Species-Dependent Mechanisms of Cardiac Arrhythmia: A Cellular Focus

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ABSTRACT: Although ventricular arrhythmia remains a leading cause of morbidity and mortality, available antiarrhythmic drugs have limited efficacy. Disappointing progress in the development of novel, clinically relevant antiarrhythmic agents may partly be attributed to discrepancies between humans and animal models used in preclinical testing. However, such differences are at present difficult to predict, requiring improved understanding of arrhythmia mechanisms across species. To this end, we presently review interspecies similarities and differences in fundamental cardiomyocyte electrophysiology and current understanding of the mechanisms underlying the generation of afterdepolarizations and reentry. We specifically highlight patently shortcomings in small rodents to reproduce cellular and tissue-level arrhythmia substrate believed to be critical in human ventricle. Despite greater ease of translation from larger animal models, discrepancies remain and interpretation can be complicated by incomplete knowledge of human ventricular physiology due to low availability of explanted tissue. We therefore point to the benefits of mathematical modeling as a translational bridge to understanding and treating human arrhythmia.

KEYWORDS: arrhythmia, species, cardiomyocyte, electrophysiology

Burden of Arrhythmia and the Role of Animal Models in Preclinical Therapeutic Testing

Cardiac arrhythmias such as ventricular tachycardia and fibrillation remain leading causes of morbidity and mortality in the Western world. Yet, most of the patients who die from sudden cardiac death do not meet criteria for defibrillator implantation,1 and available antiarrhythmic drugs have limited efficacy. Indeed, the failure of major clinical trials has been a significant discouragement to antiarrhythmic drug development efforts over the past 15 years.2 In part, these failures have been attributed to discrepancies between the experimental models used in preclinical development with human outcomes.3,4 Perhaps, most importantly, the electrophysiological bases for these failures are challenging to predict from our basic understanding of the differences among commonly used models and species for preclinical drug screening.5 Thus, development of novel treatments urgently requires improved understanding of arrhythmia mechanisms across species, including an awareness of limitations in existing data.6 Here, we review known similarities and differences in fundamental electrophysiology of common model species and discuss how these characteristics are likely to affect the utility of each species for understanding and targeting certain types of arrhythmia. We particularly focus on ventricular currents and cellular mechanisms, but also treat atrial differences where appropriate, and point to the benefits of mathematical modeling as a translational bridge to understanding and treating human arrhythmia.

Conditions for generating cardiac arrhythmia

In all cases, cardiac arrhythmias are complex electrophysiologic behaviors that represent the breakdown of coordinated electrical activity among millions to billions of cardiac cells, each of which has the ability to be independently electrically activated. To distill the mechanisms capable of eliciting these behaviors, it is common to view arrhythmia generation as a process requiring 2 components: (1) a triggering event, such as a spontaneous (ectopic) or reentrant ventricular activation, and (2) a vulnerable tissue substrate into which the event can propagate. Differences in fundamental electrophysiology provide the basis for the susceptibility and characteristics of both components across species, and we begin with a discussion of these differences as they have been described for major model species, including the mouse, guinea pig, rabbit, and dog.

Species Differences in Cardiomyocyte Electrophysiology

The action potential (AP) of the ventricular cardiomyocyte is controlled by a remarkably fine balance of transmembrane ion balances...
Molecular and functional differences in major depolarizing currents

The molecular constituents of all 3 major inward currents are thought to be relatively consistent across species. However, meaningful functional differences have been established, particularly for the calcium transporters, the largest of which occur for NCX. The exchanger was first isolated from the canine heart by Philipson et al. They subsequently cloned the first NCX isoform (NCX1), which is recognized as the dominant cardiac form in mammals. Although it is not clear how much NCX1 protein expression varies between species, it is clear that exchanger activity differs substantially, and the pattern of activity across species generally reflects the degree to which sarcoplasmic calcium fluxes support contractile requirements. At steady state, calcium efflux via NCX is approximately equal to the influx via ICaL. In the mouse and rat, this amounts to a relatively paltry 7% of the total calcium cycled during each beat, with the vast remainder being released from and resequestered into the SR. In nondiseased larger mammals, including rabbits and humans, the NCX contribution to calcium clearance is roughly 4-fold higher at 28% to 29% of total. In approximately equal parts, the reduced contribution for sarcolemmal calcium fluxes in rats results from (1) 3- to 4-fold higher SR calcium ATPase (SERCA2) activity, which favors SR calcium reuptake over NCX extrusion during diastole, and (2) fundamentally reduced NCX activity in the rat. Lesser NCX1 expression may partially explain this reduction, but in vivo, an important additional contribution is made by markedly higher intracellular [Na+] in rats and mice, which diminishes the thermodynamic driving force for Ca2+ extrusion. It is also possible that alterations in exchanger kinetics accompany differential regulation and expression of NCX1 splice variants across species, but these possibilities remain poorly explored. As we discuss later, the overall shift from sarcolemmal calcium sources toward SR-dependent calcium handling has allowed mice and rats to be relatively sensitive model species for SR-dependent arrhythmia phenotypes.

Peak ICaL is slightly reduced in rats compared with larger mammals, and in part, this is due to lower channel expression, particularly compared with guinea pig. ICaL activation in rats is negatively shifted compared with rabbits and guinea pigs, and this combines with slightly more positive inactivation to produce a marked increase in window current. Similar steady-state properties are observed when calcium is replaced by monovalent charge carriers, and the intrinsic kinetics of inactivation and recovery from inactivation are also slower in rat compared with rabbit. Because these observations were generally made under conditions in which SR calcium release had little opportunity to contribute to LTCC inactivation, they suggest that a range of properties independent of differences in calcium handling promote greater LTCC activity in rats than mammals with longer APs. Thus, given the same AP waveform, rat ICaL will be larger and longer. In vivo, this partially compensates for the much shorter rat AP and thereby better maintains LTCC-mediated Ca2+ influx. The molecular bases for these effects are not fully clear, and it is thought that the high sequence homology in critical regions for the major isoforms of both the alpha (α1C) and beta (β2α) LTCC subunits leaves little room for intrinsic differences in gating and permeation. It remains possible that differential expression of lesser β subunits or other accessory modulators may be important, but such effects are yet to be established.

