Quantitative comparison of cardiac ventricular myocyte electrophysiology and response to drugs in human and nonhuman species

Thomas O’Hara and Yoram Rudy

Cardiac Bioelectricity and Arrhythmia Center, Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, Missouri

Submitted 4 August 2011; accepted in final form 5 December 2011

O’Hara T, Rudy Y. Quantitative comparison of cardiac ventricular myocyte electrophysiology and response to drugs in human and nonhuman species. Am J Physiol Heart Circ Physiol 302: H1023–H1030, 2012. First published December 9, 2011; doi:10.1152/ajpheart.00785.2011.—Explanations for arrhythmia mechanisms at the cellular level are usually based on experiments in nonhuman myocytes. However, subtle electrophysiological differences between species may lead to different rhythmic or arrhythmic cellular behaviors and drug response given the nonlinear and highly interactive cellular system. Using detailed and quantitatively accurate mathematical models for human, dog, and guinea pig ventricular action potentials (APs), we simulated and compared cell electrophysiology mechanisms and response to drugs. Under basal conditions (absence of β-adrenergic stimulation), Na+ / K+-ATPase changes secondary to Na+ accumulation determined AP rate dependence for human and dog but not for guinea pig where slow delayed rectifier current (IKs) was the major rate-dependent current. AP prolongation with reduction of rapid delayed rectifier current (IKr) and IKs (due to mutations or drugs) showed strong species dependence in simulations, as in experiments. For humans, AP prolongation was 80% following IKr block. It was 30% for dog and 20% for guinea pig. Under basal conditions, IKs block was of no consequence for human and dog, but for guinea pig, AP prolongation after IKs block was severe. However, with β-adrenergic stimulation, IKr played an important role in all species, particularly in AP shortening at fast rate. Quantitative comparison of AP repolarization, rate-dependence mechanisms, and drug response in human, dog, and guinea pig revealed major species differences (e.g., susceptibility to arrhythmogenic early afterdepolarizations). Extrapolation from animal to human electrophysiology and drug response requires great caution.

MATERIALS AND METHODS

Mathematical models. Previously developed and extensively validated models of the human (21), dog (7), guinea pig (10), and rabbit (28) ventricular epicardial (Epi) APs were used. Epi cells were chosen (specified if otherwise) because the transient outward K+ current (Ito), an important determinant of AP morphology and rate dependence, is most prominent in Epi cells. Model codes are available on our website (http://rudylab.wusl.edu). The Shannon-Bers rabbit model was executed using CellML repository and tools (www.cellml.org).

Simulation of β-adrenergic response to extracellular application of β-agonist isoproterenol (ISO) was according to previous work in human (20) using original current formulations (21) with τs,PKA = 0.6 * τs,PKAI and GKS,PKA = 3.2 * GKS, in accord with experiments (30)).

Definitions and pacing protocol. The duration of the AP (APD) at 30, 50, 70, and 90% of complete repolarization (APD30–90, in ms) was determined. APD was measured from the time of maximum dVm/dt during the AP upstroke (Vm = membrane potential). For APD rate dependence, we paced to steady state at each cycle length (CL). Only the last or last two paced beats are shown.

RESULTS

Block of delayed rectifier K+ currents, simulating effects of drugs or mutation. Figure 1 shows simulations and corresponding experiments (18) for the species dependence of delayed rectifier K+ current block (rapid and slow, IKr and IKs, respectively) during steady-state pacing (CL = 1,000 ms). For 70% IKr block (Fig. 1A), simulated APD90 was substantially prolonged in human (77%, 172-ms prolongation). Block consequence was comparatively small for dog (34%, 65-ms APD prolongation), rabbit (32%, 67-ms APD prolongation), and guinea pig (21%, 35-ms APD prolongation). In Fig. 1B, 90% IKs block was essentially of no consequence for human (4%, 10-ms APD prolongation), dog (1%, 2-ms APD prolongation), and rabbit (3%, 8-ms APD prolongation). By contrast, only 50% IKs block (Fig. 1, right), was required to substantially prolong APD in guinea pig (27%, 44-ms APD prolongation).

