindreds familial atrial standstill co-segregates with limb-girdle myopathy; a disorder reminiscent of the Emery–Dreifus syndrome [27]. The development of genetic and genomic resources for the dog will enable cloning of the genes responsible for these disorders and allow the parallels with human syndromes to be defined at a molecular level.

3. Atrial fibrillation

Atrial fibrillation (AF) is the most common clinical arrhythmia and has been associated with many forms of human heart disease. It is possible to induce AF in larger animals with sustained or repeated rapid atrial pacing [14,16,28,29]. These models originally were thought to be removed from the initiation of human AF [30], but recent work implicating autonomous drivers suggests the pathophysiology may be more representative than previously recognized [31]. Nonetheless little is known of the basis for interindividual variability in the threshold for the arrhythmia.

3.1. Atrial remodeling

One of the key insights gained from pacing models is the complex interaction between AF and the underlying atrial myocardial substrate [15]. AF induces electrical, contractile and molecular changes in the atrial myocytes that arise within minutes [32]. These changes include shortening of the atrial effective refractory period and loss of rate adaptation [33–35]. The levels of expression and subcellular distributions of numerous ion channels, connexins and calcium handling proteins are altered by AF [36–40]. This remodeling process appears in many instances to result from compensatory responses to increased intracellular calcium, but several other mechanisms also may be involved. Pretreatment with calcium channel blockers mitigates some of this remodeling, but may actually have a net proarrhythmic effect [14,41]. The ultimate result of atrial remodeling is sarcomere loss, patchy apoptosis, focal inflammation and fibrosis in the atria: closely paralleling findings from human atria [11,15,42]. The contributions of calcium influx, inflammation and the renin-angiotensin-aldoosterone system to remodeling now are being unraveled in animal models and in humans.

While large animal models for AF have revealed little of the nature of the primary substrate for spontaneous forms of the arrhythmia, they nevertheless have proven of great clinical utility [31,43–45]. Importantly, while these insights are invaluable, reports of permanent AF are rare in these models, and in the absence of heart failure most animals spontaneously return to sinus rhythm [40,46]. Thus in addition to shedding light on atrial remodeling in AF, these models also highlight the concept that a primary predisposition likely underlies recurrent initiation and maintenance of AF in humans.

3.2. Transgenic and ‘knockout’ models of AF

Atrial fibrillation is seen in several genetically modified mouse lines [47]. Bradycardia and AF have been documented in mice overexpressing (over 20 fold above normal) the sarcoplasmic reticulum (SR) protein junctin in the myocardium [48]. These findings were associated with profound
myocardial hypertrophy, significant downregulation of other SR proteins, and prolongation of action potential duration (APD). Spontaneous AF has also been reported in several other transgenic mice, but in each case in the context of over-expression of proteins known to be important in cardiomyocyte function [49–51]. These models can be difficult to interpret as, with the exception of single families, the genetic basis of human AF remains largely unknown and the targeted over-expression of nearly any protein in the heart may have ill-defined “toxic” effects [52,53].

It is possible to glean mechanistic insights even while the genetic basis of human AF is unknown. For example, mice null for the I(KACh) channel, which mediates much of the cardiac effect of vagal stimulation, exhibit markedly reduced vulnerability to the induction of AF when compared with wild-type controls [54]. These data further buttress a key role for vagally mediated effects in the pathogenesis of atrial fibrillation, and raise the possibility that I (KACH) blockers could have a role in the treatment of the arrhythmia.

3.3. Disease models of AF

A rate-limiting step at present is knowledge of the primary causes of AF in humans. Ultimately, faithful genetic models of human disease, precisely recapitulating the molecular defects through homologous recombination or ‘knock in’ methods, will be generated [22]. The size and electrophysiological peculiarities of the mouse heart may preclude direct modeling of AF in this organism, but precise animal models have assuaged similar concerns in the study of LQT [61]. In any event techniques for the genetic manipulation of other animals are under development [55].