Surprisingly, few data are available to systematically describe functional differences in INa (or late INa) across species. In large part, this is because INa remains very difficult to measure in cardiac cells, where its magnitude and kinetics under physiologic conditions strain voltage control in micro-electrode preparations. Original studies defining cardiac INa and its pharmacology were performed in rabbit Purkinje fibers, which have several structural characteristics that allow for...
reliable measurement. However, modern microelectrode approaches in isolated myocytes still necessitate reducing the current by decreasing the extracellular Na⁺ concentration, temperature, or channel availability through partial pharmacologic blockade. All of these strategies meaningfully alter current magnitude or kinetics, and it has therefore been very difficult to compare results between studies and groups. At this time, we are unaware of any study that has systematically assessed differences in intrinsic ventricular sodium current across species. In contrast, the molecular basis of INa has been studied in a number of different species. Although Nav1.5 is the dominant alpha subunit in all cases, nontrivial and variable expression of other isoforms has been observed across species, and a number of studies have observed roles for these lesser (tetrodotoxin [TTX]-sensitive) isoforms in cardiac INa. In addition, a wide variety of accessory proteins, including β subunits, scaffolding proteins, and signaling enzymes, may contribute species-dependent effects on INa function, primarily by altering kinetics and trafficking.

For all of these reasons, it seems likely that some systematic species differences in the functional characteristics of cardiac INa (and probably also late INa) exist; however, to our knowledge, none have been clearly reported to date.

**Molecular and functional differences in repolarizing currents**

The ensemble of K⁺ currents contributing to repolarization is relatively large, and varying expression of the different K⁺ currents is the primary explanation for major species differences in cardiac electrophysiology (Figure 1). To simplify our perspective with respect to these species differences, we separate the major K⁺ currents into 2 groups: (1) those that develop rapidly during the AP (several to 10s of ms) and (2) those that develop slowly (50-100s of ms). This framework is subtly different to conventional nomenclature, which describes cardiac repolarization in terms of (1) the transient outward currents (Ito) and (2) the delayed rectifier currents (IK).

**Figure 1.** Computational models represent the most concise, precise, and simple means of storing current information of myocyte electrophysiology. They also present the most simple means of comparing dynamic characteristics between species or those resulting from disease. Human: the left ventricular epicardial model of Grandi et al. The relatively fast and large transient outward current is a characteristic of epicardial myocytes, and humans exhibit more subtle expression of the delayed rectifiers than other larger mammals. In particular, the rapidly activating delayed rectifier dominates over the slowly activating form, which has important implications for repolarization reserve. Canine: epicardial model from Hund and Rudy showing the pronounced expression of INa in these cells, which is responsible for the characteristic peak and dome action potential (AP) of this cell type and alters time courses of both INa and sarcoplasmic reticulum calcium release. Rabbit: the rabbit model of Shannon et al showing the pronounced expression of INa in these cells, which is responsible for the characteristic peak and dome action potential (AP) of this cell type and alters time courses of both INa and sarcoplasmic reticulum calcium release. Guinea pig: Luo and Rudy developed the first biophysically detailed models of ventricular myocyte electrophysiology. These models have been continually revised, and here, we show output of the Faber-Rudy model published in 2007. Note the high expression of all currents, and particularly INa, which makes a major contribution to the stability of the phase 2 plateau. Inward (reverse mode) INaCa is only substantial in this model at high intracellular Na (15 mM), and normal Na is ~7 mM. Mouse: the detailed mouse model of Morotti et al showing how high expression of both INa and INaCa overwhelms all inward currents in these cells and thereby markedly abbreviates the AP. This eliminates almost all opportunities for activation of INa and INa, both of which are included in the model.
convention is important for our purpose because it is more tailored to differentiating among species. For example, in the context of brief APs of mouse and rat myocytes, repolarization proceeds so rapidly that channel species which conduct current after 50 ms will rarely be recruited, even if they are expressed and measurable in conventional square pulse protocols. In this way, the repolarization characteristics in these ubiquitous model species are determined more by differences in the activation kinetics of the repolarizing currents (even among the rapidly activating currents) than by the inactivation kinetics that are conventionally used to separate and classify them. As we discuss below, for several of the rapidly activating currents, the details of these activation kinetics remain somewhat mysterious and they are likely to be critical for shaping and maintaining the stability of repolarization in lower species and perhaps also atrial myocytes of larger species.33

Rapidly developing repolarizing currents. A number of rapidly activating currents contribute to initial repolarization from the AP overshoot and counteract the developing I CaL early in the AP of large mammals. In the mouse and rat, these currents are generally much larger and responsible for all but terminal repolarization of the AP. This species dependence is pronounced and key for permitting the much higher heart rates in mice and rats. Interestingly, these currents have also been shown to exhibit remarkable variability among mammals with longer APs, and the reasons for this variability in terms of whole organ function are still not entirely clear.

In many but not all species, the transient outward current (Ito) dominates the early contribution to repolarization. This current was initially described as the combination of 2 components, one of which (Ito1) responded to intracellular calcium and was shown to be chloride-selective.46,47 This current is detectable and nontrivial in humans,48 rabbits,49 dogs,50,51 and cats,52 but it has not been systematically compared across species. It appears to be more highly expressed in atrial tissue, where it is distinct from the Ca2+-dependent K+ currents.53 The faster 4-aminopyridine (4-AP)–sensitive component of Ito (Ito1) is K+-selective and much better studied (see reviews of Niwa and Nerbonne53 and Patel and Campbell54). This current can itself be decomposed into 2 components that differ in their rates of inactivation and recovery from inactivation and exhibit strong transmural variation. It is common for these 2 K+-specific components to be summed and referred to simply as Ito, and there are clear species differences in this compound K+-selective current following a pattern of expression where mouse > rat > dog > rabbit > human > guinea pig. Total murine Ito is approximately 3- to 4-fold larger than rabbit,55 whereas guinea pig Ito is generally undetectable.56–59 Importantly, these differences in the compound current are averaged across regions as well as over the 2 kinetic components. Differences across species in how total Ito and the kinetic subtypes are expressed through the ventricular wall also underlie important characteristics of organ-level electrophysiology56,60 and contraction.61,62 We focus on the defining characteristics of these 2 components and key aspects of species specificity, as discussed below.