Figure 2 shows that after application of 1 μM ISO (a saturating dose), consequence of IKs block increased for human and dog. Still, block effect in human and dog remained smaller than in guinea pig (14, 26, and 61%; 33-, 45-, and 90-ms APD90 prolongation, respectively).
APD rate dependence. APD$_{90}$ was longest for human. As shown in Fig. 3, top, at CL = 1,000 ms (dashed black), APD$_{90}$ was 223 ms for human, 189 ms for dog, and 163 ms for guinea pig. Dog AP repolarization rate was the most gradual. At CL = 1,000 ms, the time difference between APD$_{30}$ and APD$_{90}$ (APD$_{90}$ - APD$_{30}$) was 70 ms for human, 101 ms for dog, and 60 ms for guinea pig (71, 94, and 40 ms at physiological resting rates for these species; Fig. 3, solid black). APD$_{90}$ occurred relatively early in dog because of the prominent phase 1 notch due to $I_{K_{1}}$, which lowered the voltage of the phase 2 AP dome, resulting in more gradual repolarization. The range of APD$_{90}$ over CLs from 300 to 2,000 ms was 54 ms for human, 45 ms for dog, and 59 ms for guinea pig. Note that 1-Hz pacing may be a poor choice for representing physiological AP dynamics in guinea pig, a species in which resting heart rates are much faster (compare solid and dashed black curves).

Intracellular sodium accumulation. We clamped Na$^{+}$ concentrations in the myoplasm and submembrane space of the models ([Na$^{+}$]$_{i}$) and [Na$^{+}$]$_{ss}$, respectively) to their value at CL = 2,000 ms during the final paced beat at CL = 300 ms. In the absence of [Na$^{+}$] clamp, [Na$^{+}$]$_{i}$ is 10.0, 10.0, and 14.5 mM at CL = 300 ms and 6.6, 7.4, and 9.7 mM at CL = 2,000 ms in human, dog, and guinea pig, respectively. Figure 4 shows the AP, the difference between slow and fast rate APs ($\Delta V_{m}$), and the Na$^{+}$/K$^{+}$-ATPase current ($I_{NaK}$) for these simulations ([Na$^{+}$]$_{i}$; Fig. 4, inset). When [Na$^{+}$] was clamped to the relatively small CL = 2,000 ms values (Na2K) during pacing at CL = 300 ms, APD increased relative to CL = 300 ms control in all species. With [Na$^{+}$] clamp, APD$_{90}$ was 26, 26, and 12% longer for human, dog, and guinea pig, respectively. For human and dog, $\Delta V_{m}$ was greatly reduced by [Na$^{+}$] clamp, indicating that rate-dependent [Na$^{+}$] changes play an important role in rate-dependent AP changes, via electrogenic $I_{NaK}$. In other words, when [Na$^{+}$] is clamped, slow and fast rate APs are similar. However, for guinea pig, $\Delta V_{m}$ remained large despite [Na$^{+}$] clamp.
Na⁺ concentrations in guinea pig were higher than in the other species [including rabbit, see Shannon et al. (28)], raising the question of whether this is an artifact of the model. However, the rate dependence of Na⁺ in the guinea pig model was validated against experiments in a previous study (8). Still, the Na⁺ concentration is notoriously difficult to measure, and fluorescence indicators may tend to overestimate its value (23).

