Rabbit models as tools for preclinical cardiac electrophysiological safety testing: importance of repolarization reserve

István Baczkó[a], Norbert Jost[a,b], László Virág[a], Zsuzsanna Bősze[c], András Varró[a,b]

[a] Department of Pharmacology & Pharmacotherapy, University of Szeged, Dom ter 12., 6720 Szeged, Hungary

[b] MTA-SZTE Research Group of Cardiovascular Pharmacology, Hungarian Academy of Sciences, Dom ter 12., 6720 Szeged, Hungary

[c] Rabbit Genome and Biomodel Group, NARIC-Agricultural Biotechnology Institute, 2100 Gödöllő, Hungary

Corresponding author

Dr. István Baczkó, Department of Pharmacology & Pharmacotherapy, University of Szeged, Dóm tér 12., H-6720 Szeged, Hungary

E-mail: baczko.istvan@med.u-szeged.hu

Abstract

It is essential to more reliably assess the pro-arrhythmic liability of compounds in development. Current guidelines for pre-clinical and clinical testing of drug candidates advocate the use of healthy animals/tissues and healthy individuals and focus on the test compound's ability to block the hERG current and prolong cardiac ventricular repolarization. Also, pre-clinical safety tests utilize several species commonly used in cardiac electrophysiological studies. In this review, important species differences in cardiac ventricular repolarizing ion currents are considered, followed by the discussion on electrical remodeling associated with chronic cardiovascular diseases that leads to altered ion channel and transporter expression and densities in pathological settings. We argue that the choice of species strongly influences experimental outcome and extrapolation of results to human clinical settings. We suggest that based on cardiac cellular electrophysiology, the rabbit is a useful species for pharmacological pro-arrhythmic investigations. In addition to healthy animals and tissues, the use of animal models (e.g. those with impaired repolarization reserve) is suggested that more closely resemble subsets of patients exhibiting increased vulnerability towards the development of ventricular arrhythmias and sudden cardiac death.

Keywords pre-clinical safety testing, cardiac arrhythmias, repolarization reserve, I_{Ks}, species differences, rabbit

Contents

1. Introduction

To markedly reduce compound attrition during drug development as well as to prevent the relegation or withdrawal of already approved drugs from the market, the efficacy of pro-arrhythmic liability assessment of drugs needs vast improvement. Many cardiovascular and non-cardiovascular compounds have been associated with provoking Torsades de Pointes (TdP) arrhythmia (Haverkamp et al, 2000; Redfern et al, 2003), a typical drug-induced chaotic ventricular tachycardia that can degenerate into ventricular fibrillation and lead to sudden cardiac death (SCD; Fenichel et al, 2004). It is unacceptable to use drugs for non life threatening pathologies that can cause SCD, however, in the clinical setting, it is very difficult to predict TdP arrhythmia due to its low incidence (Fenichel et al, 2004). Although recognized as essential to study, pro-arrhythmic potential is investigated in healthy tissue, isolated heart preparations and animals in pre-clinical safety assessment and in healthy volunteers as described by current international guidelines (ICH-S7B, 2005; ICH-E14, 2005). In addition, most of these tests concentrate on the potential of candidate compounds to block hERG current in expression systems, and on the prolongation of action potentials (AP) in cardiac tissue, manifested as QT interval prolongation on the surface electrocardiogram. However, a growing body of evidence indicates that repolarization prolongation in itself does not equal to increased pro-arrhythmic risk (Mattioni et al, 1989; Weissenburger et al, 1991; Carlsson et al, 1993; Hondeghem et al, 2001; van Opstal et al, 2001; Belardinelli et al, 2003; Thomsen et al, 2004; Anderson, 2006). Crucial issues of phase 3 repolarization disturbances, including action potential triangulation (Hondeghem et al, 2001) are not adequately addressed by pro-arrhythmic tests. It is clear, that drug-induced arrhythmia episodes occur mostly in subsets of patients that are more vulnerable to rhythm disturbances, i.e. mostly in cardiovascular and metabolic diseases that involve structural and/or electrical remodeling of the heart leading to serious impairments in conduction and/or repolarization. Such common examples are: congestive heart failure (Kjekshus, 1990), hypertrophic cardiomyopathy (Decker et al, 2009), congenital long-QT syndromes (El-Sherif and Turitto, 1999) and diabetes mellitus (McNally et al, 1999; Whitsel et al, 2005). Slowing of the upstroke of the action potential and impaired conduction also play key roles in increased arrhythmia susceptibility. Changes in connexin expression (Poelzing and Rosenbaum, 2004), structural remodeling (chamber enlargement, hypertrophy, fibrosis, etc.) significantly alter conduction

and contribute to the arrhythmia substrate and maintenance (Akar et al, 2004). The discussion of conduction disturbances is beyond the scope of this paper.

In this review, we focus on species differences in repolarizing ion currents in animals most often used for cardiac electrophysiological and pro-arrhythmic investigations, followed by a description of electrical remodeling associated with some common chronic cardiovascular diseases, also showing differences among species used in pre-clinical safety studies. We strongly argue that the choice of species will significantly influence experimental results and their extrapolation to human clinical settings.

2. Species differences in repolarizing potassium currents

The repolarization of cardiomyocytes is governed by the highly regulated and balanced activities of inward and outward currents via different ion channels and electrogenic ion pumps **(Fig. 1)**. The value of the rabbit ventricular arrhythmia and pro-arrhythmia models largely depend on their predictive capability to human. Human relevance is determined by the electrophysiological background of rabbit ventricular myocytes compared to humans and other frequently used animal preparations in experimental arrhythmia research or drug testing. Mice and rats are commonly used in arrhythmia research and to create transgenic long QT (LQT; **Table 1**) models. They have the advantage of low cost and fairly good predictive capability for ischaemia-induced arrhythmias. In small rodents, impulse conduction depending on sodium, calcium currents and connexin function, is similar to that in human. However, for studies on repolarization, mice and rats have limited value: they have a triangular AP (**Fig. 2A-E**) while humans, dogs and rabbits have a prominent plateau phase (**Fig. 2F-H**) and a rectangular AP (Yang et al, 2014; Nerbonne and Kass, 2005; Saito et al, 2009; Grandy et al, 2007). The main repolarizing currents in mice and rats are transient outward (I_{to}) and ultrarapid delayed rectifier like (I_{Kur}) potassium currents, as opposed to I_{Kr} in dogs, rabbits and humans. Inhibition of I_{to} and I_{Kur} significantly prolonged AP duration in mice and rats (**Fig. 2 B and D**). The functions of I_{Kr} and I_{Ks} are still not well explored and controversial (Nerbonne and Kass, 2005; Babij et al, 1998; Saito et al, 2009; Grandy et al, 2007; Yang et al, 2014) in mice. Acute administration of I_{Kr} blockers exert no effect on ventricular AP in mice and rats (**Fig. 2A and C**) but prolong repolarization in human, dog and rabbit **(Fig. 2F-H)**. However, transgenic LQT1 (genetic loss of I_{Ks} function; **Table 1**) and LQT2 (loss of I_{Kr} function; **Table 1**) murine models exhibited arrhythmias and prolonged QT intervals (Drici et al, 1998; Babij et al, 1998). A recent report by Yang *et al*. (Yang et al,

2014) may resolve this controversy: prolonged exposure to I_{Kr} inhibitors lengthened the AP in mice (**Fig. 2E**) and caused afterdepolarizations. However, the authors found that the I_{Kr} blocker indirectly (involving the phosphoinositide 3-kinase pathway) augmented the late component of I_{Na} and prolonged repolarization by increasing an excess of inward current. Based on the above, it is assumed that murine transgenic LQT models may have limited pharmacological and pathophysiological implications for humans. On the other hand, rabbit transgenic LQT models may have more relevance to humans, since rabbits and humans have similar cardiac ventricular action potential waveforms and potassium channel expression patterns (**Figs. 3-5**). The function and gating of different types of potassium channels are similar in rabbits and humans (**Figs. 3-5**), with the exception of I_{to}. Kv1.4 channels are responsible for I_{to} in rabbits that have slower recovery from inactivation than Kv4.3 channels expressed in human ventricle (Wang et al, 1999; and own unpublished results). These differences may lead to yet unexplored differences in Phase 1 repolarization and arrhythmia susceptibility between rabbits and humans.

In rabbits, I_{Ks} and I_{Kr} show higher current densities (**Fig. 3**) with similar gating kinetics (**Fig. 4**) compared to those in human. Also, the repolarization capacity of rabbits (**Fig. 5)** and dogs (Jost et al, 2013) due to higher I_{K1} current densities is most likely more robust than that in human. In contrast to rabbits, guinea pigs lack I_{to} (Findlay, 2003), and express very strong I_{Ks} with slow deactivation kinetics compared to rabbit and human ventricles (Lu et al, 2001). As the main phase 3 repolarizing current, I_{Kr}, is very similar in rabbits and humans, and as the other considerations above suggest, rabbits are more useful than rats, mice or guinea pigs for electrophysiological, pharmacological antiarrhythmic and pro-arrhythmic investigations.