4. Atrial flutter

Large animal models have been remarkably helpful in understanding the macro-reentrant nature of atrial flutter. Human studies by Waldo and colleagues following open-heart surgery established the importance of entrainment in understanding reentrant rhythms [56]. Multisite mapping in the dog revealed evidence of atrial activation around the tricuspid orifice, demonstrated the presence of an excitable gap, and established the need for 2 barriers for flutter initiation and propagation [57,58]. Building on these insights ablation has been developed as a definitive treatment for atrial flutter in man. To date no spontaneous or genetically engineered models of atrial flutter exist.

5. Atrioventricular block

Long-term monitoring of the murine cardiac rhythm has lead to increased recognition of conduction system disturbances in genetically altered mice. Not unexpectedly, the majority of transgenic mouse models are bradycardic at the time of death [59]. Caution must be exercised as hypoxia, acidosis, or other metabolic derangement may result in secondary bradycardia and AV block. With these caveats in mind, several models of AV block have been described.

5.1. Transgenic and ‘knockout’ models of AV block

Atrioventricular block has been reported in several mice engineered to over-express different proteins in cardiac tissue. Once again, it can be difficult to discriminate between toxicity of the transgene and a direct role in AV conduction system development or function. Both angiotensin I receptor and angiotensin converting enzyme related carboxypeptidase (ACE2) over-expression lead to first degree heart block, broadening of the QRS complex, and premature death [49]. Several knockout mouse models have resulted in the development of AV block. The transcription factor HF-1b is expressed in ventricular myocytes and the conduction system. Knockout of HF-1b results in sinus pauses, sinus bradycardia, and second or third degree AV block. These mice also develop spontaneous episodes of ventricular tachycardia. Analysis showed abnormal expression and distribution of KCNE2, connexin 40, and prolongation of the APD that was at least in part due to diminished IKs [60]. Connexin 40 is expressed throughout the mouse atria and conduction system and is directly implicated in cell-to-cell coupling and impulse propagation. Therefore it is not surprising that the connexin 40 knockout mouse has a prolonged PR interval with documented slowing of conduction in the AV node, and bundle-branch system [61]. Several elegant papers have delineated the conduction deficits in these knockout models. There is evidence of tetralogy of Fallot and double outflow right ventricle on some genetic backgrounds [62], but concrete parallels with human disease are unclear.

Murine models also may be employed to answer specific developmental questions. A ventricle-restricted knockout of the cardiac transcription factor Nkx2.5 results in an underdeveloped AV node and His bundle with late onset of complete heart block that is associated with dropout of conduction cells [63]. While Nkx2.5 previously was known to be essential for early steps in cardiac development, this model suggests an ongoing requirement for this transcription factor for the persistence of the cardiac conduction system.

5.2. Disease models of AV block

In humans, mutations in the cardiac sodium channel, SCN5A can lead not only Brugada syndrome and the LQT syndrome, but also result in isolated conduction system disease with AV block [64]. A mouse model of the latter syndrome has been developed in which the mutant animals
have one copy of the SCN5A deleted. These haploinsufficient mice develop 2:1 AV block and display decreased myocardial conduction velocities [65]. These mice and compound heterozygotes for SCN5A missense mutations exhibit fibrosis reminiscent human disease [66].

Myotonic dystrophy is the most common form of muscular dystrophy and is caused by the expansion of a trinucleotide repeat on human chromosome 19 [67]. Cardiac manifestations include varying degrees of AV block as well as sudden death. Dispute over the molecular mechanism of the cardiac defects has been addressed by the development of a mouse knockout model of the myotonic dystrophy protein kinase (DMPK). Mice deficient in DMPK display first, second and third degree AV block, while the haploinsufficient mice show first degree AV block [68]. By analogy to defects seen in the skeletal muscle of these mice, the mechanism of AV nodal pathology is thought to be due to alterations in the activation kinetics or amplitude of the ICa,L current.

A form of cardiac ‘hypertrophy’ caused by mutation of the gamma subunit of the AMP activated protein kinase is characterized by glycogen deposition in cardiac muscle, accessory pathways, and conduction system disturbances [69]. A mouse model carrying a mutation responsible for the human disease has been generated that faithfully reproduces the three characteristic traits found in the human disease [70]. In this model the annulus fibrosis that normally insulates the atria and ventricles is penetrated by glycogen filled cardiomyocytes that appear to be responsible for ventricular pre-excitation.

