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Genetically engineered mice as a model for studying cardiac arrhythmias

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1. ABSTRACT

Sudden cardiac death due to ventricular tachyarrhythmias remains an unresolved problem, probably because the mechanisms responsible for the progression of cardiac disease to electrophysiological failure are poorly understood. Genetically engineered mice, the principal mammalian model for studying cardiac electrophysiology, have contributed to the understanding of the genetic, molecular and systemic mechanisms involved in the initiation and/or maintenance of cardiac arrhythmias leading to cardiac death, e.g. cardiac excitability, conduction velocity and refractoriness. Several murine models harbouring humangene mutations leading to electrical and structural cardiac disorders have been developed, including channelopathies (long QT syndrome), familial conduction disorders, cardiomyopathies and other inherited cardiac disorders. This article reviews the results of the main genetically modified mice addressing the genesis of cardiac arrhythmias and sudden cardiac death.

2. INTRODUCTION

Cardiac arrhythmias are a leading cause of morbidity and mortality. Sudden cardiac death (SCD) due to ventricular tachyarrhythmias, and less frequently to bradyarrhythmias and pulseless electrical activity, accounts for more than 300,000 deaths annually in the United States alone and its prevention remains an unresolved problem (1). Lethal arrhythmias that cause SCD are often associated with heart diseases, such as coronary artery disease, dilated nonischemic and hypertrophic cardiomyopathy and heart failure (HF), drugs and assorted noncardiac diseases, but may also occur in structurally healthy and frequently young individuals (1). The discovery of mutations in gene encoding cardiac ion channels has emerged as the basis for a variety of inherited and acquired cardiac arrhythmias and abnormalities in ion channel expression account for the high risk of lethal arrhythmias associated to ischemia, hypertrophy and HF (2-4). However, only 20% of the family members carrying one mutant allele of an

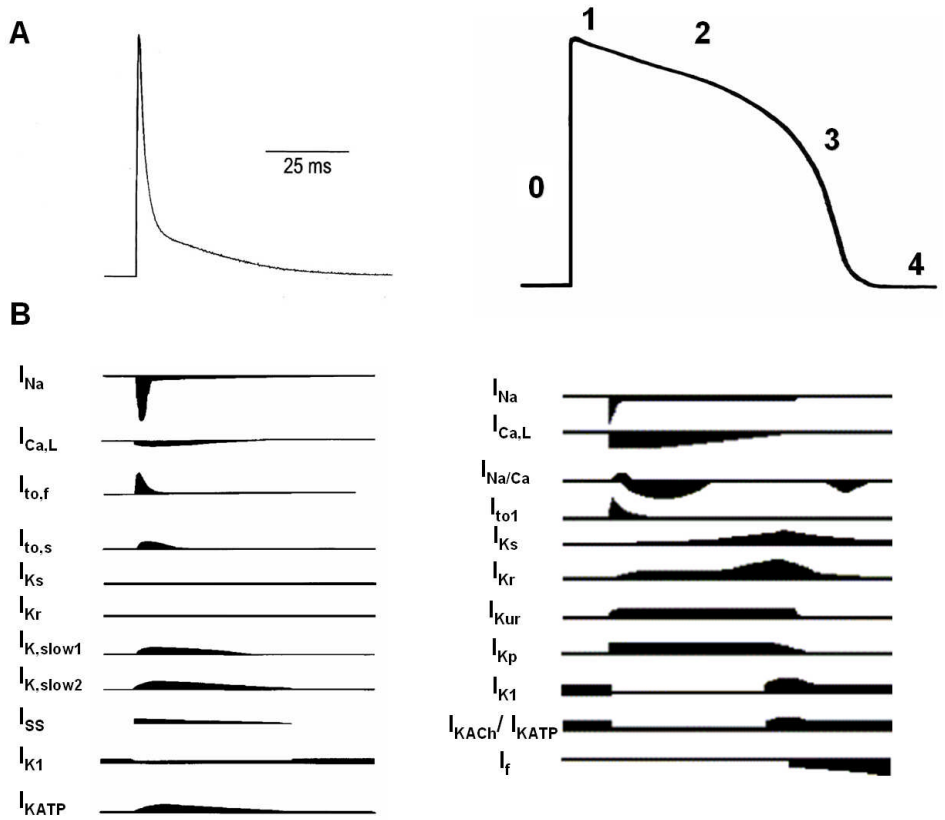

Figure 1. Schematic action potentials (A) and underlying ionic currents (B) in mouse (left) and human (right) cardiac myocytes. The amplitudes of the depolarizing (downward) and repolarizing (upward) currents are not on the same scales. Modified from Nerbonne (5) and Tamargo et al (4), respectively.

arrhythmia susceptibility gene display the SCD phenotype and diseases most frequently associated with fatal arrhythmias, including atherosclerosis and most cardiomyopathies, do not result from primary disorders in membrane current regulation, which suggest that additional pathways must be present to produce the SCD phenotype (2). Further evidence of the requirement of "additional hits" to produce lethal ventricular arrhythmias came from patients carrying mutations in some K^+ channels, who despite of a marked prolongation of the QT interval in the surface electrocardiograms (ECG) do not present ventricular arrhythmias or SCD. Thus, SCD is a complex phenotype that most likely results from the complex interactions among an underlying genetic predisposition to electrical instability, electrical (abnormal repolarization and conduction) and structural (hypertrophy, fibrosis, inflammation) remodeling, changes in autonomic tone, myocardial ischemia, metabolic abnormalities, electrolyte disturbances, enviromental triggers or drugs. A better understanding of the triggers acting on the receptive substrate and the precise molecular and cellular pathways involved in the progression of cardiac disease to electrophysiological failure are major unanswered questions in SCD research.

Genetically engineered mice, such as transgenic (TG) and knock-in/-out mice, have proved to be extremely useful for understanding the molecular correlates of ion channels involved in impulse formation and propagation and cardiac repolarization (5,6). Aditionally, some mice have been engineered as models of arrhythmias and SCD (specific genetic defects regulating ion channels, receptors, connexins-Cx, G proteins and autonomic control), others as models for arrhythmogenic cardiac diseases (congenital heart diseases, familial conduction disorders and cardiomyopathies, abnormalities of Ca^{2+} handling) and sometimes the engineered mice unexpectedly develop cardiac arrhythmias and increased mortality (mutations in transcription factors) (5,6). Tables 1 and 2 summarize genetically engineered mice models of cardiac arrhythmias and SCD. These mice display electrophysiological abnormalities that can be analyzed using ex vivo and in vivo techniques (5). The aim of this review is to analyze the results from studies of genetically engineered mice addressing the genesis of cardiac arrhythmias and SCD.

3. GENETIC MANIPULATION OF CHANNEL FUNCTION IN THE MICE

Myocardial electrical activity is evident in the form of action potentials, that reflect the secuencial activation-inactivation of Na^+, Ca^{2+} and K^+ channels (2-4,7). Figure 1 shows the relationship between a hypothetical action potential and the time course of the currents that participate in its genesis in mouse (left) and human (right) cardiac cells. The initial upstroke of human

atrial and ventricular cells (phase 0) is due to the activation of the fast inward Na^+ current (I_{Na}). Initial rapid repolarization (phase 1) is due to the rapid closure (inactivation) of Na^+ channels, and activation of transient outward K^+ current (I_{to}) and, in the atria, of the ultrarapid component of the delayed rectifier K^+ current (I_{Kur}). During phase 2 inward depolarizing currents through Na^+ (slowly inactivated) and L-type Ca^{2+} channels ($I_{Ca,L}$) are balanced by the different components of the delayed rectifier (I_{Kur}, rapid-I_{Kr} and slow-I_{Ks}), whereas phase 3 is mediated by several outward K^+ currents (I_{Kr}, I_{Ks} and the inward rectifier-I_{K1}). I_{K1} also maintains the resting membrane potential (phase 4). In human and mouse ventricular myocytes, ATP-sensitive K^+ (I_{KATP}) channels provide a link between cellular metabolism and membrane potential. Differences in expression patterns of these channels determine the differences (regional and species) in action potential morphology (4,7).