3. Electrical remodeling in cardiovascular and metabolic diseases: implications for arrhythmia susceptibility

A brief discussion of electrical remodeling associated with cardiovascular diseases is necessary to appreciate why it leads to increased sensitivity to arrhythmias and why it is not satisfactory to use only healthy animals and tissues for the assessment of TdP liability. During the course of a number of cardiovascular diseases (e.g. heart failure, atrial fibrillation, myocardial infarction, etc.), in order to maintain intracellular homeostasis and cardiac function, characteristic adaptive changes appear in the structure and electrophysiology of the heart. These changes are referred to as structural and electrical remodeling, respectively, and the longer they persist, the higher the chance for further deterioration of cardiac function and

arrhythmia development. Here we discuss the effects of electrical remodeling on repolarization in three chosen clinical entities with repolarization disturbances: congestive heart failure, hypertrophic cardiomyopathy and diabetes mellitus.

In congestive heart failure, cardiac hypertrophy is accompanied by profound electrical remodeling and it is consistently found that the I_{to} (Kaab et al., 1996; Beuckelmann et al., 1999), the I_{Ks} (Li et al, 2004; Li et al, 2002; Tsuji et al, 2000) and I_{K1} (Li et al, 2004; Rose et al, 2005) repolarizing currents are downregulated, while most investigations find that the I_{Kr} expression does not change (Li et al, 2004; Li et al, 2002; Tsuji et al, 2000). In addition, increased slowly inactivating, late sodium current ($I_{Na,late}$) has been shown to contribute to APD prolongation and arrhythmias in HF (Valdivia et al, 2005). Therefore, the repolarization capacity of failing cardiomyocytes is markedly reduced, leading to the slowing of phase 3 repolarization and prolongation of the AP and QT intervals (Nuss et al, 1999; Janse, 2004), to early afterdepolarization (EAD) formation (Janse, 2004) and to Torsades de Pointes (El-Sherif and Turitto, 1999). Accordingly, increased risk of acquired repolarization prolongation was found in patients with congestive heart failure (Lehmann et al, 1996). In a rabbit model of HF, increased Na^+/Ca^{2+} exchanger (NCX) and decreased I_{K1} expression have been shown to be important contributors to afterdepolarizations and arrhythmias (Pogwizd et al, 2001). The hyperpolarization-activated, cyclic nucleotide-gated pacemaker "funny current" (I_f), normally playing a key role in pacemaking in the sinus node (Biel et al, 2002) is upregulated in ventricular myocardium in human HF, providing arrhythmogenic triggers (Stillitano et al, 2008). Based on the above, it is not surprising that approximately 50% of HF patients are lost due to SCD resulting from ventricular fibrillation (Kjekshus, 1990), and that compounds with mild or moderate ion channel modulating effects can precipitate serious ventricular arrhythmias unexpectedly.

Hypertrophic cardiomyopathy (HCM) is a common hereditary cardiac disease caused by different mutations in genes encoding sarcomeric proteins. HCM is characterized by left ventricular hypertrophy, myocardial fibrosis and myofiber disarray that may represent an arrhythmogenic substrate of the disease (Maron 2002; Gersh et al, 2011). HCM is the most common cause of SCD in young individuals (Decker et al, 2009) and in young (<35 years) competitive athletes (Maron et al, 2009). Remodeling in HCM is progressive (Olivotto et al, 2012) and a recently a close correlation was found between the degree of adverse remodeling and increased risk for SCD in patients with HCM (Vriesendorp et al, 2014). Although the exact elements of ion channel remodeling have not been thoroughly explored in HCM,

increased $I_{Na,late}$ and beneficial effects of its inhibition have been described in human HCM (Coppini et al, 2013.).

An increased risk for SCD has been identified in patients with both adult and juvenile diabetes mellitus (McNally et al, 1999; Whitsel et al, 2005). Prolongation of the frequency corrected QT interval (QTc) along with increased QTc dispersion was found in patients with type 1 diabetes (Suys et al, 2002; Veglio et al, 2002). The reasons for repolarization prolongation and increased arrhythmia sensitivity in diabetes are poorly understood. Most of the earlier investigations in this regard were carried out in diabetic rats (Magyar et al, 1992; Rusznák et al, 1996; Shimoni et al, 1994; Shimoni et al, 1995; Xu et al, 1996; Tsuchida et al, 1997). These rat studies observed a significantly descreased amplitude of I_{to}, that was later attributed to lower expression of Kv4.2 and Kv4.3 (Qin et al, 2001). However, as it was described in section 2, rat and human ventricular repolarization are very different. Consequently, the results of studies on repolarization in diabetic rats can be misleading in the understanding of the mechanisms involved in diabetic patients. Subsequent studies have been performed in species with artificially induced diabetes that have ventricular repolarization more relevant to human. In diabetic dogs, in addition to reduced I_{to} amplitude, a decreased I_{Ks} density was found while $I_{Ca,L}$, I_{K1} and I_{Kr} were unaltered (Lengyel et al, 2007). In a study performed in diabetic rabbits, Zhang et al (2007) observed reduced I_{Kr}, however, other repolarizing currents were not investigated in their study. Lengyel *et al.* (2008) have found that I_{Ks} density was significantly reduced, however, I_{to}, I_{Kr}, I_{K1} and $I_{Ca,L}$ were not different from control in rabbits with diabetes induced by alloxan administration.

In summary, the pathological alterations in the expression of ion channels can be distinctly different in species most commonly used for arrhythmia studies. The remodeling of these repolarizing and depolarizing currents lead to decreased repolarizing capacity that in turn creates increased susceptibility to arrhythmia development („substrate") in pathological settings. Altered NCX and I_f expression can provide triggers for arrhythmia initiation. The decreased repoarizing capacity is referred to as reduced repolarization reserve, as detailed in the following section.

4. Repolarization reserve

Dr. Roden suggested the concept of repolarization reserve first (Roden, 1998), that emphasizes the redundant nature of myocardial repolarizing capacity. The loss or impaired function of one or more repolarizing potassium currents, and/or gain of function of inward

currents (e.g. LQT3, **Table 1**) does not always lead to marked repolarization prolongation on the ECG, since other currents can compensate. In these cases, however, the heart will be more susceptible to additional (even mild) repolarization inhibition and to the development of arrhythmias (Roden and Yang, 2005; Varró and Baczkó, 2011). Repolarization reserve enables the heart to withstand additional repolarization challenges, and was experimentally demonstrated in different species including rabbit (Varró et al, 2000; Biliczki et al, 2002). Several repolarizing currents have been implicated in repolarization reserve, however, I_{Ks} is critical both in experimental animals (Varró et al 2000; Lengyel et al, 2001; Volders et al, 2003; Abi-Gerges et al, 2006, Lengyel et al, 2007; Johnson et al, 2010) and in humans (Jost et al, 2005). A recent study found that I_{Ks} densities were lower in human than in rabbit and dogs (Jost et al, 2013a), suggesting a weaker repolarization reserve in humans. The kinetics of rabbit I_{Ks} is more similar to human (**Fig. 3**) compared to that in guinea-pigs or dogs (Jost et al, 2013b). The role of I_{Ks} in repolarization reserve is well characterized compared to other potassium currents. The different I_{Ks} density and kinetics data in different species frequently used in cardiac electrophysiological studies, however, caution us about the human extrapolation of the results.

Other potassium currents can also be involved in repolarization reserve: I_{to} may be a contributor in dogs (Virág et al, 2011). The I_{K1} current has been shown to be important in dogs (Biliczki et al, 2002), and in patients with LQT7 (**Table 1**) the loss of function mutations in Kir2.1 channels significantly reduce I_{K1} current and increase pro-arrhythmic risk without marked QT prolongation on the ECG (Zhang et al, 2005). However, significant species differences exist in the composition of channel subtypes that are eventually responsible for the I_{K1} current (Melnyk et al, 2002; Anumonwo and Lopatin, 2010), leading to different I_{K1} current characteristics (Dhamoon et al, 2004). These differences can lead to species dependent arrhythmia susceptibilities (Husti et al, 2015) and responses to K^+ channel blockers (Nerbonne and Kass, 2005). Recently, in line with these differences, dogs and rabbits were found to have distinct arrhythmia susceptibilities following the combined pharmacological inhibition of I_{K1}, I_{Ks} and I_{Kr} (Husti et al, 2015). It is likely that due to stronger I_{K1} and I_{Ks} found in dogs compared to rabbits and humans, dogs may possess larger repolarization reserve than rabbits and humans. In summary, rabbit pro-arrhythmia models with impaired repolarization reserve may represent enhanced arrhythmia susceptibility and may be more useful than dog models in predicting human electrophysiological changes following administration of drugs affecting repolarization.