Mechanism of \(I_{KS}\) rate dependence. In guinea pig, slow deactivation of \(I_{KS}\) results in incomplete deactivation between beats at fast pacing rate and channel accumulation in the open state. Consequently, \(I_{KS}\) accumulates to be larger during the AP, thereby shortening the APD. In human and dog, \(I_{KS}\) deactivates faster, which counteracts the possibility of accumulation in the open state. As shown previously [experiments (24) and simulations (29)], in these large mammals \(I_{KS}\) channels accumulate at fast rate in closed states that are kinetically proximal to the open state [termed “available reserve” (29)] from which they can open rapidly to generate larger current during the AP repolarization phase, contributing to APD shortening. The available reserve was shown to be augmented by \(\beta\)-adrenergic stimulation (14).

Figure 5 shows APs at slow (CL = 2,000 ms) and fast (CL = 300 ms) rates and the corresponding \(I_{KS}\) currents in the presence of ISO for human (left) and guinea pig (right). Guinea pig \(I_{KS}\) exhibits an instantaneous “jump” of current upon stimulation at fast pacing (solid arrow) but not at slow pacing (dashed arrow), revealing open state accumulation. In contrast, human \(I_{KS}\) does not display an instantaneous jump at fast rate; rather, it increases faster than at slow rate (solid arrow compared with dashed arrow) to reach a higher peak during AP repolarization, revealing the available reserve mechanism of \(I_{KS}\) participation in APD shortening. Similar behavior and mechanism are observed in the dog [shown extensively by Heijman et al. (14)]. This mechanism plays an important role in providing a repolarization reserve to ensure proper repolarization when \(I_{Kr}\) is reduced by drug block or mutations (20, 25, 29).

Rate dependence of \(\beta\)-adrenergic effects on \(I_{NaK}\) and \(I_{KS}\). One of the targets of \(\beta\)-adrenergic stimulation is \(I_{NaK}\). Specifically, phosphorylation of phospholemman (PLM) by PKA

relieves inhibition on $I_{\text{NaK}}$. This was modeled as an increase in Na$^+$ binding affinity of the pump (14). Thus it is important to ask whether PKA effects on PLM are needed to preserve APD rate dependence in the presence of $\beta$-adrenergic stimulation in human and dog. This was tested by measuring the percent APD$_{90}$ shortening at fast rate with 1 $\mu$M ISO vs. 1 $\mu$M ISO without PLM as a PKA target. In dog, APD shortening was by 26% whether or not PLM was a PKA target [analysis derived from Heijman et al. (14); Fig. 3A]. In human, it was 27% with and 21% without PLM as a PKA target (using CLs of 300 and 2,000 ms, 1 $\mu$M ISO). These results indicate that in the presence of $\beta$-adrenergic stimulation, PKA effects on $I_{\text{NaK}}$ helped preserve APD rate dependence in human but were not important in dog.

The role of $I_{\text{Ks}}$ in determining APD rate dependence rises in prominence with $\beta$-adrenergic stimulation. Experimental evidence and computer simulations suggest that fast pacing causes channel accumulation in closed states near the open state (available reserve, described above) and that $\beta$-adrenergic stimulation alters $I_{\text{Ks}}$ gating to further promote this accumulation (31, 36). Therefore, we sought to determine the degree to which $I_{\text{Ks}}$ accumulation determines APD rate dependence once $\beta$-adrenergic stimulation is applied. In the presence of 1 $\mu$M ISO, we reset $I_{\text{Ks}}$ gates to their slow rate (CL = 2,000 ms) values at the start of the last paced beat at fast rate (CL = 300 ms), eliminating the available reserve accumulation at fast rate. Under these conditions, the percent APD$_{90}$ shortening at fast rate was only 4% for dog, compared with 26% in the presence of $I_{\text{Ks}}$ available reserve accumulation. For human, it was 21%, compared with 27%. We conclude that $\beta$-adrenergic stimulation affects mechanisms of APD rate dependence in a species-dependent fashion. In human, PLM phosphorylation and $I_{\text{Ks}}$ available reserve accumulation are equally important factors, while in dog, $I_{\text{Ks}}$ accumulation in available reserve states is more important.