3.1. Functional consequences of targeting I_{to} and I_K in the mouse

I_{to} is the main repolarizing current in the mouse heart (4,6,7). Two types of I_{to} are present in the mouse: a rapid activating, inactivating and reactivating current ($I_{to,f}$) encoded by Kv4.2 and Kv4.3 α-subunits and a more slowly inactivating and recovering current ($I_{to,s}$) encoded by Kv1.4 α-subunits (6,7). Additionally, there are at least two components of the delayed rectifier in mouse ventricles, the 4-aminopyridine sensitive ($I_{K,slow1}$) and the TEA-sensitive ($I_{K,slow2}$) current, encoded by Kv1.5 and Kv2.1, respectively (6,7).

$I_{to,f}$ is selectively eliminated in atrial and ventricular myocytes isolated from TG mice overexpressing a dominant-negative pore mutant of Kv4.2 (Kv4.2W362F) (8-10). $I_{to,f}$ is also selectively eliminated in ventricular myocytes isolated from Kv4.2$^{-/-}$ mice (11). Although the action potential duration (APD) in ventricular myocytes and QT intervals in ECG recordings are markedly prolonged, these mice do not present spontaneous atrial or ventricular arrhythmias, possibly because $I_{to,s}$ is upregulated in both mice models, reducing the dispersion of ventricular repolarization and protecting from the arrhythmogenic effects of the loss of $I_{to,f}$. In contrast, the expression of a dominant-negative (DN) N-terminal fragment of the Kv4.2 (Kv4.2N) produces a heterogeneous reduction in I_{to}, APD prolongation and severe physiopathological consequences (12). At 2 to 4 weeks of age, mice develop a hypercontractile state, with significant elevations in mean arterial pressure, peak systolic ventricular pressures, and contractile force. However, by 3 to 4 months of age, they develop a marked reduction in I_{K1} density, dilated cardiomyopathy, interstitial fibrosis and congestive HF. The mechanisms by which cardiac expression of the Kv4.2N leads to cardiac hypertrophy and HF are unknown, but they can be linked to sustained elevations in $[Ca^{2+}]_i$ secondary to the reduction in I_{to} and the APD prolongation. Both $I_{to,f}$ and $I_{to,s}$ were eliminated in Kv4.2DN/Kv1.4DN mice (10). Ventricular myocytes show prolonged APD and sometimes early afterdepolarizations (EADs) and ECGs a marked prolongation of the PR and QT intervals, atrio-ventricular (AV) block, and occasionally spontaneous ventricular tachycardia (VT). However, these mice presented a decreased dispersion of repolarization (10,13). These results suggest that increased dispersion of repolarization rather that the extent of ventricular APD and QT prolongation is the primary determinant of arrhythmia susceptibility in the mice heart (5,6,10). $I_{K,slow1}$ was eliminated in mice overexpressing a truncated dominant negative Kv1.1 α-subunit (Kv1.1N206Tag), while $I_{K,slow2}$ was upregulated in apical ventricular myocytes (14,15). TG hearts exhibit a marked prolongation of APD (QT interval), less shortening of APDs with decreasing cycle length, greater gradients of refractoriness from apex to base and a significant increase in the frequency of premature ventricular contractions compared with control hearts. Optical mapping demonstrates that a premature impulse applied at the ventricular apex of these hearts produces a sustained reentrant VT by encountering functional lines of conduction block caused by enhanced dispersion of refractoriness (14,16).

Selective elimination of $I_{to,s}$ (Kv1.4$^{-/-}$ mice), $I_{K,slow}$ (Kv2.1DN), $I_{K,slow1}$ (Kv1.1DN, Kv1.5$^{-/-}$ and Kv1.5DN), $I_{K,slow2}$ (Kv2.1DN) and $I_{K,slow1}$ and $I_{K,slow2}$ (Kv1.1DN/Kv2DN) resulted in APD and QT prolongation, but the mice exhibit normal cardiac structure and do not develop spontaneous arrhythmias (6). Interestingly, mouse models in which I_{Kr} and I_{Ks} have been knocked out do not exhibit QT prolongation or proarrhythmic activity (6,8,17,18).

KChIP2 is an ancillary β-subunit that associates with Kv4 α-subunits and its expression is downregulated in cardiac hypertrophy (4). KChIP2$^{-/-}$ mice exhibit normal cardiac structure, contractile function and QT intervals, but display a prolonged elevation in the ST segment and are highly susceptible to the induction of either atrial (atrial flutter) and ventricular (polymorphic VT) arrhythmias and SCD (19). In these mice $I_{to,f}$ is eliminated and the APD is markedly prolonged in ventricular myocytes from KChIP2$^{-/-}$ mice when increasing the stimulating frequency. Moreover, the epicardial-to-endocardial transmural gradient of I_{to} expression across the left ventricle is abolished, leading to an increased dispersion of repolarization, which might cause unidirectional block and contribute to the development of reentrant circuits.

3.2. Voltage-gated channels underlying congenital long QT syndromes

The congenital long QT syndrome is a group of congenital disorders characterized by a prolongation of the QT interval and a propensity to polymorphic VT (torsades de pointes), which may lead to syncope, cardiac arrest or SCD (20). It is caused by mutations in genes encoding channels that regulate cardiac Na^+ (SCN5A-LQT3), Ca^{2+} (CACNA1C-LQT8) and several K^+ (KCNQ1-LQT1, KCNH2-LQT2, KCNE1-LQT5, KCNE2-LQT6 and KCNJ2-LQT7) currents and in a cytoskeletal gene (ankyrin B-LQT4) that may affect Na^+ channel kinetics (2,3,20).

In the heart, I_{Ks} is generated by coassembly of KCNQ1 and KCNE1 subunits. Mice expressing in the heart a dominant negative truncated splice variant of

KCNQ1-isoform 2 exhibit bradycardia, abnormal P wave morphology, prolonged PR and QT intervals and intranodal AV block leading to occasional Wenckebach phenomenon (21). These results demonstrate that KCNQ1 channels play a role not only in ventricular repolarization, but also in sinus node automaticity and propagation of impulses through the AV node. These changes are associated to a prolongation of ventricular APD and a remodeling of K^+ channel expression (downregulation of Kv4.2 and Kv1.5, upregulation of Kv4.3), but no histological or functional evidence of cardiac hypertrophy or contractile dysfunction is observed. In exon 1-KCNQ1$^{-/-}$ mice no changes in heart rate or PR and QRS intervals are observed (22). However, exon 2-KCNQ1$^{-/-}$ mice are deaf, present abnormal P- and T-wave morphologies, delayed AV conduction and prolongation of the QT and JT intervals (23). In isolated hearts of these animals, however, endocardial and epicardial monophasic action potentials and QT intervals are similar to those recorded in WT hearts. Thus, this KCNQ1$^{-/-}$ mouse is a potentially valuable animal model of Jervell and Lange-Nielsen Syndrome (23).