It is important to note that repolarization reserve changes dynamically. Xiao and co-workers (Xiao et al, 2008) have shown that in cultured adult canine ventricular myocytes a one-day incubation with the selective I_{Kr} blocker dofetilide, following an expected prolongation of the APD at the beginning, led to shortened APD, that is the repolarization prolonging effect of dofetilide was blunted. Enhanced I_{Ks} was found in dofetilide incubated cells compared to control cardiomyocytes, and the results were supported by increased KvLQT1 and MinK protein levels, while the mRNA for both of these proteins did not change, pointing to the involvement of post-transcriptional regulatory mechanisms. In fact, miR-133a and miR-133b expressions were reduced in dofetilide incubated cells. Muscle-specific microRNAs repressed I_{Ks}-encoding genes without changing the mRNA for KvLQT1 (Luo et al, 2007). The results suggest that in case of chronic administration of drugs with cardiac potassium channel blocking effects (antibiotics, antipsychotics, antihistamines, NSAIDs, etc.), a compensatory I_{Ks} upregulation may occur in normal myocardium in an attempt to restore normal myocardial repolarization. In case of I_{Ks} downregulation (e.g. cardiac hypertrophy) or genetic impairment (e.g. LQT1 syndrome), such seemingly harmless compounds may cause serious and unexpected repolarization disturbances. The loss of I_{Ks} function can be especially harmful when sympathetic tone and intracellular cAMP levels are elevated. In this case, the I_{Ks} mediated repolarization shortening is prevented but APD prolongation by cAMP enhanced $I_{Ca,L}$ is retained, leading to pro-arrhythmia (Stengl et al, 2006).

5. *In vivo* proarrhythmia models with reduced repolarization reserve: role of the rabbit

Based on the discussion above it is conceivable that in addition to models using healthy animals and tissue preparations, models with impaired repolarization reserve are needed for more reliable testing of drug-induced arrhythmia liability. As an example illustrating the clinical significance of repolarization reserve, we would like to highlight an interesting clinical study (**Fig. 6**), where patients who previously developed TdP („Study Group") due to the administration of QT prolonging compounds responded with a significantly more pronounced QTc lengthening to the same test dose of sotalol compared to patients without the history of TdP (Kääb et al, 2003). The QTc intervals were similar in both groups at baseline, and it is assumed that the markedly different responses to the I_{Kr} blocker sotalol were due to differences in repolarization reserve in the two groups.

The first large animal experimental proarrhythmia model with impaired repolarization reserve was the dog model with chronic and complete atrioventricular block (Chezalviel et al, 1995; Vos et al, 1995). This model features bradycardia, eccentric ventricular hypertrophy

(Vos et al, 1998), and a marked electrical remodeling with reduced I_{Ks} density that contributes to increased suceptibility to TdP arrhythmias (Volders et al, 1999; Thomsen et al, 2004). The advantages of this model include a stable stage of compensated myocardial hypertrophy without deteriorating into heart failure (Schoenmakers et al, 2003), high proarrhythmia reproducibility in the same animal (Verduyn et al, 2001). A number of studies used this model for the assessment of proarrhythmic side affects of drugs (Chiba et al, 2000; Sugiyama et al, 2002; Thomsen et al, 2003; Takahara et al, 2006). The disadvantages of this model are related to its high cost, a need for special expertise in performing AV ablation, and duration of the experiments since several months are needed for the ventricular hypertrophy to develop, which is a prerequisite for the increased arrhythmia susceptibility in dogs with AV block (Vos et al, 1998). In this model, marked QTc prolongation following amiodarone administration did not cause a significant increase in TdP incidence, similarly to observations in humans (van Opstal et al, 2001).

The first rabbit pro-arrhythmia model investigating the development of TdP in *in vivo* settings was established at the beginning of the nineties by Carlsson and co-workers. One of the main aspects of this model is the i.v. administration of the α_1-adrenergic agonist methoxamine, that is essential for the model's vulnerability towards ventricular arrhythmias (Carlsson et al, 1990; Carlsson et al, 1993). Therefore, it is important to note that test compounds with α-adrenergic blocking properties may register as false negative drugs in this model. The exact mechanism responsible for methoxamine-induced arrhythmia susceptibility is not established, however, α_1-adrenergic receptor mediated increase in $[Ca^{2+}]_i$ definitely plays a role, probably leading to increased triggered activity (Carlsson et al, 1996; Volders et al, 2000). The relation of this model to repolarization reserve is not clear, however, the short-term variability of the QT interval (STV_{QT}), a suggested surrogate biomarker for the prediction of ventricular arrhythmias (Berger et al, 1997; Hondeghem et al, 2001; for a comprehensive review see Varkevisser et al, 2012), was elevated and showed correlation with subsequent arrhythmia development in this model (Jacobson et al, 2011) similarly to other rabbit models with impaired repolarization reserve (Lengyel et al, 2007; Major et al, 2016, submitted). Also, extensive work has been carried out by Farkas *et al*. in Langendorff-perfused rabbit hearts to assess the value of different biomarkers for the prediction of pro-arrhythmic activities of drugs with special attention to beat-to-beat variability, also influenced by intrinsic heart rate changes, termed „absolute beat-to-beat variability (Orosz et al, 2014; Sarusi et al, 2014).

Since in the chronic AV-block dog model I_{Ks} was shown to be downregulated and additional I_{Kr} block resulted in high incidence of TdP arrhythmias, it was hypothesized that pharmacological inhibition of I_{Ks} could similarly prime the heart to arrhythmias by drugs exerting additional repolarization inhibition. Indeed, in anesthetized rabbits (and in conscious dogs as well) this study showed an increased incidence of TdP when I_{Ks} (by HMR1556) and I_{Kr} (by dofetilide) were inhibited in combination, and the increased incidence of TdP was associated with elevated beat-to-beat variability of the QT interval (Lengyel et al, 2007). In contrast to the chronic AV-block canine model, where chronic loss of I_{Ks} function is present due to I_{Ks} downregulation, this rabbit pro-arrhythmia model could refer to a clinical situation where compounds with different potassium channel blocking profiles are administered concomitantly. The acute i.v. administration of drug combinations in an anesthetized rabbit present far fewer technical challenges to investigators compared to the dog model described above (Thomsen, 2007). In this model with pharmacologically induced impairment of repolarization reserve, the non-steroidal antiinflammatory drug diclofenac increased TdP incidence, while it did not alter repolarization in normal rabbit heart tissue and did not cause arrhythmias in animals with intact repolarization reserve (Kristóf et al, 2012). In the same study diclofenac inhibited I_{Ks} and I_{Kr} and did not influence I_{to} and I_{K1} (Kristóf et al, 2012). Combined pharmacological inhibition of not only I_{Ks} and I_{Kr}, but I_{K1}+I_{Kr} and I_{K1}+I_{Ks} can also lead to impaired repolarization reserve and increased TdP incidence, with rabbits showing more sensitivity to I_{K1}+I_{Kr} and dogs to I_{K1}+I_{Ks} inhibition (Husti et al, 2015). This study concluded that the dog and rabbit exhibited different repolarization reserve and arrhythmia sensitivity profiles, with possible important relevance to human extrapolation of results obtained in these species (Husti et al, 2015).

Another experimental approach to reduce repolarization reserve is the genetic modification of ion channels involved in cardiac repolarization in transgenic animal models. These models could have clinical relevance by representing patients with similar mutations in affected ion channels, however, the choice of species – mouse versus rabbit - is very important for obtaining clinically meaningful results. The first two transgenic LQT rabbit models were created by Brunner et al (2008), overexpressing the dominant-negative mutants of the pore-forming human KvLQT1 (LQT1) or HERG channels (LQT2), respectively. These landmark transgenic rabbit models are expertly discussed in another review of this issue by Lang et al (2016, this issue, in press). These rabbit LQT models exhibited a strong phenotype, including prolongation of the action potential and QT intervals, and spontaneous SCD in LQT2 rabbits (Brunner et al, 2008). In order to achieve a somewhat milder impairment of

repolarization reserve, a model would be needed where the incidence of spontanous arrhythmias was lower with retained increased susceptibility to arrhythmias. For this purpose, targeting the β-regulatory subunit of the I_{Ks} channel, minK (encoded by *KCNE1*) was identified.

Although transgenic mouse models were previously created affecting the I_{Ks} channel, including *KCNE1* knockout (Drici et al, 1998), knock-in (Nishio et al, 2009; Rizi et al, 2008) and dominant negative loss-of-function mutation (Demolombe et al, 2001) models, mice have approximately 10 times faster heart rates and a number of important differences in their cardiac repolarization compared to humans (Nerbonne and Kass, 2005). Therefore, it is not surprising that transgenic mouse models can only mimic some aspects of the human LQT phenotype (Salama and London, 2007). To create a transgenic rabbit model with milder impairment of repolarization reserve, the heart specific overexpression of the human mutant *KCNE1,* carrying a G52R missense mutation was performed in rabbits (Major et al, 2016, in press). This mutation was first identified in a Chinese LQT family (Ma et al, 2003). In this family, seven individuals were mutation carriers, five of them were clinically affected, on the other hand, the ECG was normal in two family members (Ma et al, 2003). A dominant-negative effect of the mutant G52R-*KCNE1* was described, reducing I_{Ks} current amplitude by 50% (Ma et al, 2003). It was shown subsequently that the G52R mutation did not affect channel-subunit assembly or channel trafficking, but resulted in *KCNE1* to be unable to modulate the gating properties of *KCNQ1* (Harmer et al, 2010). These G52R-*KCNE1* overexpressing animals were subjected to a marked repolarization challenge by the i.v. administration of the I_{Kr} blocker dofetilide (Major et al, 2016, in press). At baseline, before dofetilide administration, the heart rate corrected QT-index of the LQT5 animals was mildly but significantly longer, and had a significantly higher STV_{QT}. A significantly larger number of animals developed TdP following dofetilide administration, paralleled by a further increase in STV_{QT}. Patch-clamp studies in isolated ventricular myocytes surprisingly did not show differences in I_{Ks} current amplitude, however, demostrated accelerated I_{Ks} and I_{Kr} deactivation kinetics in LQT5 transgenic rabbits compared to their wild-type littermates (Major et al, 2016, in press). It was concluded that LQT5 transgenic rabbits exhibited increased arrhythmia susceptibility and may represent a promising model for testing the pro-arrhythmic potential of candidate compounds. Further studies are needed to validate this model using drugs with proven pro-arrhythmic liability.