The generation of murine models of two immune forms of AV block has rendered the study of the genetic basis of differential sensitivity to this outcome feasible. A mouse model of Lyme disease and its cardiac effects exists and exhibits significant prolongation of the QRS complex, without frank AV block [71]. The degree of QRS prolongation correlated with the severity of the inflammation seen on pathology. Similarly, models of the neonatal AV block observed in newborns of mothers with lupus erythematosis have been generated. Congenital heart block in this model correlates with the presence of maternal antibodies to the SSA/Ro and SSB/La ribonucleoproteins. Pathologic evidence in human cases indicates that an inflammatory reaction perhaps mediated by transplacental transmission of maternal antibodies is responsible for injury to the fetal conduction system. Mice immunized with the SSA/Ro and SSB/La antigens give birth to pups with varying degrees of heart block [72].

Syncope and sudden death have been observed in certain purebred Pug dogs, and are associated with intermittent sinus pauses and paroxysmal second degree heart block on electrocardiographic study. The trait appears to be transmitted in a heritable fashion and at pathology affected dogs have been shown to have hypoplasia of the His bundle [73]. The AV node and cardiac valves were entirely normal, while there does appear to be a low incidence of atrial and ventricular septal defects. The genetic mutation responsible for this phenotype remains to be elucidated.

6. Sudden death

6.1. Ischemic VT

By now it is well-established that ventricular tachycardia (VT) in the setting of prior infarction is a reentrant arrhythmia arising from surviving myocytes in the border zone of the myocardial scar. The canine infarct model, along with studies in humans, provided useful insights leading to our current understanding of infarct related VT [74–76]. Among the advances specifically attributable to this model are the observations that individual cells in the infarct border zone have normal action potentials but display slow and discontinuous conduction — probably due to abnormal connexin expression [77]. The observed low amplitude, prolonged and multi-component electrograms that are characteristic of the VT circuit appear to be due to delayed activation of individual islets of myocytes in the border zone [78]. Studies in this model also highlighted the reproducibility of inducible VT, and provided support for the evolving concept of reentrant rhythms. There is some evidence that the susceptibility to sudden death in the context of ischemia has a heritable basis. Small animal models of infarct related VT are now also being developed and may enable studies of the basis of this diathesis [77,79,80]. More recently large animal infarct models have proven useful for the evaluation of new antiarrhythmic regimens, as well as invasive modalities including cryoablation, laser ablation, pacing and surgical techniques [81].

6.2. Hypertrophic and dilated cardiomyopathy including ARVC

Several arrhythmic syndromes including sudden death are prominent features of the human cardiomyopathies [17]. The genes for many of these disorders have been identified through human genetic studies and models in mouse or other organisms have been generated.

In only one of the murine models of hypertrophic cardiomyopathy (the α myosin R403G knockin) has sudden death been observed [82]. Electrophysiologic studies in these mice have demonstrated QT dispersion, heterogeneous ventricular conduction and inducible ventricular arrhythmias, all of which are more marked in female mice [18]. Limited vulnerability to ventricular arrhythmias at electrophysiologic study also has been demonstrated in a mouse model of myosin binding protein-C related hypertrophic cardiomyopathy, but homozgyotes for the same allele revealed dilated cardiomyopathy and markedly increased vulnerability to such arrhythmias [83]. These disparate phenotypes suggest that the complex overlap between
hypertrophic and dilated forms of cardiomyopathy may be recapitulated in murine models. Ongoing efforts using these mice are focused on determining the links between the primary mutations and specific arrhythmias. Spontaneous arrhythmias have not yet been demonstrated in other mouse cardiomyopathy models, but again many of these models are only indirectly reflective of human disease.