In the adult mouse heart, KCNE1 is expressed in pacemaker cells (sinus node and AV node and proximal conducting system) and in the atria (17,24). In KCNE1$^{-/-}$ myocytes I_{Ks} is absent and I_{Kr} is significantly reduced, indicating that KCNE1 modulates both I_{Ks} and I_{Kr} (17,25). Moreover, KCNE1$^{-/-}$ mice do not display a prolongation of endocardial action potentials, QT interval or other ECG phenotype (25) and there is no evidence of arrhythmias or SCD (i.e. targeting the mouse KCNE1 gene leads to a mild cardiac phenotype)(17,25). However, the QT-RR adaptation is significantly increased in another KCNE1$^{-/-}$ mice as observed in LQT1 patients, leading to a prolonged QT interval during bradycardia that may trigger EADs and increase the propensity for torsades de pointes (26).

While loss of KCNE1 function prolongs ventricular APD, some mutations in either KCNQ1 or KCNE1 subunits can generate gain-of-function defects in the expressed K^+ current and a shortening of APD predisposing to cardiac arrhythmias, including atrial fibrillation (AF). KCNE1$^{-/-}$ mice have normal atrial size and structure but show a shortening of the atrial action potentials that enhances susceptibility to AF which can be abolished by isoproterenol (24). This shortening is attributed to an increase in outward K^+ current (total and that sensitive to the KCNQ1 blocker chromanol 293B) and to a marked accumulation of I_{Ks} in atrial myocytes at rapid driving rates.

Mutations in *SCN5A* are associated with three forms of primary electrical disorders: long QT syndrome 3 (LQT3), Brugada syndrome, and cardiac conduction defects (27). Mutations in SCN5A, the gene that encodes the α subunit of the Na^+ (SCN5A) channel result in an increase (gain of function) in the late sodium current (I_{Na}) responsible for LQT3 (20). In LQT3, TdP occur preferably at rest and some patients may suffer from resting bradycardia, in addition to QT prolongation and develop functional AV block immediately before the onset of TdP. Mice with a heterozygous knock-in ΔKPQ SCN5A$^{\Delta/+}$ deletion (SCN5A-Tg) show bradycardia, prolonged APD, increased dispersion of APD and EADs leading to polymorphous VT (28,29). Adrenergic agonists and ventricular pacing reduce APD dispersion, suppress EADs and prevents VT in these mice. Mexiletine partly suppresses the arrhythmia, possibly by preventing APD prolongation at long cycle lengths and by producing postrepolarization refractoriness, but aggravates bradycardia. These findings provide a pathophysiological rationale for pacing in LQT3.

In addition, loss-of-function mutations in *SCN5A* have been described in patients with phenotypic characteristics of bradycardia, AV block and ventricular fibrillation (VF) (27). Even when the mechanism of arrhythmias in these conditions remains unresolved, VF could result from delayed conduction, unidirectional block, and reentrant excitation. Thus, slow conduction as a result of Na^+ channel dysfunction represents a critical target for understanding and managing cardiac arrhythmias. Homozygous SCN5A$^{-/-}$ mice die embryonic day (E) 10.5 due to severe defects in ventricular morphogenesis. Heterozygous SCN5A$^{+/-}$ mice show a reduction in Na^+ conductance, impaired AV conduction and delayed intramyocardial conduction (as evidenced by prolongation of the P wave and PR interval and rightward shift in the QRS axis), increased ventricular refractoriness, and VT with characteristics of reentrant excitation (30). However, the QT interval remains unchanged, suggesting that noninactivating Na^+ channels are unlikely to influence significantly the duration of ventricular repolarization.

Furthermore, the SCN5A$^{+/-}$ mice recapitulate the idiopatic progressive cardiac conduction disease (Lenegre's disease) phenotype (31). These animals show age-related lengthening of the P-wave and PR and QRS intervals, and old mice show extensive ventricular fibrosis, heterogeneous expression of Cx43 and a decrease in Cx40 expression in the atria. However, animals conserved cardiac function with aging and lack ventricular hypertrophy.

KCNH2 gene encodes the pore-forming α-subunit of I_{Kr}. Mice with a complete knockout of mERG1 gene result in intrauterine death (32). I_{Kr} is absent and the APD prolonged in ventricular myocytes from KCNH2G628S mice. However, this prolongation is not observed in intact ventricular strips studied at more physiological rates and temperature (200 to 400 bpm, 37ºC) (33). Moreover, these mice have normal QT intervals, which confirms that, in contrast to large animals, I_{Kr} is not an important K^+ repolarizing current in the adult mouse heart. I_{Kr} is also undetected in myocytes isolated from mice with a targeted deletion of the N-terminal splice variant of mERG1B$^{-/-}$ (34). These mice develop the bradycardia phenotype observed in some patients with LQT2, even when no changes in PR and QT intervals are observed.

TG mice overexpressing KCNH2 specifically in the heart present normal cardiac structure and normal PR, QRS and QT intervals (35). In WT, but not in TG mice,

$BaCl_2$ induced short runs of fast VT and rapid atrial pacing non-sustained atrial tachycardia and/or VF. TG mice also present post-repolarization refractoriness, which may result from the substantial amount of I_{Kr} remaining after repolarization, and when pretreated with dofetilide are as sensitive to arrhythmias as WT mice. Thus, cardiac overexpression of KCNH2 can protect against experimental arrhythmias, an effect that can be associated to increased repolarization reserve.

Human mutations of the gene encoding the membrane adaptor ankyrin-B (AnkB2) causes LQT4 and varying degrees of cardiac dysfunction, including bradycardia, sinus arrhythmia, idiopathic VF, catecholaminergic polymorphic VT and SCD in response to exercise or emotional stress (36). Loss-of-function mutation AnkB2E1425G results in loss of expression and mis-localization of ankyrin-B-binding proteins [Na^+/K^+-ATPase, Na^+/Ca^{2+} exchanger and inositol-1,4,5-trisphosphate (IP3) receptors] at transverse-tubule/sarcoplasmic reticulum (SR) sites. Cardiomyocytes from Ank-$B^{+/-}$ mice show a normal APD, decreased spontaneous contraction and prolonged Ca^{2+} transients and after exposure to isoproterenol develop both early and delayed afterdepolarizations (37). $AnkB2^{+/-}$ mice present bradycardia, abrupt sinus slowing, intermittent AV dissociation, PR and QTc prolongation and polymorphic VT and died after exercise combined with adrenaline (Table 2).

3.3. Targeting other K^+ channels

I_{K1} regulates cardiac excitability by controlling the membrane potential and is encoded by KCNJ2 and KCNJ12 genes (4). Mutations in KCNJ2 gene are associated with Andersen syndrome, an autosomal dominant disease characterized by periodic paralysis, skeletal developmental abnormalities and QT prolongation (LQT7) with ventricular arrhythmias (38). KCNJ2 is the major component of murine I_{K1}. In fact, ventricular myocytes from $KCNJ2^{-/-}$ mice lack detectable I_{K1} and present a prolonged APD and more frequent spontaneous action potentials than WT myocytes (39). These animals present marked bradycardia and a prolongation of the PR, QRS and QT intervals, but no ectopic beats or reentry arrhythmias are observed. Thus, despite the ablation of I_{K1} and the marked effects on APD and spontaneous activity in isolated myocytes, the consequences for the intact heart are surprisingly mild. $KCNJ12^{-/-}$ mice show a 50% reduction in I_{K1}, raising the possibility that it can also contribute to the native I_{K1} (39). To assess the role of I_{K1} in cardiac excitability, TG mice expressing in the heart dominant-negative Kir2.1 channel subunits with a mutated signature sequence (AAA for GYG substitution) were engineered (40). In ventricular myocytes isolated from this mouse, I_{K1} is reduced by 95%, leading to a significant prolongation of the APD and ECG recordings show bradycardia and prolongation of the PR, QRS and QT intervals. Overexpression of KCNJ2 in TG mice led to two viable mice lines, with high (line 2) and low level of expression (line 1) (41). Line 1 presents cardiac hypertrophy and normal life span, while line 2 presents marked hypertrophy, multiple arrhythmias, including bradicardia, AV block, AF and premature ventricular beats and 60-70% of animals die between day 10 and 60. In both cell lines increased I_{K1} density markedly shortens the APD (QTc intervals) leading to neuron-like action potentials, hyperpolarizes the resting membrane potential and leads to a compensatory increase in I_{Ca} density, Ca^{2+} load of intracellular stores and, ultimately, cardiac hypertrophy. Thus, I_{K1} upregulation may be considered a model of the short QT syndrome. Moreover, these findings demonstrate that I_{K1} plays a significant role in control of action potential repolarization and major ECG intervals in the intact heart.