It has to be mentioned that the anesthetic protocol used in the rabbit *in vivo* pro-arrhytmia studies can have profound effects on the development of arrhythmias (Odening et

al, 2008; Vincze et al, 2008). In this regard, xylazine/ketamine-anaesthesia can be recommended, since it does not seem to affect repolarizing currents (Odening et al, 2008).

As described in section 3, hypertrophic cardiomyopathy is associated with increased incidence of SCD, and the assessment of SCD risk is incomplete since established SCD risk factors demonstrated only a low positive predictive value (McKeown and Muir, 2013). Therefore, for estimation of HCM associated arrhythmia risk, an animal model of HCM in a species that has similar repolarization and repolarization reserve profile to humans would be very useful. Interestingly, transgenic rabbit models of HCM were described as early as 1999 (Marian et al, 1999). However, few studies investigating arrhythmia mechanisms in transgenic HCM rabbits can be found in the literature, mainly concentrating on pathological aspects of conduction in this model (Ripplinger et al, 2007; Lombardi et al, 2009). A recent clinical study from our group found increased short-term variability of the QT interval that highly correlated with indices of left ventricular hypertrophy in patients with HCM (Orosz et al, 2015). Therefore, it is recommended that further studies are performed in transgenic rabbits with HCM regarding repolarization abnormalities and sensitivity of the animals to drug-induced arrhythmias.

6. Conclusions

Current guidelines for pre-clinical and clinical testing of drug-induced arrhythmia liability mostly utilize healthy animals or individuals and concentrate on whether the test compound prolongs ventricular repolarization, or blocks the hERG current in cellular expression systems. Significantly less attention is paid to key issues of phase 3 repolarization disturbances such as AP triangulation, reverse use dependence of AP prolongation and spatial dispersion and temporal instability of repolarization. Also, in addition to K^+ current disturbances, alterations (increases and reductions) in inward currents can also destabilize repolarization and provoke TdP (Hondeghem et al, 2010). For pre-clinical testing, several animal species are used. In this review, we have discussed important species differences in cardiac ventricular repolarizing currents, as well as the substantial structural and electrical remodeling associated with chronic cardiovascular diseases, leading to very different cardiac ion channel and/or transporter expression in pathological settings and to reduction of the repolarizing capacity of the heart, i.e. impairment of repolarization reserve. Remarkably, these pathological expression changes can also show differences among species used in pre-clinical safety studies.

Therefore, the choice of species markedly influences experimental outcome and extrapolation of results to human clinical settings. We argue that based on cellular ventricular electrophysiology, the rabbit should be a useful species for electrophysiological, pharmacological antiarrhythmic and pro-arrhythmic investigations. In particular, the repolarization reserve of the rabbit resembles human repolarization reserve similarly as that found in dogs.

However, for practical reasons, advantages of rabbit models should be mentioned compared to canine models, these include reduced cost (breeding, animal keeping), the already existing and proven technology for the creation of transgenic animals and favorable ethical considerations (the rabbit is not a pet animal), in spite of the fact that the size of the heart and heart rates of the dog are similar to those in humans.

In conclusion, in cardiac safety testing, although the investigations on healthy animals and individuals are still important, the addition of models recapitulating human disease are definitely needed. These additions are justified in order to test compounds in models that more closely resemble patient subpopulations with increased vulnerability to ventricular arrhythmias and sudden cardiac death, such as congestive heart failure, hypertrophic cardiomyopathy, congenital LQT syndromes and diabetes mellitus.

Figure 1. Illustration of the main transmembrane ionic currents and electrogenic pumps shaping the ventricular cardiac action potential. Specific blockers are also included. The transmembrane currents shown are not proportional to the actual current densities. Channel proteins that mediate these currents are also indicated. Reproduced from Varró and Baczkó, 2011, with permission.

Figure 2. Representative action potential recordings from different species frequently used in cardiac electrophysiological research. (**A**) The lack of effect of acute administration of the I_{Kr} blocker dofetilide, and the prolonging effect of 4-aminopyridine (4-AP) (**B**) on action potentials in mice. (**C**) Lack of effect of the I_{Kr} blocker sotalol on the AP in a rat papillary muscle preparation. (**D**) The significant AP prolonging effect of the combined I_{to} and I_{Kur} inhibitor AVE0118 in rat. (**E**) Extended (5 h) application of dofetilide prolonged the AP in mice. Sotalol exerts significant AP lengthening effect in (**F**) humans, (**G**) dogs, and (**H**) rabbits. Panels **A, B** and **E** reproduced from Yang et al, 2014; panel **D** from Nagy et al, 2009; panel **F** from Jost et al, 2013; panel **G** from Varró et al, 2000, with permission. Panels **C** and **H** show unpublished results from our laboratory.

Figure 3. (**A**) Original recordings of E-4031 sensitive (I_{Kr}, left) and L-735.821 (I_{Ks}, right) rapid and slow delayed rectifier potassium currents in undiseased human (top), and rabbit (bottom) ventricular myocytes. Nisoldipine (1 μM) was used to block L-type inward calcium current (I_{CaL}) and L-735,821 (100 nM) or E-4031 (1 μM) to block I_{Kr} or I_{Ks} currents, respectively. (**B)** The peak I_{Kr} (left) and I_{Ks} (right) tail current-voltage (I-V) relationship in undiseased human (triangles) and rabbit (diamonds) ventricular myocytes. I_{Kr} and I_{Ks} currents were examined in isolated human or rabbit ventricular myocytes using test pulses of 1000 ms (I_{Kr}) or 5000 ms (I_{Ks}) in duration to between -20 mV and +50 mV from the holding potential of -40 mV. The pulse frequency was 0.05 Hz (I_{Kr}) or 0.1 Hz (I_{Ks}). Unpublished data from our laboratory.

Figure 4. Activation and deactivation kinetics of I_{Kr} (**A**) and I_{Ks} (**B**), in undiseased human (left) and rabbit (right) ventricular myocytes. Activation kinetics of I_{Kr} and I_{Ks} were measured as tail currents at -40 mV, after test pulses to +30 mV with duration gradually increasing between 10 and 5000 ms. Deactivation kinetics of I_{Kr} and I_{Ks} outward tail currents were measured at -40 mV, after a 1000 ms (for I_{Ks}) or 5000 ms (for I_{Ks}), respectively, long test

pulse to +30 mV. Average I_{Kr} and I_{Ks} activation and deactivation time constants (τ) values±SEM are given in insets. Adapted from Iost et al, 1998; Virag et al, 2001 and Lengyel et al, 2001, with permission.

Figure 5. (**A**) Original recordings of inward rectifier potassium (I_{K1}) currents in undiseased human (left), and rabbit (right) ventricular myocytes. I_{K1} current was studied by measuring the steady-state current level at the end of the 400 ms long voltage pulse in the voltage range of -120 to 0 mV with a pulse frequency of 0.33 Hz. The holding potential was -90 mV. Nisoldipine (1 μM) was used to block L-type inward calcium current. (**B)** Current-voltage relationships of I_{K1} current in human (circle) and rabbit (triangle) ventricular myocytes measured after depolarizing voltage steps beetween -80 mV to 0 mV (outward range of I_{K1} current). Values represent mean±SEM. Insets show applied voltage protocols on both panels. Adapted from Jost *et al*., 2013, with permission and unpublished data from our laboratory.

Figure 6. Clinical significance of repolarization reserve. Changes in individual QTc intervals in control patients (**A**) and in patients with suspected acquired long QT syndrome (**B**, Study Group) following the i.v. administration of sotalol. The dotted line represents the cut-off value of 480 ms that differentiated between the study group and the control group. From Kääb et al, 2003, with permission.

Acknowledgments

This work was supported by the Hungarian Scientific Research Fund (OTKA NN 110896 and OTKA K 109610 to I.B., OTKA NN 109904 to N.J., OTKA NK 104397 to Z.B , OTKA NK 104331 to A.V.), by the National Office for Research and Technology – Baross Programme (REG-DA-09-2-2009-0115-NCXINHIB), and by the Hungarian Academy of Sciences.