The fast component (I to,fast or Ito,f) is distinguished by its very rapid recovery from inactivation (τ, 20–50 ms), but also inactivates with a time constant of ~10 ms (50–80 ms in room temperature recordings), and is therefore often separated from other components by these decay kinetics.63,64 The molecular basis of this current is now thought to involve both homooligomerization and heterooligomerization of Kv4.2/4.3 pore-forming alpha subunits.55,63 These pores may be regulated by a number of accessory subunits, including the K channel–interacting proteins (KChIPs), MinK–related peptides (MiRPs), Kvβ 1–3 family proteins,53 and di-aminopeptidyl transferase-like protein 6 (DPP6).65 Functionally, high expression of Ito,f in the epicardium relative to endocardium underlies a large portion the transmural Ito gradient in most species, and in the dog, this gradient is very large and completely due to region-specific expression of Ito,f.54

The slow component of Ito (Ito,slow or Ito,s) inactivates 5– to 8-fold more slowly than Ito,f and is defined for its much slower recovery from inactivation (τ, 1 s), which can have important implications for the frequency dependence of repolarization. Kv1.4 is the dominant alpha subunit, and it generally exhibits an opposite transmural gradient to Ito,f (endocardium > epicardium). The rabbit is a noteworthy exception where it provides the bulk of total Ito throughout the heart and is expressed at higher levels in the epicardium presumably to maintain the overall transmural gradient in Ito.66 It remains unclear whether there are any important regulatory subunits for this current,52 but it is at least known to be regulated by calcium/calmodulin-dependent protein kinase II (CaMKII).55

The final major rapidly activating outward current is the ultrarapidly activating delayed rectifier (IKur), which has been known by a variety of other names (IKs, I Kaslow, IKur) as its existence and properties have been established by different methods and in different species and regions of the heart.51 This current is prominent in the atria of large mammals51,52 and throughout the hearts of mice and rats,64,66–70 where it makes a major contribution to the more rapid repolarization and shaping the triangular AP waveform in these cells. It is distinguished from the transient outward currents by markedly slower inactivation, and for this reason, deactivation is probably the dominant process terminating IKur during repolarization in vivo. In part, this is because the cells in which IKur is strongly expressed also repolarize relatively rapidly (Figure 1). IKur in mice was originally known as IKur, but it has now been shown that there are 2 components with differing activation kinetics (IKur,fast/IKur and IKur,slow).71 The more rapidly activating and inactivating component is virtually identical to IKur observed in other species and tissues and is carried by Kv1.5. C-type inactivation of this channel is
cooperative and importantly regulated by β-subunit interaction with the C-terminal domain. For this reason, in vivo inactivation is generally thought to be considerably faster than is observed in heterologous expression of alpha subunit alone. The more slowly activating $I_{\text{K}_{\text{slow2}}}$ exhibits differing pharmacology and is sensitive to millimolar tetraethylammonium, but not low-concentration ($\mu$M) 4-AP, and appears to be encoded by Kv2.1. Depending on temperature and the method used to separate these currents, it is likely that this more slowly activating component often contributes to a large proportion of the pedestal current, which is often named the steady-state voltage-dependent current ($I_{\text{L}_\text{p}}$). The similar inactivation characteristics but differing activation kinetics of the various rapidly activating currents can give the overall current ensemble differing decay kinetics depending on the relative expression of the components and due to temperature. This can be exaggerated by differences in β-subunit expression, which is important for both Kv1.5 inactivation and Kv2.1 activation. Together, these characteristics make kinetically based separation of these currents very challenging. Finally, it should be noted that another rapidly activating and slowly inactivating current ($I_{\text{K}_{a}}$) has been described in guinea pig but is probably identical to $I_{\text{Kur}}$.81

**Slowly developing repolarizing currents.** The remaining delayed rectifier currents, which are known as the rapidly activating ($I_{\text{Kr}}$) and slowly activating ($I_{\text{Ks}}$) forms, exhibit much slower current development. As described below, the role of these currents in rats and mice remains somewhat controversial, but under normal circumstances, it is unlikely that they make more than a minor contribution to repolarization in these species. In contrast, important differences exist for the balance of $I_{\text{K}_{a}}$ and $I_{\text{Ks}}$ among mammals with longer APs, where they determine the trajectory and duration of the phase 2/3 plateau. As the delayed rectifiers slowly push repolarization out of the $I_{\text{C,L}}$ activation range, the voltage-independent inward rectifier ($I_{\text{Kr}}$) is also recruited to drive terminal repolarization in ventricles of all species and in most other types of myocyte in the heart. This current also exhibits differing expression across species, which modifies both the trajectory of late repolarization and the stability of resting potential.