Cardiac ATP-sensitive K^+ (K_{ATP}) channels results from the coassembly of four inwardly rectifying channel α-subunits (KCNJ11) α-subunits with four regulatory SUR2A (ABCC9) β-subunits (4). K_{ATP} channels adjust membrane excitability to cellular metabolic demands and are required for adequate adaptation to stress**.** $KCNJ11^{-/-}$ mice show a maladaptive phenotype characterized by a lower exercise tolerance, impaired cardiac performance, Ca^{2+} overload, QT prolongation, development of ventricular arrhythmia and increased mortality under sympathetic stimulation (42). Moreover, following β-adrenergic stimulation, $KCNJ11^{-/-}$ mice present inconsistent AV conduction and diastolic Ca^{2+} load which act as a trigger for EADs leading to fatal ventricular tachyarrhythmias (43). Moreover, in these animals the protective effect of ischemic preconditioning is abolished and following global ischemia/reperfusion, the increase of left ventricular end-diastolic pressure during ischemia was more marked, and the recovery of contractile function was worse than in WT hearts (44).

4. SINOATRIAL AND ATRIOVENTRICULAR NODAL DYSFUNCTION

Activation of cardiac muscle is initiated and coordinated by the specialized electrical system, comprising the sinoatrial (SA) and the AV nodes, the activation of which depends on the $I_{Ca,L}$ and of the His-Purkinje system, the activation of which depends on the I_{Na}. Heart rate is determined by the depolarization rate of pacemaker cells in the SA node and can be modulated by β-adrenergic, M2-muscarinic and A1-adenosine receptors. M2 and A1 receptor stimulation activates the inward rectifier K^+ current $I_{K,Ach}$ (or $I_{K,Ado}$), inhibits the β-adrenergic effects on I_{CaL} in the SA node cells and produces bradycardia and AV block.

Acetylcholine (Ach) released on vagal stimulation slows heart rate through activation of M2-receptors on the sinus nodal cells and subsequent opening of atrial muscarinic KAch channels activated by G protein βγ-subunits resulting from heteromeric assembly of KCNJ3 and KCNJ5 subunits (4). In animal models increased vagal tone shortens atrial APD and refractoriness and predisposes the atria to fibrillation, while vagal denervation prevents the induction of AF (45). $KCNJ5^{-/-}$ mice have normal basal heart rates, but exhibit an altered heart rate variability and a decreased negative chronotropic response after vagal stimulation or adenosine administration (46). Carbachol shortens atrial APD and

initiates AF in WT mice but not in KCNJ5$^{-/-}$ mice (47). Moreover, after carbachol, sinus node recovery time and sinus node cycle lengths are shorter and ventricular refractory periods longer in KCNJ5$^{-/-}$ than in WT mice. However, there are no differences between KO and WT mice in AV node function and ventricular tachyarrhythmias could not be induced in either type of mice. These results suggest that $I_{K(Ach)}$ plays a major role in the regulation of heart rate, both at rest and after vagal stimulation.

Reduced parasympathetic heart rate modulation has been observed in TG mice with a reduction of neuronal nitric oxide synthase (NOS1$^{-/-}$) (48) or of membrane-bound G-protein β-subunits in atrial cardiomyocytes (49). Reduction of functional Gβγ protein by > 50% blunted the the bradycardia and the AF induced by carbachol and the increases in time- and frequency-domain indexes of heart rate variability and baroreflex sensitivity (49). In addition, sinus node recovery time and inducibility of atrial arrhythmias were reduced in these mice. Thus, Gβγ plays a crucial role for parasympathetic heart rate control, sinus node automaticity, and atrial arrhythmia vulnerability.

Further analysis of the role of autonomic nervous system on sinoatrial nodal cells and heart rate has been analyzed in TG overexpressing β1-adrenoceptors (50) and the α-subunit of GTP-binding protein ($G_s\alpha$)(51); both mice exhibit tachycardia and a pronounced depression in heart rate variability.

The role of adenosine on sinus node or AV nodal dysfunction has been analyzed in TG mice with heart-directed overexpression of A1 and A3 receptors. Mice overexpressing A1 adenosine receptors (A1ARs) present sinus nodal dysfunction (bradycardia, a decrease in the chronotropic response to exercise), first-degree AV block and spontaneous supraventricular arrhythmias at baseline or after exposure to adenosine (52). Low-level expression of A3ARs reduces infarct size after ischemia-reperfusion, while high levels of expression (A3^{high}) leads to cardiac dilatation and fibrosis, ventricular systolic dysfunction, hypotension, bradycardia, progressive AV block, and incessant bradycardia–tachycardia syndrome (53,54). These effects are attenuated during exercise. Atrial arrhythmias present before atrial cardiomyopathy were attributed to intermittent activity of the sinus node or EADs, but activation of I_{KAdo} by A3R overexpression may also participate by decreasing the threshold of atrial tachyarrhythmias. Cardiac G_i-coupled receptors, such as the A1 adenosine and the M2 muscarinic receptors, decrease intracellular cAMP levels, contractility and heart rate and aberrant G_i signaling has been implicated in the etiology of human idiopathic dilated cardiomyopathy. Conditional expression of a G_i-coupled receptor (Ro1) in the adult mouse heart causes a lethal cardiomyopathy (90% of the animals die after 8 weeks) with systolic dysfunction, LV chamber dilation and a variety of cardiac arrhythmias characterized by wide QRS complexes in the days before death (55). Suppression of Ro1 expression after 8 week protected mice from further mortality and allowed partial improvement in systolic function.

Hyperpolarization-activated cation (HCN) channels generate a mixed Na^+ and K^+ inward current (I_h) which contributes to the cardiac pacemaker activity of the SA node. HCN4$^{-/-}$ mice die in utero, while the contraction rate of isolated HCN4-deficient hearts is reduced by 40% (56). HCN2$^{-/-}$ mice are viable but exhibit cardiac sinoatrial dysrhythmia, reduced locomotor activity and spontaneous absence seizures (56). HCN2$^{-/-}$ sinoatrial cells show a reduction in I_h and a hyperpolarization of the maximum diastolic potential and the mice show sinoatrial node dysfunction, but normal PQ, QRS, and QT intervals. Moreover, heart rate at rest and during activity and in response to isoproterenol is similar in HCN2$^{-/-}$ and WT mice. Thus, the role of HCN2 in sinoatrial cells is the stabilization of a normal diastolic membrane potential that is required for regular cardiac rhythmicity. Moreover, the finding that loss of HCN2 does not abolish the spontaneous activity of cardiac pacemaking cells strongly supports that pacemaking is a multifactorial mechanism relying on the interaction of several currents, including that generated by HCN4 channels (57).