Editors' note

Please see also related communications in this issue by Arevalo et al. (2016) and Lang et al. (2016).

References

Abi-Gerges N, Small BG, Lawrence CL et al. (2006) Gender differences in the slow delayed (I_{Ks}) but not in inward (I_{K1}) rectifier K^+ currents of canine Purkinje fibre cardiac action potential: key roles for I_{Ks}, ß-adrenoceptor stimulation, pacing rate and gender. Br J Pharmacol 147: 653-660.

Akar FG, Spragg DD, Tunin RS et al. (2004). Mechanisms underlying conduction slowing and arrhythmogenesis in nonischemic dilated cardiomyopathy. Circ Res 95: 717-725.

Anderson ME (2006). QT interval prolongation and arrhythmia: an unbreakable connection? J Intern Med 259: 81-90.

Anumonwo JM, Lopatin AN (2010) Cardiac strong inward rectifier potassium channels. J Mol Cell Cardiol 48: 45-54.

Atiga WL, Calkins H, Lawrence JH et al. (1998) Beat-to-beat repolarization lability identifies patients at risk for sudden cardiac death. J Cardiovasc Electrophysiol 9: 899-908.

Arevalo H, Boyle PM, Trayanova N (2016). Computational Rabbit Models to Investigate the Initiation, Perpetuation, and Termination of Ventricular Arrhythmia. Prog Biophys Mol Biol, *in press, this issue*

Babij P, Askew GR, Nieuwenhuijsen B et al. (1998) Inhibition of Cardiac Delayed Rectifier K+ Current by Overexpression of the Long-QT Syndrome HERG G628S Mutation in Transgenic Mice. Circ Res 83: 668-678.

Barhanin J, Lesage F, Guillemare E et al. (1996) K(V)LQT1 and lsK (minK) proteins associate to form the I(Ks) cardiac potassium current. Nature 384: 78-80.

Batey AJ, Coker SJ (2002) Proarrhythmic potential of halofantrine, terfenadine and clofilium in a modified in vivo model of torsade de pointes. Br J Pharmacol 135: 1003-1012.

Berger RD, Kasper EK, Baughman KL et al. (1997). Beat-to-beat QT interval variability: novel evidence for repolarization lability in ischemic and nonischemic dilated cardiomyopathy. Circulation 96: 1557–1565.

Belardinelli L, Antzelevitch C, Vos MA (2003) Assessing predictors of drug-induced torsade de pointes. Trends Pharmacol Sci 24: 619-625.

Beuckelmann DJ, Nabauer M, Erdmann E. (1993) Alterations of K^+ currents in isolated human ventricular myocytes from patients with terminal heart failure. Circ Res 73: 379-385.

Biel M, Schneider A, Wahl C (2002) Cardiac HCN Channels: structure, function, and modulation.Trends Cardiovasc Med 12: 206-212.

Biliczki P, Virág L, Iost N et al. (2002) Interaction of different potassium channels in cardiac repolarization in dog ventricular preparations: role of the repolarization reserve. Br J Pharmacol 137: 361-368.

Brennan M, Palaniswami M, Kamen P (2001) Do existing measures of Poincare plot geometry reflect nonlinear features of heart rate variability? IEEE Trans Biomed Eng 48: 1342-1347.

Brunner M, Peng X, Liu GX et al. (2008) Mechanisms of cardiac arrhythmias and sudden death in transgenic rabbits with long QT syndrome. J Clin Invest 118: 2246-2259.

Carlsson L, Almgren O, Duker G. (1990) QTU-prolongation and torsades de pointes induced by putative class III antiarrhythmic agents in the rabbit: etiology and interventions. J Cardiovasc Pharmacol 16: 276-285.

Carlsson L, Abrahamsson C, Andersson B et al. (1993) Proarrhythmic effects of the class III agent almokalant: importance of infusion rate, QT dispersion, and early afterdepolarisations. Cardiovasc Res 27: 2186-2193.

Carlsson L, Drews L, Duker G. (1996) Rhythm anomalies related to delayed repolarization in vivo: influence of sarcolemmal Ca++ entry and intracellular Ca++ overload. J Pharmacol Exp Ther 279: 231-239.

Chezalviel-Guilbert F, Davy JM, Poirier JM, Weissenburger J. (1995) Mexiletine antagonizes effects of sotalol on QT interval duration and its proarrhythmic effects in a canine model of torsade de pointes. J Am Coll Cardiol 26: 787-792.

Chiba K, Sugiyama A, Satoh Y et al. (2000) Proarrhythmic effects of fluoroquinolone antibacterial agents: in vivo effects as physiologic substrate for torsades. Toxicol Appl Pharmacol 169: 8–16.

Coppini R, Ferrantini C, Yao L et al. (2013) Late sodium current inhibition reverses electromechanical dysfunction in human hypertrophic cardiomyopathy. Circulation 127: 575-584.

Decker JA, Rossano JW, Smith EO et al. (2009) Risk factors and mode of death in isolated hypertrophic cardiomyopathy in children. J Am Coll Cardiol 54: 250-254.

Demolombe S, Lande G, Charpentier F et al. (2001) Transgenic mice overexpressing human KvLQT1 dominant-negative isoform. Part I: Phenotypic characterisation. Cardiovasc Res 50: 314-327.

Dhamoon AS, Pandit SV, Sarmast F et al. (2004) Unique Kir2.x properties determine regional and species differences in the cardiac inward rectifier K+ current. Circ. Res. 94:1332-1339.

Drici MD, Arrighi I, Chouabe C et al. (1998) Involvement of IsK-associated K^+ channel in heart rate control of repolarization in a murine engineered model of Jervell and Lange-Nielsen syndrome. Circ Res 83: 95-102.

El-Sherif N, Turitto G. (1999) The long QT syndrome and torsade de pointes. Pacing Clin Electrophysiol 22: 91-110.

Fenichel RR, Malik M, Antzelevitch C et al. (2004) Drug-induced torsades de pointes and implications for drug development. J Cardiovasc Electrophysiol 15: 475-495.

Findlay I. (2003) Is there an A-type K^+ current in guinea pig ventricular myocytes? Am J Physiol Heart Circ Physiol 284: H598-H604.

Gersh BJ, Maron BJ, Bonow RO et al. (2011) 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. Circulation 124: e783-831.

Grandy SA, Trépanier-Boulay V, Fiset C (2007) Postnatal development has a marked effect on ventricular repolarization in mice. Am J Physiol Heart Circ Physiol 293: H2168-H2177.

Harmer SC, Wilson AJ, Aldridge R et al. (2010) Mechanisms of disease pathogenesis in long QT syndrome type 5. Am J Physiol Cell Physiol 298: C263-273.

Hinterseer M, Beckmann BM, Thomsen MB et al. (2009) Relation of increased short-term variability of QT interval to congenital long-QT syndrome. Am J Cardiol 103: 1244-1248.

Hinterseer M, Beckmann BM, Thomsen MB et al. (2010) Usefulness of short-term variability of QT intervals as a predictor for electrical remodeling and proarrhythmia in patients with nonischemic heart failure. Am J Cardiol 106: 216-220.

Hondeghem LM, Carlsson L, Duker G (2001) Instability and triangulation of the action potential predict serious proarrhythmia, but action potential duration prolongation is antiarrhythmic. Circulation 103, 2004-2013.

Hondeghem LM, Dumotier B, Traebert M (2010) Oscillations of cardiac wave length and proarrhythmia. Naunyn Schmiedebergs Arch Pharmacol 382, 367-376.

Huang W, Wang Y, Cao YG et al. (2013) Antiarrhythmic effects and ionic mechanisms of allicin on myocardial injury of diabetic rats induced by streptozotocin. Naunyn Schmiedebergs Arch Pharmacol 386: 697-704.

Husti Z, Tábori K, Juhász V et al. (2015) Combined inhibition of key potassium currents has different effects on cardiac repolarization reserve and arrhythmia susceptibility in dogs and rabbits. Can J Physiol Pharmacol 93: 535-544.

ICH-S7B (2005). International Conference on Harmonisation; guidance on S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals; availability. Notice Fed Regist 70: 61133-61134.

ICHE14 (2005). International Conference on Harmonisation: Guidance on E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs; availability. Notice Fed Regist 70: 61134-61135.

Iost N, Virág L, Opincariu M, et al. (1998) Delayed rectifier potassium current in undiseased human ventricular myocytes.Cardiovasc Res 40: 508-515.

Janse MJ (2004) Electrophysiological changes in heart failure and their relationship to arrhythmogenesis. Cardiovasc Res 61: 208-217.

Jacobson I, Carlsson L, Duker G. (2011) Beat-by-beat QT interval variability, but not QT prolongation per se, predicts drug-induced torsades de pointes in the anaesthetised methoxamine-sensitized rabbit. J Pharmacol Toxicol Methods. 63(1): 40-46.

James J, Sanbe A, Yager K et al. (2000) Genetic manipulation of the rabbit heart via transgenesis. Circulation 101: 1715-1721.

Johnson DM, Heijman J, Pollard CE et al. (2010) I_{Ks} restricts excessive beat-to-beat variability of repolarization during beta-adrenergic receptor stimulation. J Mol Cell Cardiol 48: 122-130.