Among these slowly developing currents, the species dependence of the delayed rectifiers has particular importance for repolarization reserve and susceptibility to long QT (LQT)–associated arrhythmias such as torsades de pointes. Because $I_{\text{Kr}}$ is also now recognized as a critical target for understanding the cardiotoxic outcomes of many drugs, the question of which species provides the best foundation for assessing delayed rectifier block has been brought strongly into focus over the past 10 years. $I_{\text{Kr}}$ is functionally unique among cardiac currents in that its contribution to repolarization results largely from channels that are recovering from inactivation, rather than proceeding in a canonical activation to inactivation sequence. This behavior is due to the very rapid form of C-type inactivation of hERG tetramers (human ether-a-go-go-related gene), which greatly outstrips their more moderate activation kinetics. In this way, hERG channels accumulate in the inactivated conformations at the AP peak and early plateau, and as potential returns toward rest, they recover through the open state before deactivating, often with multiple slower kinetic components. These characteristics contrast with $I_{\text{K}_{a}}$, for which the KvLQT1 (KCNJ1) pore activates and deactivates very slowly ($\tau_{\text{act}}$, 1 s; $\tau_{\text{deact}}$, 100-300 ms) and exhibits only very slight inactivation, which is completely eliminated on coassembly with minK (KCNE1) β subunits. These differentiating kinetics have a number of implications for species differences, but 2 of the most important are as follows. (1) the kinetics of $I_{\text{Kr}}$ cause it to accumulate in or near activation at higher heart rates or during prolonged depolarization. This allows it to provide a reserve of outward current that can be recruited with diminution of other outward currents (most notably $I_{\text{K}_{a}}$) and also underlies its contribution to steep restitution of AP duration in species exhibiting an AP plateau. These roles have very important implications for pharmacologic characterization in different species. (2) In myocytes with brief APs, the window-like activation characteristics of $I_{\text{Kr}}$ allow it to make a small contribution to repolarization when hERG is expressed. This contrasts with $I_{\text{K}_{a}}$, for which activation kinetics are too slow to contribute to repolarization in species and tissues exhibiting shorter APs, at least for as long as repolarization remains stable.

As mentioned above, there remains some debate surrounding the role of these currents in mice and rats because murine knockout of KCNQ1/KCNE1 causes exaggerated rate-dependent and adrenergic slowing of repolarization. In larger mammals, these changes are indicative of reduced repolarization reserve. However, these animals exhibit little change in AP characteristics and minimally detectable $I_{\text{Kr}}$ under normal conditions. One interpretation of these results is that the $I_{\text{Kr}}$ in small rodents can only be unmasked during adrenergic modulation, which both prolongs the murine AP secondary to exaggerated Ca$^{2+}$ cycling and is well known to increase $I_{\text{K}_{a}}$. E-4031-sensitive hERG currents can be detected in mice, but hERG knockout does not elicit measurable changes to the electrocardiogram or arrhythmia susceptibility in vivo.

Together, these findings support the general perspective that the delayed rectifiers play only very minor roles in mice, and probably also rats.

There are many established differences in both expression and kinetics of the delayed rectifiers across the larger mammals exhibiting long APs. Of greatest importance for our discussion is the role that the balance of $I_{\text{Kr}}$ and $I_{\text{Ks}}$ plays in defining the susceptibility of these species to AP prolongation and LQT-associated arrhythmia. Differences in this balance are most strongly observed and clinically relevant under conditions straining repolarization. In species with robust $I_{\text{Kr}}$, particularly guinea pig (Figure 1) and also dogs, inhibition of $I_{\text{Kr}}$ causes...
voltage-dependent recruitment of $I_{Ks}$, thereby resisting further AP and QT prolongation. Species with less pronounced $I_{Ks}$ but not sheep. Among species that are commonly used to model human physiology, both the rabbit and dog exhibit larger ventricular $I_{K1}$ expression than humans, whereas rectification properties are relatively similar. Both species exhibit 2- to 3-fold larger currents within the range relevant to repolarization, and this appears to extend to the inward portion of the voltage range. The mouse is unique among species in that the tissue distribution of $I_{K1}$ appears to be the opposite of larger species. Marine $I_{K1}$ is at least as large in the atria compared with ventricles, with the left atrium having slightly higher expression at potentials negative to $E_K$. In sum, these characteristics may leave human myocytes more susceptible to diastolic depolarization accompanying events such as spontaneous calcium release and slightly reduce the rate of terminal repolarization compared with other large mammals (Figure 1).

### Species-Dependent Mechanisms Underlying Early Afterdepolarizations

Early afterdepolarizations (EADs) are oscillations in membrane potential occurring prior to terminal repolarization of the AP, which can occur either during the plateau region of the AP (phase 2) or during late repolarization (phase 3). Classically, these events are thought to occur anytime the balance of currents contributing to the plateau is disrupted and AP duration is prolonged, ie, when outward currents are reduced and/or inward currents are increased. As we describe below, this perspective remains informative because it indicates the origin of the dysfunction, but it does not offer a precise account of the mechanisms governing EAD generation and may therefore have limited ability to guide effective therapeutic interventions. A more detailed account takes into consideration how the trajectory of repolarization interacts with the kinetics of the contributing currents to determine stable and unstable regimes of cardiac repolarization. Because AP shape varies markedly, it is perhaps not surprising that fundamentally different mechanisms underlie EAD generation across species.

### EADs in large mammals

As detailed above, ventricular cardiomyocytes from large species including humans, dogs, sheep, guinea pigs, and rabbits exhibit prolonged APs with clear phase 2/3 plateau. Early afterdepolarizations observed in these species are generally initiated late in the plateau, and early studies of EAD mechanisms established that no matter which current is the proximal source destabilizing repolarization, reactivation of the LTCC pool provides most of the current during the EAD upstroke. Over subsequent years, the range of stimuli and currents capable of destabilizing repolarization in these species has been extended as different experimental maneuvers have been tested. Aside from $I_{NaCa}$, the most important destabilizing currents are forward-mode $I_{NaCa}$ resulting from exaggerated or disorganized SR calcium release (Figure 2A) and
increased late sodium current.\textsuperscript{109,110} Similarly, maneuvers that reduce K$^+$ channel conductance and repolarization reserve have been known to elicit EADs for some time.\textsuperscript{79,103,105} Together, these observations contributed to the concise and satisfying interpretation that EADs are a straightforward extension of reduced repolarization reserve and excessive AP prolongation. Although this perspective is useful in that EADs are correlated with both AP prolongation and reduced repolarization reserve, there are several exceptions to this premise that required further explanation. Two of the most compelling are as follows: (1) AP duration can be markedly increased without resulting in EADs, whereas AP triangulation (independent of AP duration) strongly predicts EAD generation,\textsuperscript{111–113} and (2) brief APs with ample repolarization reserve and little or no plateau, as occurring in rodents or the atria of large mammals, still exhibit EADs during a range of challenges.\textsuperscript{86,114–118}