Autonomic nervous system imbalance is recognized to have a significant impact on arrhythmia susceptibility. Nhlh is a basic helix-loop-helix transcription factor that participates in cardiac development. Nhlh1$^{-/-}$ mice present depression of parasympathetic tone (evidenced by a reduction in heart rate variability and baroreceptor sensitivity and a loss of the diving reflex), stress-induced arrhythmias (evidenced by premature ventricular contractions during swimming) and an increased rate of unexpected premature death (58). These mice exhibit normal heart rates and QT intervals, but present unstable cardiac repolarization, manifested by an increase in the beat-to-beat QT interval fluctuations, which facilitates the induction of functional reentry and lethal ventricular tachyarrhythmias. These findings suggest that nervous system imbalance in situations of stress autonomic and/or exercise may increase susceptibility to lethal ventricular arrhythmias in the Nhlh1-null mouse.

The Klotho mutant mice (*kl/kl*), a model of human aging, when subjected to restrain stress show bradyarrhythmias resulting from sinus arrest or sinoatrial block, higher susceptibility to overdrive suppression, smaller positive chronotropic effects of isoproterenol, greater negative chronotropic effects of Ach and two thirds of animals die (59). Since Klotho gene is expressed only in the SA node, these results indicate that it may play a role in regulating its pacemaker function under stress conditions.

Mutations in the T-box gene Tbx5 are associated with Holt-Oram syndrome, characterized by upper limb malformations and cardiac septation defects (60). Mice with Tbx5 haploinsufficiency (*Tbx5*$^{del/+}$ mice) exhibit embryonic lethality, present forelimb and congenital heart malformations (including atrial and ventricular septation defects and conduction system disease) and ECG changes, including broader P-wave consistent with the presence of atrial enlargement, prolonged PR intervals, AV block and sinoatrial pauses (61). The pronounced decrease in Cx40 transcription, provides an explanation for the conduction system defects.

Mutations in the gene encoding the cardiac transcription factor Nkx2.5 have been identified in patients

with progressive AV block, HF and atrial and ventricular septal defects (62). Targeted disruption $Nkx2.5^{-/-}$ causes early embryonic lethality, while in $Nkx2.5^{+/-}$ mice the conduction system has half the normal number of cells and a population of $Cx40^{-}/Cx45^{+}$ cells is missing in the AV node; in contrast, the expression of Cx40 in the Purkinje fibers is unaffected, consistent with normal conduction times through the His-Purkinje system (63). TG mice expressing the mutation Csx/Nkx2.5(I183P) appear normal at 1 week of age, but rapidly develop profound defects in the conducting system and HF (64). At 2 weeks of age ECGs show bradycardia and prolongation of PR, QRS and QT intervals, at 4 weeks complete AV block and a progressive disturbance of intraventricular conduction, and most animals died before 12 weeks of age. Expression of Cx40 and Cx43 is markedly decreased 3 weeks after birth, which may contribute to the conduction defects. However, Nkx2.5(I183P) mice do not show the atrial or ventricular septal defects observed in patients.

5. ALTERATIONS IN THE CARDIAC CONDUCTION SYSTEM

The Wolff-Parkinson White syndrome (WPW) is caused by accessory muscular pathways providing direct continuity between atrial and ventricular myocardium. Electrical impulses traversing these tracts bypass the physiological AV node delay and produce ventricular preexcitation. Mutations in the gene encoding the γ_2 regulatory subunit (PRKAG2) of adenosine monophosphate-activated protein kinase (AMPK) are responsible for a cardiomyopathy characterized by ventricular hypertrophy, WPW syndrome and conduction defects (65,66). TG mice expressing a mutant human PRKAG2 gene (TG^{N488I}) show elevated AMPK activity, ventricular preexcitation, as evidenced by a short PR interval and slurred QRS complex (delta wave) on the ECG, cardiac dilatation, hypertrophy and glycogen accumulation (30-fold) and a decrease of contractile function (66,67). Immediately after birth no pups show evidence of pre-excitation, but after 1-4 weeks ECGs show spontaneous episodes of sinus bradycardia (<300 bpm) and various escape rhythms, including paroxysmal supraventricular tachycardia and AF; moreover, ECG monitoring of stressed mice suggested severe and persistent sinus bradycardia as the cause of death. Programmed electrical stimulation and intravenous procainamide prolong the PR interval, narrow the QRS complex and lengthen the AV Wenckebach cycle length in the TG mice. These findings suggest that the extrastimuli are blocked in the accesory pathway and conduct through the AV node. However, intravenous adenosine during atrial pacing results in AV block in the WT mice, but not in the TG mice with preexcitation. These findings further confirm the presence of a separate AV connection in TG mice (67). Cardiac histopathology reveals that the annulus fibrosis that insulated the atria and the ventricles is disrupted by glycogen-filled myocytes, providing the anatomic substrate for ventricular preexcitation (68).

Mice expressing the mutation R302Q show a decreased AMPK enzymatic activity, excessive cardiac glycogen, ventricular hypertrophy, slower heart rate, ventricular preexcitation, atrial premature beats and/or tachycardia and electrical stimulation induced supraventricular tachycardia consistent with orthodromic AV reentrant tachycardia (69). Again, atrial pacing and procainamide blocked the accesory pathway and abolished ventricular preexcitation, resulting in a sudden lengthening of the PR interval and abbreviation of QRS duration. Although the mechanism whereby AMPK mutations induce accessory pathways and subsequent WPW is uncertain, it has been proposed that the accessory pathways normally undergo apoptosis and AMPK mutations inhibit this process or induce de novo development of accessory pathways (69).

A new genetic pathway for cardiac arrhythmias leading to SCD implicates defects in the transition between ventricular and conduction system cell lineages (70). HF-1b is an SP-1-related transcription factor expressed in ventricular myocytes and the specialized conduction system. $HF\text{-}1b^{-/-}$ mice have normal cardiac structure and function at birth, but show defects in the cardiac conduction system including sinus bradycardia, high-degree AV block, spontaneous VT and an increased postnatal mortality, suggesting that HF-1b plays an important role in the development of the cardiac conducting system. Ventricular myocytes of these mice show a decrease in $I_{K,slow}$ density, which seems to be largely responsible for the prolongation and heterogeneity of APD (9), and fewer Purkinje cells express Cx40 and Cx43, which offer an anatomical substrate for arrhythmogenesis in these mice. These data point to chaotic impulse propagation in the distal Purkinje system and ventricular cells as the anatomical substrate for SCD.