Jost N, Virág L, Hála O, et al. (2004) Effect of the antifibrillatory compound tedisamil (KC-8857) on transmembrane currents in mammalian ventricular myocytes Curr Med Chem 11: 3219-3228.

Jost N, Virag L, Bitay M et al. (2005) Restricting excessive cardiac action potential and QT prolongation: a vital role for IKs in human ventricular muscle. Circulation 112: 1392-1399.

Jost N, Virág L, Comtois P et al. (2013) Ionic mechanisms limiting cardiac repolarization reserve in humans compared to dogs. J Physiol 591:4189-4206.

Jost N, Kohajda Zs, Corici C et al. (2013) The comparative study of rapid and slow delayed rectifier currents in undiseased human, dog, rabbit and guinea pig ventricular myocytes. Europace, 15 Suppl 2: ii1-ii9.

Kääb S, Nuss HB, Chiamvimonvat N et al. (1996) Ionic mechanism of action potential prolongation in ventricular myocytes from dogs with pacing-induced heart failure. Circ Res 78: 262-273.

Kääb S, Hinterseer M, Näbauer M et al. (2003) Sotalol testing unmasks altered repolarization in patients with suspected acquired long-QT-syndrome – a case-control pilot study using i.v. sotalol. Eur Heart J 24: 649-657.

Kjekshus J (1990) Arrhythmias and mortality in congestive heart failure. Am J Cardiol 65: 421-481.

Kristóf A, Husti Z, Koncz I et al. (2012) Diclofenac prolongs repolarization in ventricular muscle with impaired repolarization reserve. PLoS One 7: e53255.

Lang CN, Koren G, Odening KE (2016) Transgenic rabbit models to investigate long QT syndrome. Prog Biophys Mol Biol, *in press, this issue*

Lehmann MH, Hardy S, Archibald D et al. (1996) Sex difference in risk of torsade de pointes with d,l-sotalol. Circulation 94: 2535-2541.

Lengyel C, Iost N, Virág L et al. (2001) Pharmacological block of the slow component of the outward delayed rectifier current (I_{Ks}) fails to lengthen rabbit ventricular muscle QT_c and action potential duration. Br J Pharmacol 132: 101-110.

Lengyel C, Varró A, Tábori K et al. (2007). Combined pharmacological block of I_{Kr} and I_{Ks} increases short-term QT interval variability and provokes torsades de pointes. Br J Pharmacol 151: 941-951.

Lengyel C, Virág L, Kovács PP et al. (2008) Role of slow delayed rectifier K^+-current in QT prolongation in the alloxan-induced diabetic rabbit heart. Acta Physiol (Oxf). 192: 359-368.

Li GR, Lau CP, Ducharme A et al. (2002) Transmural action potential and ionic current remodelling in ventricles of failing canine hearts. Am J Physiol Heart Circ Physiol 283: H1031-H1041.

Li GR, Lau CP, Leung TK et al. (2004) Ionic current abnormalities associated with prolonged action potentials in cardiomyocytes from diseased human right ventricles. Heart Rhythm 1: 460-468.

Lombardi R, Rodriguez G, Chen SN et al. (2009) Resolution of established cardiac hypertrophy and fibrosis and prevention of systolic dysfunction in a transgenic rabbit model of human cardiomyopathy through thiol-sensitive mechanisms. Circulation 119: 1398-1407.

Lu Z, Kamiya K, Opthof T et al. (2001). Density and kinetics of I_{Kr} and I_{Ks} in guinea pig and rabbit ventricular myocytes explain different efficacy of I_{Ks} blockade at high heart rate in guinea pig and rabbit: implications for arrhythmogenesis in humans. Circulation 104: 951-956.

Luo X, Xiao J, Lin H et al. (2007) Transcriptional activation by stimulating protein 1 and post-transcriptional repression by muscle-specific microRNAs of I_{Ks}-encoding genes and potential implications in regional heterogeneity of their expressions. J Cell Physiol 212: 358-367.

Ma L, Lin C, Teng S et al. (2003) Characterization of a novel Long QT syndrome mutation G52R-KCNE1 in a Chinese family. Cardiovasc Res 59: 612-619.

Magyar J, Rusznák Z, Szentesi P (1992) Action potentials and potassium currents in rat ventricular muscle during experimental diabetes. J Mol Cell Cardiol 24: 841-853.

Major P, Baczkó I, Hiripi L et al. (2016) A novel transgenic rabbit model with reduced repolarization reserve: long QT syndrome caused by a dominant-negative mutation of *KCNE1* gene. Br J Pharmacol *in press*

Maron, B.J. (2002) Hypertrophic cardiomyopathy: a systematic review. JAMA 287: 1308-1320.

Maron BJ, Doerer JJ, Haas TS et al. (2009) Sudden deaths in young competitive athletes: analysis of 1866 deaths in the United States, 1980-2006. Circulation 119: 1085-1092.

Mattioni TA, Zheutlin TA, Sarmiento JJ et al. (1989) Amiodarone in patients with previous drug-mediated torsade de pointes. Long-term safety and efficacy. Ann Intern Med 111: 574-580.

McKeown PP, Muir AR (2013) Risk assessment in hypertrophic cardiomyopathy: contemporary guidelines hampered by insufficient evidence. Heart 99: 511-513.

McNally PG, Lawrence IG, Panerai RB et al. (1999) Sudden death in type 1 diabetes. Diabetes Obes Metab 1: 151-158.

Melnyk P, Zhang L, Shrier A et al. (2002) Differential distribution of Kir2.1 and Kir2.3 subunits in canine atrium and ventricle. Am J Physiol Heart Circ Physiol. 283: H1123-1133.

Nagy N, Szuts V, Horváth Z et al. (2009) Does small-conductance calcium-activated potassium channel contribute to cardiac repolarization? J Mol Cell Cardiol 47: 656-663.

Nerbonne JM, Kass RS (2005) Molecular physiology of cardiac repolarization. Physiol Rev 85: 1205-1253.

Nuss HB, Kaab S, Kass DA et al. (1999) Cellular basis of ventricular arrhythmias and abnormal automaticity in heart failure. Am J Physiol 277: H80-H91.

Odening KE, Hyder O, Chaves L et al. (2008) Pharmacogenomics of anesthetic drugs in transgenic LQT1 and LQT2 rabbits reveal genotype-specific differential effects on cardiac repolarization. Am J Physiol Heart Circ Physiol 295: H2264-2272.

Olivotto I, Cecchi F, Poggesi C et al. (2012) Patterns of disease progression in hypertrophic cardiomyopathy: an individualized approach to clinical staging. Circulation. Heart Failure 5: 535-546.

Orosz A, Baczkó I, Nagy V et al. (2015) Short-term beat-to-beat variability of the QT interval is increased and correlates with parameters of left ventricular hypertrophy in patients with hypertrophic cardiomyopathy. Can J Physiol Pharmacol 93: 765-772.

Orosz S, Sarusi A, Csík N et al. (2014) Assessment of efficacy of proarrhythmia biomarkers in isolated rabbit hearts with attenuated repolarization reserve. J Cardiovasc Pharmacol. 64(3): 266-276.

Poelzing S, Rosenbaum DS (2004) Altered connexin43 expression produces arrhythmia substrate in heart failure. Am J Physiol Heart Circ Physiol 287: H1762-H1770.

Pogwizd SM, Schlotthauer K, Li L et al. (2001) Arrhythmogenesis and contractile dysfunction in heart failure: roles of sodium-calcium exchange, inward rectifier potassium current, residual beta-adrenergic responsiveness. Circ Res 88: 1159-1167.

Qin D, Huang B, Deng L et al. (2001) Downregulation of K+ channel genes expression in type I diabetic cardiomyopathy. Biochem Biophys Res Commun 283: 549-553.

Ripplinger CM, Li W, Hadley J et al. (2007) Enhanced transmural fiber rotation and connexin 43 heterogeneity are associated with an increased upper limit of vulnerability in a transgenic rabbit model of human hypertrophic cardiomyopathy. Circ Res 101: 1049-1057.

Roden DM (1998). Taking the "idio" out of "idiosyncratic": predicting torsades de pointes. Pacing Clin Electrophysiol 21: 1029-1034.

Roden DM, Yang T (2005) Protecting the heart against arrhythmias: potassium current physiology and repolarization reserve. Circulation 112: 1376-1378.

Rose J, Armoundas AA, Tian Y et al. (2005) Molecular correlates of altered expression of potassium currents in failing rabbit myocardium. Am J Physiol Heart Circ Physiol 288: H2077-H2087.

Rusznák Z, Cseresnyés Z, Sipos I et al. (1996) Characteristics of the ventricular transient outward potassium current in genetic rodent models of diabetes. Gen Physiol Biophys 15: 225-238.

Saito T, Ciobotaru A, Bopassa JC, et al. (2009) Estrogen contributes to gender differences in mouse ventricular repolarization. Circ Res 105: 343-352.

Salama G, London B. (2007) Mouse models of long QT syndrome. J Physiol 578: 43-53.