Figure 2. Early afterdepolarization (EAD) mechanisms differ across species and tissues. (A) Schematic representation of EAD mechanisms in large mammal ventricular myocytes. The long action potential plateau observed in ventricular myocytes from humans and other large mammals promotes EADs that initiate within the activation range of I_{CaL}. As a result, the broad range of maneuvers or defects that can destabilize repolarization in these cells converge at I_{CaL} reactivation, which carries most of the inward current in all cases. In some cases, discoordinated intracellular calcium release (systolic calcium waves) can act as the initiating factor and generate sufficient I_{NaCa} to drive these reactivation events. Because I_{NaCa} is favored at negative potentials, these types of events are more likely to generate an EAD during terminal repolarization and still I_{CaL} carries most of the current during the EAD upstroke. (B) Murine EADs involve fundamentally different mechanisms. At left, an action potential clamp experiment showing the lidocaine-sensitive current during an EAD waveform is recorded in a mouse ventricular myocyte. At these concentrations, lidocaine binds primarily to inactivated Na$^+$ channels, and this current indicates a reactivating component of I_{Na}. Because this component is sensitive to the trajectory of repolarization, it represents nonequilibrium dynamics rather than window-range reactivation. At right, the same EAD waveform is imposed on a mouse ventricular myocyte model, which shows that compared with the other major inward currents, I_{Na} is by far the greatest contributor to EADs in mice. (C) EAD mechanisms associated with reinitiation of atrial fibrillation (AF) in a human atrial myocyte model. Rapid pacing and acetylcholine administration can be used to simulate atrial fibrillation in atria of large mammals (AF\textsuperscript{119}). When the pacing is stopped to simulate termination of AF, spontaneous EADs occur at sinus rates, as has been observed in canine atria\textsuperscript{119}. These events result from I_{Na} reactivation very similar to that occurring in the mouse ventricle. This may be important in certain specific contexts of human atrial disease.
mammals: (1) AP prolongation does not elicit EADs if it occurs at positive potentials because \( I_{CaL} \) recovery is limited and slow in that potential range, and (2) AP triangulation is highly predictive of EADs and associated arrhythmias because it lengthens the time spent in potential ranges where \( I_{CaL} \) can recover and grow rapidly while \( I_K \) deactivates.\(^{112,113}\) Finally, this explanation of EAD mechanisms constitutes a form of dynamic chaos that is also capable of explaining the irregular nature of EAD appearance under some conditions and which was otherwise attributed to stochasticity in channel gating.\(^{122,123}\) As such, this conceptual and quantitative framework provides our most complete account of EAD mechanisms in APs from large mammals and reconciles contradictory aspects of prior conceptual models. These mechanisms extend to EADs in myocytes from smaller mammals and other more rapidly repolarizing cardiac cell types, but these cells also house additional EAD dynamics that are not readily observed in large mammalian myocytes.

**EADs in mice and large mammalian atria**

As described above, the rapidly repolarizing potassium currents, \( I_{Na} \) and \( I_{Kur} \), are highly expressed in the mouse and rat, and this affords those species much more rapid repolarization. \( I_{Kur} \) is also expressed in atrial tissue of larger mammals and thereby results in a more triangular atrial AP with a much more negative phase 2/3 plateau compared with ventricular tissue. We have recently shown that the rapid repolarization of mouse ventricle fundamentally alters EAD dynamics\(^{86}\) because the mouse myocyte repolarizes through the \( I_{CaL} \) activation range within 5 to 10 ms. This simultaneously prevents \( I_{CaL} \) from entering the unstable regime accessible in larger mammals and facilitates a regime in which \( I_{Na} \) is capable of reactivating (Figure 2B). The limited time spent at positive potentials largely prevents accumulation of sodium channels in the slowly recovering states of intermediate and deep inactivation and deposits the membrane potential in a range where a very small portion of channels can rapidly recover from fast inactivation and undergo reactivation events. Although this interaction between \( I_{Na} \) and \( I_K \) has not yet been assessed in quantitative detail, this nonequilibrium reactivation of \( I_{Na} \) appears to be the dominant mechanism of EAD initiation in mice (Figure 2B).

Once initiated, murine EADs may reach potentials that recruit further reactivation of \( I_{CaL} \) and exhibit dynamics much more similar to large mammals.\(^{86}\) Interestingly, mouse-like EAD dynamics have also been observed in canine atrial preparations when repolarization is accelerated via dual pharmacologic challenge by acetylcholine and \( \beta \)-adrenergic agonists or by autonomic nerve stimulation.\(^{115,116,119}\) Computational analyses involving a human atrial model suggest that \( I_{Na} \) reactivation is the initiating current in these EADs, secondary to prolongation of the low phase 3 plateau, just as in the mouse (Figure 2C).\(^{114}\) With this similarity noted, it is important to reinforce that the atria of humans and other large mammals do not normally repolarize on the same timescale as the mouse. Because the atrial AP of larger mammals (including humans) is much longer (~200 vs 50 ms in mouse), it is also likely that these dynamics are relatively specific to the particular conditions used in these studies. Thus, the repolarization trajectory of large mammalian atria can allow a range of EAD regimes including both \( I_{CaL} \) and nonequilibrium \( I_{Na} \) reactivation, but ventricle-like \( I_{CaL}^{-1}I_K \) dynamics are more probable in most pathologic circumstances.

Finally, despite unique EAD mechanisms present in mice, this species is now often used to examine arrhythmia mechanisms and interventions.\(^{124}\) Most often, the sensitivity of murine EADs to various maneuvers is then interpreted in the context of the established dynamics for large mammals.\(^{118}\) This is likely to incorrectly emphasize the role of \( I_{CaL} \) in the murine mechanism and to contribute to the poor translation of findings across levels of preclinical and clinical studies.