6. ALTERATIONS IN CALCIUM HANDLING AND CARDIAC ARRHYTHMIAS

Abnormalities of Ca^{2+} handling and Ca^{2+}-dependent signaling pathways play a pivotal role in the development of ventricular tachyarrhythmias associated to cardiac hypertrophy and HF and in some inherited syndromes leading to SCD (71). Upregulation of Ca^{2+}/calmodulin-dependent protein kinase (CaMK) type II seems to be a general feature of cardiomyopathy in humans and mice. TG mice overexpressing CaMKII present cardiac hypertrophy, longer QT intervals and more arrhythmias than WT mice and arrhythmias are additionally increased by isoproterenol (72). TG cardiomyocytes show longer APD, an increase in the open probability of L-type Ca^{2+} channels and frequent EADs. The CaMK inhibitor KN-93 significantly reduces arrhythmia severity in TG mice but not in isoproterenol-treated WT mice and the CaMKII inhibitory peptide AC3-I, but not a peptide antagonist of the Na^{+}/Ca^{2+} exchanger current (XIP), eliminate the increased open probability of the L-type Ca^{2+} channel and the EADs. These data support that CaMKII is a proarrhythmic signaling pathway in this model. However, the EAD frequency is unchanged after isoproterenol, suggesting that its proarrhythmic action occurs perhaps by enhancing EAD propagation. To test the potential role of CaMKII in regulating AV nodal

conduction, a mice with genetic CaMKII inhibition by cardiac-specific expression of anticamptide 3 inhibitory peptide (AC3-I) was engineered. These mice show a prolongation of the PR and atria-His intervals and enhanced Wenckebach-like AV conduction after isoproterenol and isolated hearts have prolonged Wenckebach cycle lengths and AV nodal effective refractory periods (73). These findings indicate that CaMKII is a proarrhythmic signaling molecule in cardiac hypertrophy and suggest that it is required for normal and catecholamine-stimulated AV conduction.

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an arrhythmogenic disorder characterized by syncopal events and SCD at a young age during physical stress or emotion, in the absence of structural heart disease. Mutations in two genes encoding Ca^{2+}-handling proteins, the ryanodine receptor 2 gene (*RYR2*), which encodes a cardiac SR Ca^{2+} release channel, and the calsequestrin 2 gene (CASQ2), are associated with CPVT (74-76). Calstabin2 (FKBP12.6) binds to the RyR2 complex, stabilizes the channel in a closed state and prevents leakage of SR Ca^{2+} into the cytoplasm during the diastole (77). However, in patients with CPVT, phosphorylation by cAMP-dependent protein kinase A (PKA) of RyR2 channels at Ser^{2809} during exercise or sympathetic stimulation partially dissociates calstabin2 from the channels, increasing their open probability during the diastole. This causes Ca^{2+} release from the SR, which can initiate delayed afterdepolarizations, ventricular arrhythmias and SCD. Hearts of calstabin2$^{-/-}$ and calstabin2$^{+/-}$ mice are structurally normal as assess by echocardiography and histology and no ECG abnormalities are observed and RyR2 channels from calstabin2$^{-/-}$ mice and CPVT-mutants (S2246L, R2474S, R4497C) exhibit normal single channel properties (76,77). However, calstabin2$^{-/-}$ and calstabin2$^{+/-}$ present episodes of polymorphic VT or syncopal events and almost all mice died during strenuous exercise and despite there are no changes on action potential morphology, delayed afterdepolarizations are observed in ventricular cardiomyocytes from these mice (77). Ventricular tachycardias and SCD in calstabin2$^{+/-}$ mice are reversed by JTV519, which increases the affinity of calstabin 2 for PKA-phosphorylated RyR2 channels, decreases open channel probability and ventricular arrhythmias, providing a molecular mechanism to treat the disorders that trigger SCD (78). After injection of isoproterenol, VT was induced by rapid pacing in 71% of calstabin$^{+/-}$ but not in WT mice and animals pretreated with JTV519 are less susceptible to develop VT. However, 100% of calstabin$^{-/-}$ mice treated with JTV519 developed VT and died when subjected to exercise testing, which indicates that the ability of JTV519 to induce rebinding of calstabin to RyR2 underlies its antiarrhythmic actions.

Calcineurin is a Ca^{2+}/calmodulin-activated phosphatase located in the lumen of the cardiac SR that dephosphorylates nuclear factor of activated cells (NF-AT3), which then translocates into the nucleus and interacts with transcription factors that activate a range of genes involved in hypertrophy. Mice overexpressing calcineurin present a decrease in $I_{to,f}$, $I_{to,s}$ and I_{Kslow} densities, prolongation of the sinus node recovery time, AV node conduction and ventricular APD (PR and QT intervals), cardiac hypertrophy and fibrosis, congestive HF, bradycardia and high degree AV block that preceded the development of sustained VT (in 70% of the mice at 30 days of age) and SCD (79). Mice overexpressing NF-AT3 present cardiac hypertrophy and sinus node dysfunction, but not AV block, prolongation of ventricular refractoriness and propensity to VT (80). Thus, calcineurin-induced phenotype is partly independent of the NF-AT3 signaling pathway.

Junctin is a 26-kDa integral membrane protein, colocalized with the RyR2, calsequestrin and triadin at the luminal face of junctional SR. Cardiac-specific overexpression (24 folds) of junctin is associated with atrial and ventricular enlargement, hypertrophy and fibrosis, impaired left ventricular systolic function and several arrhythmias, such as bradycardia, AF, ventricular premature beats and sinus pauses (81). Moreover, overexpression of junctin leads to down-regulation of RyR2 channels and an increase in $I_{Ca,L}$ density and APD prolongation which may account for the bradycardia in the TG heart.

Mice overexpressing calreticulin, a Ca^{2+}-binding chaperone located in the lumen of the cardiac SR, exhibit cardiac dilatation, decreased systolic function, bradycardia associated with synus node dysfunction and progressive prolongation of the PR interval followed by complete AV block and SCD (82). However, there was no evidence of myocyte disarray, necrosis or myocarditis. Myocytes have a decreased $I_{Ca,L}$ density and low levels of Cx40 and Cx43. These results suggest that calreticulin is essential for the development of the SA and AV nodes that require $I_{Ca,L}$ for activation.

TG mice overexpressing the inflammatory cytokine tumor necrosis factor-alpha (TNFα) in the heart develop a cardiomyopathy characterized by ventricular hypertrophy and dilatation, decreased LV ejection fraction, interstitial inflammatory infiltrates and fibrosis, attenuation of ß$_1$-adrenergic responsiveness, inducible atrial and ventricular arrhythmias and marked increase in mortality (83). Ventricular myocytes from these mice have prolonged APD, without increased dispersion of refractoriness, elevated diastolic and depressed systolic [Ca^{2+}] and prolonged Ca^{2+} transients. Premature beats have diminished action potential amplitudes and conduct in a slow, heterogeneous manner, leading to areas of conduction block and the initiation of reentrant nonsustained ventricular tachyarrhythmias (84). Lowering extracellular [Ca^{2+}] normalizes conduction and prevents inducible tachyarrhythmias despite APD prolongation. Thus, both APD prolongation and abnormal Ca^{2+} handling may contribute to the initiation of reentrant arrhythmias in this model of HF.

7. ROLE OF THE RENIN ANGIOTENSIN SYSTEM

Angiotensin II (AII) via AT1 receptors produces both cardiac electrical and structural remodeling which may underlie ventricular arrhythmias in hypertension, HF

and cardiac hypertrophy (1,85). TG mice overexpressing the AT1 receptors selectively in the heart, but not in other tissues (liver, lung, kidney), present massive atrial enlargement with myocyte hyperplasia at birth (86). TG mice have significant lower heart rate, prolongation of the PR interval, widening of the QRS which suggests a decrease in conduction velocity and die within the first weeks after birth. TG mice with cardiac-specific overexpression of AT1 receptors lacking $G\alpha q/G\alpha 1$ coupling (AT1-i2m) exhibit cardiac hypertrophy, increased LV end-systolic and end-diastolic dimensions, bradycardia and AV block with widened QRS complex, these changes being more severe than in WT mice overexpressing the AT1R (Tg-WT) (87). AT1-i2m mice have a decreased peak $I_{Ca,L}$ density which may contribute to bradycardia, and exhibit less severe fibrosis and apoptosis than Tg-WT mice. Acute AII administration increases heart rate only in Tg-WT mice, while chronic AII infusion induces a greater cardiac hypertrophy in Tg-i2m mice. These results suggest that AT1R mediates signaling mechanisms via $G\alpha q/G\alpha 1$-dependent and –independent mechanisms, which in turn modulate hypertrophy with distinct phenotypes.