Sanguinetti MC, Curran ME, Zou A et al. (1996) Coassembly of K(V)LQT1 and minK (IsK) proteins to form cardiac I_{Ks} potassium channel. Nature 384: 80-83.

Sarusi A, Rárosi F, Szűcs M, et al. (2014) Absolute beat-to-beat variability and instability parameters of ECG intervals: biomarkers for predicting ischaemia-induced ventricular fibrillation. Br J Pharmacol 171(7): 1772-1782.

Schoenmakers M, Ramakers C, van Opstal JM, et al. (2003) Asynchronous development of electrical remodeling and cardiac hypertrophy in the complete AV block dog. Cardiovasc Res 59: 351–359.

Shimoni Y, Firek L, Severson D et al. (1994) Short-term diabetes alters K^+ currents in rat ventricular myocytes. Circ Res 74: 620-628.

Shimoni Y, Severson D, Giles W (1995) Thyroid status and diabetes modulate regional differences in potassium currents in rat ventricle. J Physiol 488: 673-688.

Stengl M, Ramakers C, Donker DW et al. (2006) Temporal patterns of electrical remodeling in canine ventricular hypertrophy: focus on I_{Ks} downregulation and blunted beta-adrenergic activation. Cardiovasc Res 72: 90-100.

Stillitano F, Lonardo G, Zicha S et al. (2008) Molecular basis of funny current (If) in normal and failing human heart. J Mol Cell Cardiol 45: 289-299.

Sugiyama A, Satoh Y, Shiina H, et al. (2002). Torsadegenic action of the antipsychotic drug sulpiride assessed using in vivo canine models. J Cardiovasc Pharmacol 40: 235-245.

Suys BE, Huybrechts SJ, De Wolf D, et al. (2002) QTc interval prolongation and QTc dispersion in children and adolescents with type 1 diabetes. J Pediatr 141: 59-63.

Takahara A, Sugiyama A, Ishida Y, et al. (2006) Long-term bradycardia caused by atrioventricular block can remodel the canine heart to detect the histamine H1 blocker terfenadine-induced torsades de pointes arrhythmias. Br J Pharmacol 147: 634-641.

Thomsen MB, Volders PG, Stengl M, et al. (2003) Electrophysiological safety of sertindole in dogs with normal and remodeled hearts. J Pharmacol Exp Ther 307: 776-784.

Thomsen MB, Verduyn SC, Stengl M et al. (2004) Increased short-term variability of repolarization predicts d-sotalol-induced torsades de pointes in dogs. Circulation 110: 2453-2459.

Thomsen MB. (2007) Double pharmacological challenge on repolarization opens new avenues for drug safety research. Br J Pharmacol 151(7): 909-911.

Tsuchida K, Watajima H. (1997) Potassium currents in ventricular myocytes from genetically diabetic rats. Am J Physiol 273: E695-E700.

Tsuji Y, Opthof T, Kamiya K et al. (2000) Pacing-induced heart failure causes a reduction of delayed rectifier potassium currents along with decreases in calcium and transient outward currents in rabbit ventricle. Cardiovasc Res 48: 300-309.

Valdivia CR, Chu WW, Pu J et al. (2005) Increased late sodium current in myocytes from a canine heart failure model and from failing human heart. J Mol Cell Cardiol 38: 475-483.

van Opstal JM., Schoenmakers M., Verduyn SC et al. (2001) Chronic amiodarone evokes no torsade de pointes arrhythmias despite QT lengthening in an animal model of acquired long-QT syndrome. Circulation 104: 2722-2727.

Varkevisser R, Wijers SC, van der Heyden MA et al. (2012) Beat-to-beat variability of repolarization as a new biomarker for proarrhythmia in vivo. Heart Rhythm 9: 1718-1726.

Varró A, Lathrop DA, Hester SB et al. (1993) Ionic currents and action potentials in rabbit, rat, and guinea pig ventricular myocytes. Basic Res Cardiol 88: 93-102.

Varro A, Baczko I (2011) Cardiac ventricular repolarization reserve: a principle for understanding drug-related proarrhythmic risk. Br J Pharmacol 164: 14-36.

Varro A, Balati B, Iost N et al. (2000) The role of the delayed rectifier component IKs in dog ventricular muscle and Purkinje fibre repolarization. J Physiol 523: 67-81.

Veglio M, Giunti S, Stevens LK et al. (2002) Prevalence of Q–T interval dispersion in type 1 diabetes and its relation with cardiac ischemia: the EURODIAB IDDM Complications Study Group. Diabetes Care 25: 702-707.

Verduyn SC, van Opstal JM, Leunissen JD, Vos MA. (2001) Assessment of the pro-arrhythmic potential of anti-arrhythmic drugs: an experimental approach. J Cardiovasc Pharmacol Ther 6: 89-97.

Vincze D, Farkas AS, Rudas L et al. (2008) Relevance of anaesthesia for dofetilide-induced torsades de pointes in alpha1-adrenoceptor-stimulated rabbits. Br J Pharmacol. 153(1): 75-89.

Virág L, Iost N, Opincariu M et al. (2001) The slow component of the delayed rectifier potassium current in undiseased human ventricular myocytes.Cardiovasc Res 49: 790-797.

Virág L, Jost N, Papp R et al. (2011) Analysis of the contribution of I_{to} to repolarization in canine ventricular myocardium. Br J Pharmacol. 164: 93-105.

Volders PG, Sipido KR, Vos MA et al (1999) Downregulation of delayed rectifier K^+ currents in dogs with chronic complete atrioventricular block and acquired torsades de pointes. Circulation 100: 2455-2461.

Volders PG, Vos MA, Szabo B, et al. (2000) Progress in the understanding of cardiac early afterdepolarizations and torsades de pointes: time to revise current concepts. Cardiovasc Res 46: 376-392.

Volders PG, Stengl M, van Opstal JM et al. (2003) Probing the contribution of I_{Ks} to canine ventricular repolarization: key role for ß-adrenergic receptor stimulation. Circulation 107: 2753-2760.

Vos MA, Verduyn SC, Gorgels AP, et al. (1995) Reproducible induction of early after depolarizations and torsade de pointes arrhythmias by d-sotalol and pacing in dogs with chronic atrioventricular block. Circulation 91: 864-872.

Vos MA, de Groot SH, Verduyn SC et al. (1998) Enhanced susceptibility for acquired torsade de pointes arrhythmias in the dog with chronic, complete AV block is related to cardiac hypertrophy and electrical remodeling. Circulation 98: 1125-1135.

Vriesendorp PA, Schinkel AF, de Groot NM et al. (2014) Impact of adverse left ventricular remodeling on sudden cardiac death in patients with hypertrophic cardiomyopathy. Clinical Cardiology 37: 493-498.

Weissenburger J, Davy JM, Chezalviel F et al. (1991) Arrhythmogenic activities of antiarrhythmic drugs in conscious hypokalemic dogs with atrioventricular block: comparison between quinidine, lidocaine, flecainide, propranolol and sotalol. J Pharmacol Exp Ther 259: 871-883.

Wang Z, Feng J, Shi H et al. (1999) Potential molecular basis of different physiological properties of the transient outward K+ current in rabbit and human atrial myocytes. Circ Res 84: 551-561.

Whitsel EA, Boyko EJ, Rautaharju PM et al. (2005) Electrocardiographic QT interval prolongation and risk of primary cardiac arrest in diabetic patients. Diabetes Care 28: 2045-2047.

Xiao L, Xiao J, Luo X et al. (2008) Feedback remodeling of cardiac potassium current expression: a novel potential mechanism for control of repolarization reserve. Circulation 118: 983-992.

Xu Z, Patel KP, Rozanski GJ (1996) Metabolic basis of decreased transient outward K+ current in ventricular myocytes from diabetic rats. Am J Physiol 271: H2190-H2196.

Yang T, Chun YW, Stroud DM et al. (2014) Screening for acute I_{Kr} block is insufficient to detect Torsades de Pointes liability: role of late sodium current. Circulation. 130: 224-234.

Zhang L, Benson DW, Tristani-Firouzi M et al. (2005) Electrocardiographic features in Andersen-Tawil syndrome patients with KCNJ2 mutations: characteristic T-U-wave patterns predict the KCNJ2 genotype. Circulation 111: 2720-2726.

Zhang Y, Xiao J, Lin H et al. (2007) Ionic mechanisms underlying abnormal QT prolongation and the associated arrhythmias in diabetic rabbits: a role of rapid delayed rectifier K^+ current. Cell Physiol Biochem 19: 225-238.