### Ca\(^{2+}\) Waves and Delayed Afterdepolarizations

Spontaneous RyR Ca\(^{2+}\) release, which can be visualized as a propagating Ca\(^{2+}\) wave, produces a phasic depolarization of the membrane potential as released Ca\(^{2+}\) is extruded by NCX. As noted above, Ca\(^{2+}\) waves occurring late in the APs of large species can elicit EADs, which may subsequently recruit LTCCs. However, in both small and large species, spontaneous Ca\(^{2+}\) waves can occur at resting membrane potential due to spontaneous RyR openings, with resulting NCX current eliciting delayed afterdepolarizations (DADs). When of sufficient magnitude, DADs can trigger sufficient Na\(^+\) channel opening to elicit a spontaneous AP. Although the basic mechanisms of Ca\(^{2+}\) wave and DAD generation are shared across species, susceptibility to these events is dependent on species-specific parameters, including RyR sensitivity, cellular Ca\(^{2+}\) flux balance, cellular ultrastructure, and heart rate (diastolic interval).

RyR opening is steeply dependent on cytosolic [Ca\(^{2+}\)], although the precise nature of this relationship is also determined by other factors including [Mg\(^{2+}\)], pH, and SR luminal [Ca\(^{2+}\)].\(^{125}\) In bilayer studies mimicking physiologic conditions, most reports indicate that half maximal activation of RyRs occurs within a range of 2 to 10 \( \mu \)M cytosolic [Ca\(^{2+}\)] (reviewed in a study by Bers\(^{126}\)). However, there appear to be species-dependent differences as recent studies have demonstrated that RyRs from rat hearts exhibit substantially lower Ca\(^{2+}\) sensitivity than those from human, sheep, and dog.\(^{127-129}\) This finding is not generally supported by studies examining Ca\(^{2+}\) spark probability in cardiomyocytes; indeed, Satoh et al.\(^{130}\) observed higher spark rates in rat than rabbit cardiomyocytes. This discrepancy is likely due to differences in luminal [Ca\(^{2+}\)], on which Ca\(^{2+}\) spark frequency has a near-exponential dependence.\(^{131}\)

Following termination of electrical pacing, rabbit cells exhibit rapid loss of SR Ca\(^{2+}\) due to prominent Ca\(^{2+}\) extrusion by NCX. In rat cells, however, SR Ca\(^{2+}\) reuptake by SERCA dominates
over NCX function, and SR Ca\(^{2+}\) content is maintained.\(^{130}\) This high luminal SR Ca\(^{2+}\) sustains high RyR open probability, despite the reported low sensitivity of the channel to cytosolic Ca\(^{2+}\).\(^{127,128}\) In agreement with this observation, unstimulated cells from rats and mice exhibit regular Ca\(^{2+}\) waves during conditions of Ca\(^{2+}\) overload, whereas in rabbit cells sparks and waves rapidly cease following cessation of electrical pacing.\(^{130,152,153}\) Guinea pig cardiomyocytes also exhibit a pronounced loss of SR Ca\(^{2+}\) content during rest, whereas ferret and human cardiomyocytes may show a slow decline or increase in releasable Ca\(^{2+}\).\(^{134-136}\) Such interspecies differences should be carefully considered when interpreting Ca\(^{2+}\) wave susceptibility in resting cells.

Species differences in Ca\(^{2+}\) wave generation are also apparent in the more physiologic context of electrical stimulation. Indeed, pacing frequency is a key regulator of RyR Ca\(^{2+}\) sensitivity. Although SR content is high in cardiomyocytes from small rodents, it is relatively maintained across the physiologic range of pacing frequencies.\(^{137}\) In contrast, SR content is lower in large species but increases with pacing rate, which is expected to augment the potential for waves. Simultaneously, there is accumulation of diastolic [Ca\(^{2+}\)] at increasing frequency, as SERCA and NCX become unable to keep up with Ca\(^{2+}\) influx. Rising cytosolic Ca\(^{2+}\) levels increase the likelihood of waves both directly, by triggering RyR opening, and indirectly, by activating CaMKII, which phosphorylates the RyR and increases its Ca\(^{2+}\) sensitivity. These proarrhythmic, RyR-sensitizing effects of high heart rates are tempered somewhat by RyR “refractoriness.”\(^{71,126}\) This term refers to the fact that following each Ca\(^{2+}\) transient RyRs exhibit a brief period of reduced availability. Thus, at high pacing frequencies, there is less diastolic interval when RyRs are available to be activated. For this reason, it might be envisioned that animals with high heart rates such as mice have limited susceptibility to Ca\(^{2+}\) waves in vivo.

Species differences in SR and sarcolemmal Ca\(^{2+}\) fluxes have consequences not only for determining Ca\(^{2+}\) wave susceptibility but also for the subsequent generation of DADs. Because SERCA and NCX compete for the same pool of Ca\(^{2+}\), high NCX activity and/or low SERCA activity reduce SR Ca\(^{2+}\) content, making spontaneous Ca\(^{2+}\) release less likely.\(^{106,138}\) However, when a wave does occur in this scenario, proportionally greater released Ca\(^{2+}\) is extruded due to prominent NCX activity.\(^{139}\) Thus, in large species with robust transsarcolemmal Ca\(^{2+}\) cycling, larger proarrhythmic DADs are expected per unit released Ca\(^{2+}\). In contrast, healthy mice and rats exhibit prominent SR reuptake which more easily loads the SR, but also draws Ca\(^{2+}\) away from the NCX during a wave, minimizing DAD amplitude. Such opposing effects complicate prediction of arrhythmic potential in different species and also in disease scenarios where SERCA and NCX expression are often inversely regulated.\(^{140-142}\)