TG mice with genetically clamped elevations in AII (RenTgMK) have high blood pressure and concentric cardiac hypertrophy, fibrosis, bradycardia, prolongation of PR and QT intervals and progressive QRS widening and approximately 60% of male mice die suddenly between 6 and 8 months of age (88). Genetic studies reported a link between angiotensin-converting enzyme (ACE) polymorphisms and the incidence of cardiac hypertrophy, SCD and acute coronary events (89). To investigate the cardiac effects of Ang II, a mouse model (ACE 8/8) was developed with 100-fold increase in cardiac ACE, but no increased expression of ACE in kidney, gut, vascular endothelium and brain (90). After 3 weeks of age hearts of ACE 8/8 mice show atrial enlargement and fibrosis with normal ventricular and renal morphology and function and near normal blood pressure levels. ACE 8/8 mice have low QRS and T wave voltages, irregular RR intervals consistent with AF, prolongation of the PR interval and a high incidence of SCD. ECG monitoring showed a ventricular escape rhythm preceding death. Thus, cardiac overexpression of AII in the heart participates in the pathogenesis of atrial dysfunction, cardiac arrhythmias and SCD. Mice overexpressing ACE2 in myocardial cells show normal ECG at 1 week of age, but developed progressive conduction disturbances (prolonged PR and QRS durations by 2 weeks of age), which progressed to complete AV block by 3-4 weeks of age), ventricular ectopy and premature SCD due to sustained VT and VF (91). The hearts of these mice present a prolongation of atrial and ventricular refractoriness and a down-regulation of Cx40 and Cx43 that may account for conduction slowing and ventricular arrhythmias observed in this model. Spontaneous downregulation of the ACE2 transgene in surviving older animals correlate with restoration of nearly normal conduction, rhythm, and connexin expression.

To specifically assess the role of the mineralocorticoid receptor (MR) in the heart in the absence of changes in aldosteronemia, a TG mice model (DT mice) with conditional cardiac-specific overexpression of the human MR was generated (92). Surface ECGs show prolongation of AV (PR interval) and ventricular (QRS interval) conduction times and ventricular repolarization (QT intervals) and spontaneous and triggered ventricular arrhythmias. The prolongation of the APD is associated with ion channel remodeling, namely downregulation of I_{to} and upregulation of $I_{Ca,L}$. Most of these electrophysiological changes are reversed with spironolactone. However, no cardiac fibrosis, apoptosis and inflammatory cell infiltrate are observed in the DT mice.

8. HYPERTROPHIC AND DILATED CARDIOMYOPATHIES

Several cardiomyopathies are associated with arrhythmias and SCD. Male mice with a missense mutation Arg403Gln (α-MHC$^{403/+}$) in the α-myosin heavy chain display histological (myocyte disarray, hypertrophy and fibrosis) and hemodynamic abnormalities characteristic of familial hypertrophic cardiomyopathy, electrophysiological abnormalities (prolongation of the sinus node recovery time, JT and QT intervals and ventricular repolarization, right axis deviation and slower and heterogeneous ventricular conduction) and during programmed ventricular stimulation inducible VT and SCD during exercise (93). The electrophysiological abnormalities and the inducible VT are more evident in male than in female mice. Homozygous mutant mice expressing a truncated form of myosin-binding protein C (MyBP-C$^{t/t}$) develop severe dilated cardiomyopathy and regions of fibrosis, whereas MyBP-C$^{t/+}$ mice develop a mild hypertrophic cardiomyopathy, but both mice exhibit normal atrial, AV and ventricular conduction and refractoriness (94). However, MyBP-C$^{t/t}$ mice present right-axis deviation, ventricular extrasystoles and inducible nonsustained VT more easily than MyBP-C$^{t/+}$ mice both before and after isoproterenol infusion.

Mutations in the LMNA gene encoding for the nuclear-envelope proteins lamin A and lamin C cause Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy, progressive conduction-system disease (sinus bradycardia, AV block, or atrial arrhythmias), HF and SCD (95). A mouse model carrying the LMNAH222P mutation develop muscular dystrophy and dilated cardiomyopathy associated with conduction defects (prolongation of the PR and QRS intervals, frequent sinoatrial blocks), ventricular extrasystoles and all of them die by 9 months of age. Thus, this mouse represents a good model for studying laminopathies (96).

9. MODELS OF ATRIAL FIBRILLATION

We have already mentioned models that reproduce AF-induced electrical and structural remodeling (Tables 1 and 2). Mice overexpressing a constitutively active mutant form of TGF-ß$_1$ present atrial interstitial fibrosis, atrial conduction abnormalities and enhanced susceptibility to AF in the absence of abnormalities in atrial APD, surface ECG, ventricular size and histology or

connexin protein distribution (97). Thus, this model shows that atrial fibrosis may be sufficient to increase AF inducibility.

Transcription factors of the cAMP-responsive element modulator family (CREM) play an important role in cardiac gene regulation contributing to HF. TG mice with heart-directed expression of CREM-IbDeltaC-X, a human cardiac CREM isoform, display atrial enlargement and hypertrophy, AF with rapid ventricular response and die prematurely (98). Hearts of these mice present an increased basal and isoproterenol-stimulated contractility probably due to a selective up-regulation of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) and β1-adrenoceptor density. These changes are associated with increased activity of serine-threonine protein phosphatase 1 and a decrease in messenger RNAs encoding transcription factor dHAND and small G-protein RhoB. Thus, CREM regulates cardiac morphology, function, and gene expression.

10. ROLE OF CONNEXINS IN CARDIAC CONDUCTION AND DEVELOPMENT

Connexins (Cxs) facilitate rapid and coordinated electrical excitation, a prerequisite for normal rhythmic contraction. Reduced expression and altered distribution of Cxs underlie the high incidence of arrhythmias associated with many forms of heart disease, including HF and hypertrophic, ischemic, and dilated cardiomyopathies (1,99). Three major Cxs are expressed in the heart: Cx43 is found in atrial and ventricular myocytes, Cx40 in the atria and the His-Purkinje system and Cx45 in the AV node and adjoining His bundles and electrically insulate the central conductive tissues from the working myocardium. Results from genetically engineered mice demonstrate that Cxs are involved in impulse propagation and cardiogenesis and that $Cx40^{-/-}$ and $Cx43^{-/-}$ mice develop lethal ventricular tachyarrhythmias (99). $Cx40^{-/-}$ mice show prolongation of the PR, QRS and QT intervals, increased AV Wenkebach cycle length and a higher incidence of inducible intra-atrial reentrant tachycardia and cardiac abnormalities (hypertrophy, common AV junction, ventricular septal defects) (99-101). However, a reduction in Cx40 expression greater than 50% is needed to cause a conduction defect. As already mentioned, dominant mutations of Tbx5 (61) and Nkx-5 (64) and overexpression of calreticulin (82) result, in both humans and mice, in a cardiac phenotype similar to that of Cx40-deficiente mice. $Cx45^{-/-}$ mice have abnormal cardiac development and die in utero at E10 (102), suggesting that Cx45 has an indispensable role in cardiac conduction during very early stages of heart development.