Up to 9 disparate disorders including Emery–Dreifuss muscular dystrophy (EDMD), Hutchinson–Gilford progeria syndrome (HGPS) and dilated cardiomyopathy with conduction-system disease can result from different mutations in the lamin A/C gene [84]. Interestingly, a ‘knock-in’ mouse that was created with the aim of producing an EDMD model instead displayed a phenotype most consistent with human HGPS, including reduction in growth rate, and disorders of bone, muscle, skin, and early death at 4 weeks [85]. Similarly, a lamin A/C mutation that causes EDMD was recently introduced into mice with the resulting phenotype mimicking not just EDMD, but also dilated cardiomyopathy and heart block [86]. These early investigations into the structure-function relationships of the lamin A gene in mice are just the first steps in unraveling how different mutations in the same gene can have such protean effects on the whole organism.

Occasionally genetically modified animals may suggest candidate genes for human conditions. Thus, the observation of arrhythmogenic right ventricular dysplasia in mice null for the desmosomal gene plakophilin 2 led to the identification of mutations in the human orthologs of the same gene in as many as 30% of probands with this disorder [87,88]. To date no arrhythmias have been observed in the mouse model, but once again these are homozygous null mice, while the human mutations are heterozygous and likely dominant negative in their mode of action.

Spontaneously occurring forms of cardiomyopathy exist in several species. Mutations in the delta-sarcoglycan gene cause both hypertrophic and dilated cardiomyopathy in the Syrian hamster, modeling human myopathies resulting from sarcoglycan defects [89]. A number of electrophysiologic abnormalities have been demonstrated downstream of these cytoskeletal abnormalities, but spontaneous arrhythmias have not been observed. Sudden death in the context of stress has recently been described, suggesting that modeling of arrhythmias may be feasible in this organism [90]. Hypertrophic cardiomyopathy is found in several cat breeds, and may well be as heterogeneous as human forms of this disease. In some cases there is associated syncope, but studies of arrhythmias are limited [91].

Several forms of dilated cardiomyopathy are found in dogs but in most cases direct correlations with specific human disorders have yet to be made [26]. Exceptions are an X-linked dystrophin-associated Duchenne-like cardiomyopathy in Golden Retrievers and the cardiomyopathy seen in Boxers which displays the overlap between dilated cardiomyopathy and arrhythmogenic right ventricular dysplasia seen in some human kindreds.

6.3. Long QT syndrome

The genetic dissection of the human long QT syndrome has been one of the major advances in our understanding of arrhythmias. Numerous mouse models have been generated to study LQT mechanisms, but their utility has been hampered by peculiarities of murine electrophysiology which lead to concerns over the applicability of the organism as a model of human cardiac repolarization. The mouse has a very rapid heart rate and a short action potential. Unlike humans, mouse repolarization is dominated by the transient outward current, Ito [92]. The arrhythmias that have been recorded in these LQT model mice are sinus bradycardia, type I second degree AV block, monomorphic and polymorphic VT. With a few notable exceptions, the ventricular arrhythmias are transient possibly reflecting the small size of the mouse heart. In general, these models have been useful tools for exploring the ionic mechanisms that determine repolarization in the mouse, but their applicability to human disease states has been somewhat less straightforward. We will briefly review the salient characteristics of these models and refer the reader to prior reviews for a more detailed analysis [59,93–95].

Mutations in the cardiac sodium channel SCN5A, can result in AV block, Brugada syndrome or the LQT 3 syndrome in humans. In what is perhaps the best genetic reproduction of the human disease, a long QT mouse model has been generated by ‘knock-in’ of the KPQ deletion of the SCN5A gene [23]. This same mutation in humans causes LQT3 with spontaneous arrhythmias especially at low heart rates. The ‘knock-in’ mice show prolongation of APD especially with sudden acceleration of the heart rate or with premature beats. They also display spontaneous polymorphic VT. An advantage of the ‘knock-in’ strategy is that the expression of the mutant gene has the best chance to mimic the spontaneously occurring mutation seen in humans and avoids potential non-specific effects of over-expression under an alternative promoter (Fig. 2).