Transmural cell types. Species dependence of APs and rate dependence of APD are shown for different transmural cell types in Fig. 6. Simulations show that transmural dispersion (difference between maximum APD$_{90}$ and minimum APD$_{90}$) is least for dog and greatest for guinea pig, especially at slow pacing rates. In dog, the absence of large $I_{\text{o}}$ in endocardium cells shortened APD relative to Epi, as seen experimentally (19). This was not true in the other species, where Epi APD was shortest. In dog, the $I_{\text{o}}$ influence is not direct; rather it is caused by an indirect effect on the voltage dependence of $I_{\text{CaL}}$ during its plateau phase (7, 15). The ever increasing M-cell
APD in guinea pig at slow rates was due to loss of $I_{Ks}$ accumulation associated with slow pacing (29), on top of reduced conductance (expression) of $I_{Ks}$ in M cells. In fact, early afterdepolarizations (EADs) developed at slow pacing rates in guinea pig M cells (Fig. 4, inset); note that these rates are much slower than the physiological range for guinea pig.

EADs may form with slow pacing (CL = 2,000 ms) and block of repolarizing K⁺ currents [e.g., experiments (2, 13)]. In Fig. 7, simulations under these conditions caused EAD formation with 90% $I_{Kr}$ block in dog but not human or guinea pig. EADs developed in guinea pig with 90% $I_{Ks}$ block. However, the same degree of $I_{Ks}$ block did not generate EADs in human or dog. These results demonstrate that the development of EADs is species dependent.

**DISCUSSION**

Different mammalian species show different mechanisms of response to pacing and channel block. Quantitative species comparison revealed the following: 1) $I_{Kr}$ reduction prolonged human AP substantially more than APs of dog and guinea pig. 2) Under basal conditions (absence of β-adrenergic stimulation), $I_{Ks}$ reduction had almost no effect on human and dog APs, but it severely prolonged the guinea pig AP. 3) In the presence of β-adrenergic stimulation, $I_{Ks}$ reduction affected all species to substantial degree. 4) Rate-dependent $I_{NaK}$ changes play a major role in APD rate dependence in human and dog. The role of $I_{NaK}$ is secondary to [Na⁺] accumulation at fast rates. 5) For guinea pig, $I_{Ks}$ dominates APD rate dependence via its open-state accumulation at fast rate, while in human and dog under β-adrenergic stimulation, accumulation of $I_{Ks}$ in available reserve closed states is important for proper AP repolarization and APD shortening at fast rate. 6) EAD formation due to delayed rectifier K⁺ current block at slow pacing rates is species dependent.

We did not include mouse or rat APs in this comparison, although there are models available that reproduce a wide array of physiological and drug block effects in these species [e.g., mouse(4) and rat (22)]. This choice was based on the fact that AP morphology, physiological heart rate, and rate dependence...
in these smaller mammalian species are set apart due to lack of plateau phase and a different cellular ion-channel profile. However, mouse and rat are comparable to the other species with respect to whole organ and physiome related investigations (e.g., conduction velocity and its restitution), which we cannot address here. Also not addressed is the important issue of differences in arrhythmic reentry across species. The ratios of AP wavelength to heart substrate area are widely different among species, affecting arrhythmia formation and sustenance.

The finding that APD shortening at fast rates was caused primarily by $I_{NaK}$ increase secondary to intracellular $[Na^+]$ accumulation in human and dog, but not guinea pig, has been reported previously for human (12, 21), dog (7), and guinea pig (8) using other protocols. Modeling studies are not always in agreement. For example, the Iyer-Winslow human ventricular model places a greater emphasis on the role of $I_{NaCa}$ in rate dependence of APD (16). The ten Tusscher-Panfilov human ventricular model (33) [and updated version (34)] emphasizes $I_Ks$ (under basal conditions). The direct and quantitative comparison between species presented here yields novel insights.