Finally, accumulating evidence indicates that the precise arrangement of dyads is an important determinant of Ca\(^{2+}\) wave propagation and DAD generation. In both small and large species, RyRs are localized with a regular striated appearance along z-lines (reviewed in a study by Louch et al\(^{143}\)). Small rodents have a high density of similarly organized T tubules, meaning that a large proportion of RyRs are present in or very near dyads.\(^{144-146}\) Thus, when spontaneous Ca\(^{2+}\) release occurs, nearby NCX in the T tubules is well positioned to mediate Ca\(^{2+}\) extrusion during Ca\(^{2+}\) sparks or waves.\(^{147,148}\) In larger species, such as pigs and humans, T-tubule density is considerably lower.\(^{144,149}\) Thus, a large fraction of RyRs can be considered extradyadic or “orphaned.”\(^{146}\) with NCX molecules located considerably distant. In such cells, Ca\(^{2+}\) is expected to be quickly removed from RyR release sites in dyads and more slowly from orphaned RyRs. In agreement with this prediction, Biesmans et al\(^{147}\) observed a bi-exponential time course of NCX current following Ca\(^{2+}\) release. Based on these observations, it has been hypothesized that low T-tubule density may limit DAD generation during Ca\(^{2+}\) waves.\(^{150}\) However, the orientation of dyads should also be considered, as not all dyads are oriented transversely along z-lines. Especially in rodents, a fairly high proportion of T tubules and RyRs are positioned longitudinally (axially) along the cell, spanning sarcomeres.\(^{146,151}\) Mathematical modeling has predicted that RyRs in these dyads may abet Ca\(^{2+}\) wave propagation.\(^{152,153}\) In addition to interspecies considerations, changing dyadic orientation is likely important for determining arrhythmic potential in diseases, such as heart failure, where there is loss of transversely oriented dyads at z-lines but formation of longitudinally oriented dyads.\(^{145,146,151}\)

**Tissue-Level Considerations**

Although we have focused this review on the cellular mechanisms that differentiate arrhythmia susceptibility among common model species, very important differences also exist at the tissue and organ levels and warrant a cursory treatment at least. These differences can be broadly classified as either structural or functional, and the manner in which they affect arrhythmogenesis results from their influence on arrhythmogenic tissue substrate or the interaction between cellular triggers of arrhythmia and that substrate. Although a number of structural (eg, tissue size and conduction system structure) and physiologic (gap junction conductance, heterogeneity in current expression, diastolic interval and restitution properties, alternans susceptibility, etc.) characteristics may contribute to these differences, we reduce the complexities to those contributing to either reentry or ectopy.

**Tissue size as a limitation to reentry**

The length of a propagating wave in cardiac tissue is given by the product of the conduction velocity of the wavefront and the effective refractory period (CV-ERP). In the mouse, ventricular ERP is approximately 30 ms.\(^{154}\) Assuming a bulk myocardial conduction velocity of 50 cm/s, this yields a wavelength of...
approximately 1.5 cm, which is close to the distance that the wavefront traverses during activation of an adult mouse heart. This simple calculation is commonly used to argue that the mouse heart is too small to permit sustained reentry, and both early experiments\textsuperscript{155} and more recent theoretical treatments\textsuperscript{156} helped this idea to gain general acceptance. However, experiments involving optically recorded arrhythmia then convincingly have shown that reentry is possible in the mouse heart and that reentrant vortices can be significantly smaller than the wavelengths predicted from CV and ERP during normal pacing in the same tissue (15–30 mm\textsuperscript{157}). The explanation provided for this effect was that a stable circuit could be generated by dispersion of action potential duration (APD) moving from short APs at the rotor core to longer APs at more distant sites.\textsuperscript{157} This dynamic stabilization fundamentally modified arguments surrounding limits to reentry and reinforces the importance of dynamic substrates in arrhythmia. It has also been suggested that certain conditions that push cardiac tissue away from properties of a continuous medium toward a system of discrete electrical components (cells) can facilitate reentry on a much smaller scale. These behaviors have been termed microreentry and are thought to occur in conditions involving severe local tissue heterogeneity (eg, fibrosis) or markedly reduced and heterogeneous intercellular coupling as in ischemia.\textsuperscript{158}

Susceptibility to dynamic functional substrates

Disregarding structural substrates, dispersion of repolarization due to ischemia or dynamic mechanisms is the most important form of arrhythmia substrate in the heart and can arise in otherwise healthy myocardium. However, the degree to which this dispersion is able to contribute to arrhythmia in vivo depends greatly on its degree, the intrinsic heart rate of the animal, and the spatial scale of the tissue, as described above. In humans and large mammals, dispersion of repolarization has long been considered an important precursor of arrhythmia in a range of congenital and acquired arrhythmias.\textsuperscript{159,160} The largest regional differences in AP duration in large mammals occur transmurally, and APD dispersion across the left ventricle (LV) wall is thought to be one of the most important sources of arrhythmogenic substrate. As such, the existence and importance of different transmural cell types to repolarization dynamics have been the focus of spirited investigation and debate, some of which probably rest on the specific characteristics of the canine wedge preparation.\textsuperscript{159,161–163} Certain characteristics of the canine wedge, particularly the existence of M cells, have received support from studies of human tissues,\textsuperscript{164,165} but a large portion of the transmural variation in underlying currents remains unknown in humans. As such, it is difficult to compare the value of large animal models in this respect. As discussed for the arguments related to reentry, the size of the mouse heart imposes some limits on the degree to which dispersion can provide sufficient substrate for initiation or maintenance of arrhythmia. However, heterogeneity in APD has also been observed in mice,\textsuperscript{154} and several investigations have shown that manipulation of repolarizing currents can exacerbate this to an extent that promotes arrhythmia.\textsuperscript{166,167} Given the very thin mouse ventricular free wall, it is probable that these heterogeneities must occur in an apicobasal rather than transmural basis to permit reentry, but we are unaware of any study that has assessed this directly.