More than 20 different mice with altered potassium channel expression have been generated. In models of LQT1 syndrome, both targeted deletion of KCNQ1 as well as overexpression of a dominant negative mutation have been achieved. Varying results have been reported with the knockout mice: one group reporting no effect on ECG [96], while another group has found QT prolongation in the mouse, but not in isolated hearts [21]. The dominant negative transgenic (Fig. 2), however, is reported to have QT prolongation and bradycardia, with reduced levels of both IKs and Ito, highlighting the issues of interpretation in knockout and dominant negative models [97]. Most recently several KCNQ1 “knock-in” mutant mice have been generated that show mutation specific QT prolongation that is more pronounced in homozygotes without observation of spontaneous arrhythmias [22]. The KCNE1 (minK) knockout mouse has been somewhat controversial with one of two groups reporting QT prolongation at slow heart rates that
paradoxically became shorter than controls at high heart rates while the other reported no effect on repolarization [98,99].

Mouse models of LQT2 include a KCNH2 (mERG) knockout mouse which is embryonic lethal in homozygous form. Overexpression of a KCNH2 dominant negative mutation yielded APD prolongation in myocytes isolated from transgenic mice under certain conditions, but no ECG changes or spontaneous arrhythmias [100]. A targeted deletion of mERG1b has been generated that lacks IKr in adult myocytes [101]. These mice show no QT prolongation but have abrupt episodes of sinus bradycardia, paralleling the cognate human knockout [102].

Mouse models of LQT4 have been created that have provided insights into novel mechanisms of arrhythmogenesis. Mice heterozygous for a null mutation in ankyrin-B display disruption in the subcellular distribution of the sodium pump, the sodium/calcium exchanger, and inositol-1,4,5-trisphosphate receptors (all ankyrin-B-interacting proteins), with reduced targeting of these proteins to the transverse tubules as well as lower overall protein levels [20,103]. There was no change in APD and subtle changes in QT interval that were likely due to delayed conduction. Changes in calcium handling and extrasystoles also were observed in adult mice and may play a role in arrhythmogenesis.

Mutations of IRK1 lead to decreased levels of IK1 and cause Andersen’s syndrome. Mice with a targeted deletion of IRK1 die shortly after birth due to cleft palate and have been shown to have prolonged cardiac action potentials, although no arrhythmias have been observed [104,105].

Given the importance of Ito and IKslow to mouse repolarization, several genetic models have been generated that disrupt these currents. These animals have proven useful in understanding the cellular electrophysiology of murine repolarization, but their direct relevance to human disease is somewhat less well established. These experiments have been reviewed extensively elsewhere [94].
6.4. Brugada syndrome

The Brugada syndrome is characterized by ST elevation in the right precordial leads and is associated with idiopathic VT/VF. Mutations in the cardiac sodium channel SCN5A have been identified in 10–30% of affected individuals [2]. In most cases studied to date, the mutations result in reduced sodium current in the myocardium, with several examples of mutant channels that fail to reach the sarcolemma due to defective intracellular trafficking.

While the ex vivo canine right ventricular wedge preparation has been central to our understanding of the conditions favoring transmural dispersion of repolarization and the associated triggering of arrhythmias, in vivo models so far have proven less useful [106]. The only genetic model of the Brugada Syndrome to date is the SCN5A knockout mouse. The heterozygous SCN5A null allele results in impaired AV conduction, delayed intramyocardial conduction, increased ventricular refractoriness, and ventricular tachycardia [65]. There is no report of abnormal ST segments in the right precordial leads, which may be due to the different repolarization currents that predominate in the mouse.

Repolarization abnormalities and sudden death in the absence of any myocardial structural pathology are found in the spontaneously occurring German shepherd canine model. Triggered activity has been directly implicated in the ventricular arrhythmia seen in this model and studies of the role of autonomic effects, heterogeneity of Purkinje fiber automaticity and cardiomyocyte development have been performed. Once again the precise parallels with human disease await a molecular understanding of the primary defect in these dogs.

6.5. Catecholaminergic polymorphic ventricular tachycardia

Human genetic studies have identified mutations in the genes for the cardiac isoforms of the ryanodine receptor (Ryr2) and calsequestrin (Casq2) in families in which syncope or sudden death occur in the context of exercise or other adrenergic stimuli [107,108]. There is some clinical overlap with the ARVC syndrome. In vitro data suggest that these mutations cause inappropriate calcium leak from the sarcoplasmic reticulum during diastole, which is accentuated by adrenergic stimuli [109]. The resultant delayed after depolarizations trigger ventricular arrhythmias. Animal models of this form of sudden death are just beginning to emerge. Indirect evidence of a role for calcium leak in arrhythmogenesis had been seen in mice null at the Fkbp12.6 gene encoding a protein known to regulate the ryanodine receptor [110]. Heterozygotes for a precise knock-in of a human Ryr2 mutation appear to recapitulate the key features of the arrhythmia and will be a powerful tool for understanding the disease mechanisms and testing potential therapies [111].