We found that in human β-adrenergic stimulation elevated the role of $I_Ks$, available reserve accumulation in determining APD rate dependence to match that of PKA effects on $I_{NaK}$ via PLM phosphorylation. By contrast, in dog with β-adrenergic stimulation, $I_Ks$ accumulation in available reserve states was more important than PLM phosphorylation. This subtle, but significant, finding has not been shown previously. Such a result would be difficult to measure experimentally because of practical methodological limitations. One possible implication of this finding is that in human heart failure, depressed QT rate dependence observed in vivo (6) may be caused by reduced β-adrenergic capacity (11) and a consequent failure to remove PLM inhibition of $I_{NaK}$, coupled with weak $I_Ks$ accumulation in available reserve. Importantly, the PLM effect on APD may be human specific.

AP morphotype and its rate dependence are determined by a time-dependent balance between inward and outward currents. For guinea pig, rate-dependent changes in $I_{NaK}$ secondary to $[Na^+]$ changes were large, indeed larger than for human or dog. However, as Faber and Rudy showed (8), there are even larger rate-dependent changes in guinea pig $I_Ks$. In contrast to dog and human, this makes $I_{NaK}$ only a minor participant in rate dependence of the guinea pig AP (8).

Human and dog repolarization is due mainly to $I_Kr$, which is not rate dependent (17). The same is true for rabbit in both experiments and in simulations using the Shannon-Bers rabbit model (Fig. 1A). In these species, rate dependent $I_Ks$ is small relative to guinea pig without β-adrenergic stimulation (Fig. 1B). Interestingly, rate-dependent increase of $I_Ks$ at fast rates employs different mechanisms in human and dog as compared with guinea pig. In guinea pig, there is open state accumulation that increases (the already large) current from the start of the AP. In human and dog, accumulation occurs in available reserve closed states from which transitions to the open state cause a gradual increase in current during the AP, reaching a peak during phase 3 repolarization, when even a small current can shift the delicate balance of currents in the repolarization direction and influence repolarization effectively. As shown by Silva and Rudy (29), with this strategy that conserves current during the early AP to maximize it late, $I_Ks$ can provide the necessary repolarization reserve to compensate for reduction in $I_Kr$ due to mutations or drugs. Importantly, $I_Ks$ is regulated by the β-adrenergic pathway and plays a critical role in proper repolarization during exercise and emotional stress (20, 36).

Mechanistically, β-adrenergic stimulation increases $I_Ks$ by augmenting the available reserve (14).
$I_{Kr}$ and $I_{KS}$ are critical for AP repolarization in the ventricle. Mutations that reduce these currents cause the majority of cases of inherited long QT syndrome (27), leading to lethal arrhythmia. Acquired long QT syndrome, caused by block of $I_{Kr}$ by any of a variety of pharmacological agents [including clinically useful noncardiac drugs terfenadine, fexofenadine, risperidone, sertindole, erythromycin, and cisapride (5)], can also lead to fatal arrhythmias (25). The dangers of proarrhythmic effect and promiscuity of $I_{Kr}$ block was not uniform across species. It is in this context that these issues need to be addressed quantitatively and caution used when extrapolating results of drug effects tested in nonhuman species to safety and efficacy in human clinical application.

ACKNOWLEDGMENTS

We thank the members of the laboratory of Y. Rudy for helpful discussion.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants R01-HL-049054-19 and R01-HL-R0103343-27 (to Y. Rudy) and American Heart Association Predoctoral Fellowship 0815539G (to T. J. O’Hara). Y. Rudy is the Fred Saigh Distinguished Professor at Washington University.

DISCLOSURES

No conflicts of interest, financial or otherwise, are disclosed by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: T.O. and Y.R. conception and design of research; T.O. performed experiments; T.O. analyzed data; T.O. and Y.R. interpreted results of experiments; T.O. prepared figures; T.O. drafted manuscript; T.O. and Y.R. edited and revised manuscript; T.O. and Y.R. approved final version of manuscript.

REFERENCES