In addition to intrinsic heterogeneity in APD, dynamic instabilities can also produce reentrant substrate. APD alternans is the best described of these behaviors and is observed as a period 2 variation in APD (short-long-short-long), which is typically induced by rapid pacing. Because this behavior involves complex interaction of sarcolemmal current carriers and the intracellular calcium machinery, it is generally studied in species with similar current ensembles as humans or at least with prominent phase 2/3 plateau.\textsuperscript{168–171} To our knowledge, no study has attempted to describe the importance of species differences in current expression or calcium handling for alternans mechanisms, although excellent reviews of general alternans mechanisms are provided elsewhere.\textsuperscript{172,173} With this in mind, we highlight 2 key aspects of these general mechanisms that are likely to be strongly influenced by known species differences: (1) destabilization of calcium handling is likely to be the proximal cause of the cellular behavior in large mammals under physiologic conditions,\textsuperscript{174,175} and (2) the ability of alternation in APD to create substrate for reentry depends on the alternans becoming spatially discordant or out of phase in adjacent regions of tissue.\textsuperscript{173} Given the marked differences in calcium handling (described above), APD restitution,\textsuperscript{154} heart size (described above), and intrinsic heart rate, it is very likely that the small rodents exhibit differing mechanisms of cellular alternans and differing susceptibility to alternans–dependent arrhythmia than larger species. Even among larger mammals, smaller differences in these characteristics are likely to set up important distinctions that muddy translation from preclinical to clinical studies.\textsuperscript{176}

Tissue-level determinants of ectopy

In contrast to determinants of reentry, it is likely that smaller cardiac dimensions render the heart more susceptible to ectopy and premature ventricular contractions (PVCs). This property results from the pronounced effect that tissue volume has on electronic load. Xie et al\textsuperscript{177} nicely demonstrated this relationship in 1-, 2-, and 3-dimensional simulations, where the corresponding number of contiguous cells required to generate a propagating event increased from 10s to 1000s to 100 000s. No experimental study has systematically assessed species differences in the number of cells required to generate PVCs, largely because experimental approaches to estimating these quantities involve many challenges. However, separate investigations using different approaches have described approximate source-sink characteristics in rabbits\textsuperscript{178} and mice.\textsuperscript{179} Although the different approaches (local β-adrenergic stimulation in rabbit vs
optogenetic stimulation in mouse) introduce additional uncertainty, the number of cells required for murine PVCs (100-2000) was markedly less than in the rabbit (~10,000 to million). As suggested by Xie et al., this large difference is likely to indicate a major effect of tissue geometry that had previously only been assessed by simulations. In addition, both studies observed a marked LV/right ventricle (RV) difference in the number of cells required to initiate ectopy. Ablation of the RV Purkinje network by the Lugol solution eliminated this difference in mice, but not in rabbits. In the mouse, this was taken to indicate that the Purkinje network can play an important role in determining PVC requirements, and the authors showed that the primary difference between the RV and LV was that the activating light was not able to fully penetrate the thicker LV free wall and reach the endocardial Purkinje network. Separate investigations of murine ectopy support this role for Purkinje cells. The explanation of interventricular differences in the rabbit requires further explanation but may simply be due to the thinner RV wall, which more closely approximated a 2-dimensional current sink in their experiments. Together, these tissue-level determinants along with the increased reliance on intracellular calcium cycling in small rodents make them quite a sensitive model for arrhythmias associated with spontaneous SR calcium release and DADs.

Summary and Recommendations

In general, the small size of the mouse and rat hearts combines with their differing potassium current profiles and limits their utility for reflecting diseases of human ventricular repolarization across all levels of observation. At the cellular level, their repolarization dynamics differ enough that they are relatively poor models of general AP changes and EAD dynamics in the human ventricle. At the tissue level, they appear to lack the size and specific patterns of heterogeneity thought to underlie important mechanisms of substrate generation in humans and other large mammals. However, these small rodent models may be more applicable to repolarization dynamics in the human atria, where their AP dynamics can more reasonably be regarded as exaggerated versions of the human atrial myocyte. In addition, the heightened dependence of small rodent myocytes on intracellular calcium cycling and the small size of mouse and rat hearts make them relatively sensitive models of spontaneous calcium release at the cellular level and calcium-driven focal arrhythmia in tissue.

Large mammals remain the first choice for addressing questions of specific tissue dynamics in arrhythmias of all types, and many large animal models of specific arrhythmogenic conditions have been created. Because expression of the delayed rectifiers is relatively similar between nondiseased rabbits (as well as heart failure dogs) and humans, these models have been very instructive for understanding the roles of IKr and IKr in determining repolarization reserve and susceptibility to LQTs arrhythmia. Similarly, the balance of sarcolemmal and intracellular calcium fluxes in rabbits, dogs, sheep, and pigs is relatively similar to humans, thus allowing them to be relatively good models of the interaction between calcium-sensitive transporters (particularly NCX and ICaL) and calcium-insensitive modulators of the AP. As such, these species remain the best models for studying these subtleties in detail, particularly at the cellular level and in the context of LQT-associated drug screening. Of course, the specifics of regional heterogeneities in current expression described above must be carefully considered when addressing questions specific to arrhythmogenic substrate. Due to limited tissue availability, human-specific regional heterogeneity remains a work in progress. As these data become available, the models appropriate for specific clinically oriented questions will become clear.

Finally, the wealth of information that has been collected to describe these differences and similarities across species is naturally, efficiently, and usefully stored in the mathematical models that have been developed to represent them. These models constitute the most systematic means at our disposal for interpreting results across species. Thus, we recommend continued interaction among modelers, experimentalists, and clinicians to define the following: (1) the shortcomings of existing models across species, (2) where a lack of reliable experimental data precludes quality model development, and (3) the utility and limits of modeling for linking arrhythmia dynamics observed in experiments with the broad range of criteria and therapeutic usages used to classify and treat patients.

Author Contributions

AGE and WEL drafted and revised the article text. AGE performed mathematical simulations and prepared the figures.

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