6.6. Drug-induced arrhythmias

Perhaps one of the most instructive uses of animal models is in the study of acquired arrhythmia resulting from drug-induced cardiac repolarization abnormalities [12,112]. This type of adverse drug event has been the focus of increasing regulatory attention, as several pharmaceutical agents have been withdrawn from the market in recent years, due to the risk of Torsade des Points (TdP) [12]. Drug-induced arrhythmias share much of their pathophysiology with the inherited LQT syndrome, and an inherited predisposition is suspected to play a significant role in their genesis. Virtually all of the drugs implicated in the genesis of TdP have been shown to have some inhibitory effect on Ik, in vitro. While the rate of activity in hERG assays may be as high as 10% of compound libraries (NIH Workshop on Predictive Drug Toxicology, 2004), some drugs with substantial Ik inhibition may have no clinical toxicity [113]. There are also examples of drugs with borderline preclinical testing that have profound toxicity problems [114]. This lack of predictive utility has led to the proliferation of model systems to screen for repolarization effects and other forms of cardiotoxicity.

6.6.1. Preclinical models for cardiac repolarization

Several approaches have been developed to prospectively identify drugs associated with a risk of TdP [13,115]. In vitro approaches have focused on direct or indirect measurements of Ik, in a variety of cellular systems, but these do not reliably recapitulate the presence of drug-induced proarrhythmia, suggesting that only an intact organism will allow the problem to be comprehensively understood.

A number of animal models including the anesthetized guinea pig, rabbit or trained dogs with chronic AV block have been used to allow more physiologic assessment of the risk of TdP prior to clinical testing [13]. The importance of repolarization reserve and the role of drug effects on other currents including Ica have recently been demonstrated in such models. These new data have led to the concept of systematic approaches capable of addressing all of the potential contributors to the arrhythmia. However, the models noted are low throughput and not amenable to genetic modification or screening for multiple drug–drug interactions. To overcome these limitations investigators have begun to explore the use of the larval zebrafish as a model for this and other human arrhythmias [116,117]. The zebrafish is genetically tractable, has been established as a model for studies of development and is now being explored for physiologic studies. Transgenesis is efficient, and genes can be knocked down readily using antisense morpholino technologies. If it is possible to develop representative models for arrhythmias in the fish, such an organism could
be used for unbiased approaches to the analysis of normal and abnormal cardiac electrical function.

7. Conclusions

Animal models have been central to the advances in our understanding of the mechanisms of human arrhythmia, but have also highlighted issues fundamental to all forms of disease modeling. In any complex process it is preferable to recapitulate as much of the causal pathway as possible, rather than to empirically model individual components. The mechanistic insights that have been gained over the last few decades emphasize the complexity of the pathogenesis of clinical dysrhythmia. Models capable of integrating the effects of both genetic and epigenetic modifiers will be required to dissect the multi-step pathways involved which include myocyte heterogeneity, channel processing, and downstream signaling, to name but a few.

Acknowledgements

The authors would like to thank Drs. Patrick Ellinor and Moussa Mansour as well as the three anonymous reviewers for their thoughtful comments and suggestions on the manuscript.

References

remodeling in atria of the goat. J Mol Cell Cardiol 2001;33: 2083–94.


[90] Zaitzky JJ, Redell JB, Tempel BL, Schwarz TL. The consequences of disrupting cardiac inwardly rectifying K+ current (I(K1)) as revealed by the targeted deletion of the murine Kir2.1 and Kir2.2 genes. J Physiol 2001;533:697– 710.


[95] Marks AR, Priori S, Memmi M, Kontula K, Laitinen PJ. Involvement of the cardiac ryanodine receptor/calcium release channel in...