$Cx43^{-/-}$ mice died at birth (103,104), while $Cx43^{+/-}$ mice survive and show active membrane properties, Na^+ channel activity and action potentials similar to WT mice (99,105). Under physiological conditions, $Cx43^{+/-}$ mice show no apparent abnormalities in cardiac structure and function or in sinus node, AV node and atrial conduction, but exhibit a significant slowing of intraventricular conduction and more stable and inducible ventricular arrhythmias leading to SCD (103-106). Moreover, in perfused $Cx43^{+/-}$ hearts exposed to acute regional coronary occlusion increase the incidence, frequency and duration of ventricular arrhythmias (107). To circumvent perinatal death, mice were engineered with cardiac-restricted inactivation of Cx43 (KCO mice) (103) or with a 4-hydroxytamoxifen-induced deletion of the Cx43 gene at the adult stage ($Cx43^{Cre\text{-}ER(T)/flox}$) (108). The heart of KCO has a normal structure and contractile performance, by all develop spontaneous VT and succumb to sudden cardiac arrest by 2 months of age (103,104).

Very recently, a heart–specific Cx43-deficient subline (O-CKO mice) was developed in which the loss of Cx43 occurs more gradually throughout the ventricular myocardium during postnatal stages (109,110). These mice present a decrease in QRS amplitude, slowing of conduction velocity and multiple sites of activation of the ventricular myocardium leading to spontaneous or inducible incessant VT and SCD. The finding of multiple sites of activation in the setting of a normal cardiac conduction system suggests that uncoupling must increase the proportion of Purkinje-ventricular junctions across which conduction succeeds due to improvements in current source and current load matching.

In chimeric $Cx43^{-/-}$ hearts with highly abnormal, well-circunscribed foci of Cx43-deficient myocytes, systolic function is depressed and present corresponding focal areas of conduction delay associated with an increase in spontaneous ventricular extrasystoles and runs of nonsustained VT (111). However, these mice do not present spontaneous or inducible sustained VT, which suggests that the spatial pattern of uncoupling must play a key role in determining the phenotype.

Induced $Cx43^{Cre\text{-}ER(T)/flox}$ mice develop SCD initiated by severe tachyarrhythmias, based on a stable reentry circuit in the right ventricle and fibrillatory conduction in the LV (108). Interestingly, despite the marked decrease in Cx43 expression (90%), intraventricular conduction velocity is slowed in most cases by only ≈50%. This observation is consistent with modeling, suggesting that the safety factor for conduction is paradoxically increased with reduced gap junctional coupling. However, heterogeneity in Cx43 expression in concert with increased collagen deposition may be responsible for increased arrhythmogenicity in diseased hearts (104). The mechanisms leading to SCD are uncertain. However, reduced and heterogeneous distribution of Cx43 contributes to electrical uncoupling between myocytes and together with the slowing of conduction velocity lead to the nonuniformities required to initiate breaks within a propagated wave front and to the formation of unidirectional block and reentry (103,104). Chimeric mice with patchy Cx43 expression also exhibit spontaneous tachyarrhythmias and areas of conduction abnormalities (104). Thus, reduction of Cx43 expression, and consequently, of the electrical coupling, may play a critical role in ventricular arrhythmogenesis.

N-cadherin, a member of a family of cell surface glycoproteins that mediate Ca^{2+}-dependent cell adhesion, is involved in the formation and/or function of gap junctions.

Postnatal cardiac-restricted deletion of N-cadherin gene in the adult heart leads to disassembly of the intercalated disc structure (including adherens junction and desmosomes) with a modest dilated cardiomyopathy and reduction of LV ejection fraction, cardiac output and Cx40 and Cx43 expression in the atria (112). ECG recordings show wider P waves, longer PR intervals, QRS of reduced amplitude consistent with loss of electrical ventricular coupling, slow ventricular conduction velocity and ventricular extrasystoles inducing VT that subsequently degenerated into VF (113,114).

TG mice overexpressing the transcription enhancer factor-1-related factor (RTEF-1) which mediates α_1-adrenergic signaling in cardiac myocytes show a prolongation of the PR and QRS intervals associated with an upregulation of protein phosphatase 1β and dephosphorylation of Cx40 and Cx43. Optical mapping show slow atrial and ventricular conduction accompanied by/or transient episodes of supraventricular arrhythmias (114) and atrial dilatation, but not hypertrophy.

11. THE MOUSE AS A MODEL FOR ARRHYTHMIAS

Genetically engineered mice have improved our understanding of the pathophysiology of cardiac arrhythmias and allowed us to identify new mechanisms that lead to SCD. However, the mouse is not an ideal model for human cardiac arrhythmias and SCD because of its rapid basal heart rate (> 600bpm), short APD (< 30 ms, e.g. around 10% of that in humans even when corrected for heart rate) and small heart size that may limit reentrant circuits. Moreover, the K^+ currents determining the ventricular repolarization in humans and mice are distinct, so that I_{Kr} and I_{Ks} are the main determinants in humans, while I_{to} (Kv4.2, Kv4.3 and Kv1.4) is the main repolarizing current in the mice. This can explain why mice that harbor mutant channels identical to those found in the cLQTS do not display a SCD phenotype. These differences must be kept in mind before extrapolating the results obtained in mice models to human cardiac electrophysiology. However, and despite of these disadvantages, the mouse genome can be easily manipulated and because of gestation times, technical feasibility, expense and ethics, the mouse has become the species of choice to study cardiac electrophysiology (5,6). Moreover, some genetically modified mouse models present spontaneous and/or inducible of arrhythmias and offer the opportunity to elucidate the physiological correlates of altered expression of ion channels and other regulatory proteins and the physiopathological mechanisms that couple gene products and mutations to the genesis of cardiac arrhythmias and SCD. In addition, mice models facilitate the use of genomic strategies to identify new downstream target genes and signaling pathways for the genesis of cardiac arrhythmias and SCD. Moreover, recent advances in experimental methodology permits to perform electrophysiological studies in the conscious mouse, both at rest and during exercise, avoiding the confounding effects of restraint and anesthesia (5). Nowadays, a range of physiological measures can be used to assess cardiac shape and function (echocardiography, magnetic resonance imaging, sonomicrometry, conductance volumetry), hemodynamics, flow (Doppler echocardiography) and electrophysiology phenotyping (high-resolution optical mapping, ambulatory electrocardiographic telemetry) (5,115).

12. CONCLUSIONS

Despite recent advances in preventing SCD due to cardiac arrhythmia, its incidence in the population remains unacceptably high. Thus, a major goal in SCD research is to get a better understanding of the interaction among various functional, structural, and genetic factors underlying the susceptibility to, and initiation of, fatal arrhythmias. The findings obtained in genetically engineered mice confirmed the complexity of the pathophysiology of cardiac arrhythmias and emphasize the need to integrate the molecular and genetic mechanisms with the specific arrhythmogenic substrate characteristic of the cardiac diseases frequently associated with SCD. In the future, mice models should be based on the combined application of inducible promoters and cardiac-specific deletion of the genes to allow a better control of the level and the timing of transgene expression. Identification of the trigger mechanisms and of the primary causes of cardiac arrhythmias has opened the possibility to develop genetic models reproducing both inherited and acquired arrhythmogenic substrates (including myocardial infarction, cardiomyopathies and other cardiac disease states). These models should allow us to understand the molecular mechanisms involved in the electrical and structural cardiac remodeling during normal (ageing) and pathological conditions that create an electrically unstable heart that increase the susceptibility to develop lethal cardiac arrhythmias. Finally, a better understanding of the specific pathways involved in arrhythmogenesis might be the basis for the development and testing of new therapeutic strategies for preventing SCD.

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