Type	Affected gene	Affected protein – ionic current	Mutation result
LQT1	*KCNQ1*	K_VLQT1 - I_{Ks}	loss of function
LQT2	*KCNH2*	K_V11.1 - I_{Kr}	loss of function
LQT3	*SCN5A*	Na_V1.5 - I_{Na}	gain of function
LQT4	*ANKB*	Ankyrin-B – I_{NCX}, I_{NaK}	loss of function
LQT5	*KCNE1 (minK)*	MinK - I_{Ks}	loss of function
LQT6	*KCNE2 (MiRP1)*	MiRP1 - I_{Kr}	loss of function
LQT7	*KCNJ2*	Kir2.1 - I_{K1}	loss of function
LQT8	*CACNA1c*	Ca_V1.2α1 - $I_{Ca,L}$	gain of function
LQT9	*CAV3*	Caveolin- I_{Na}	
LQT10	*SCN4B*	Na_vβ4- I_{Na}	gain of function
LQT11	*AKAP9*	Yotiao – I_{Ks}	loss of function
LQT12	*SNTA1*	α-syntrophin - I_{Na}	gain of function
LQT13	*KCNJ5*	Kir3.4 - $I_{K,ACh}$	loss of function

Table 1. List of congenital long QT syndromes, affected genes, proteins and ionic currents.

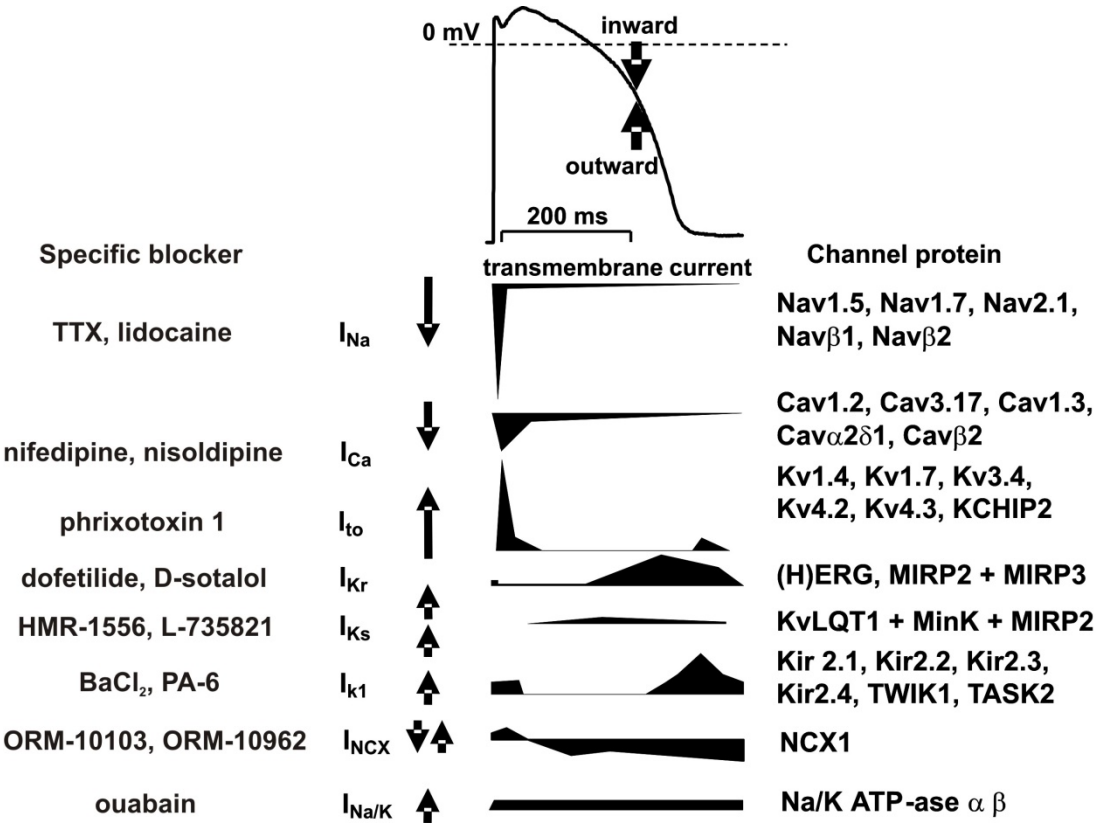

Figure 1. Illustration of the main transmembrane ionic currents and electrogenic pumps shaping the ventricular cardiac action potential. Specific blockers are also included. The transmembrane currents shown are not proportional to the actual current densities. Channel proteins that mediate these currents are also indicated. Modified from Varró and Baczkó, 2011, with permission.

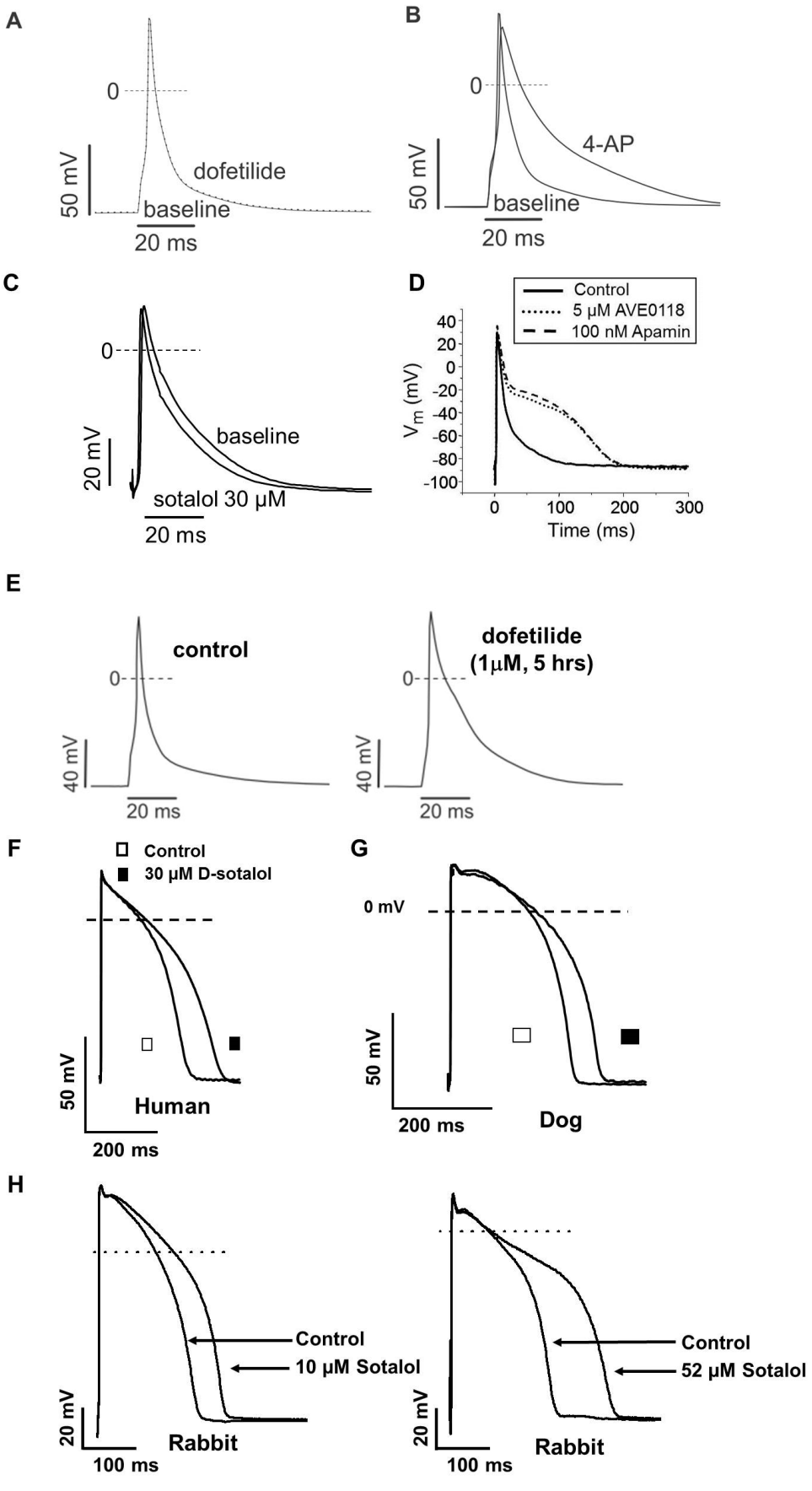
A
0
50 mV
dofetilide
baseline
20 ms
B
0
4-AP
50 mV
baseline
20 ms
C
0
baseline
20 mV
sotalol 30 μM
20 ms
D
Control
5 μM AVE0118
100 nM Apamin
40
20
0
-20
-40
-60
-80
-100
Vm (mV)
0
100
200
300
Time (ms)
E
control
0
40 mV
20 ms
dofetilide
(1μM, 5 hrs)
0
40 mV
20 ms
F
Control
30 μM D-sotalol
50 mV
Human
200 ms
G
0 mV
50 mV
200 ms
Dog
H
Control
10 μM Sotalol
20 mV
Rabbit
100 ms
Control
52 μM Sotalol
20 mV
Rabbit
100 ms

Figure 2. Representative action potential recordings from different species frequently used in cardiac electrophysiological research. (**A**) The lack of effect of acute administration of the I_{Kr} blocker dofetilide, and the prolonging effect of 4-aminopyridine (4-AP) (**B**) on action potentials in mice. (**C**) Lack of effect of the I_{Kr} blocker sotalol on the AP in a rat papillary muscle preparation. (**D**) The significant AP prolonging effect of the combined I_{to} and I_{Kur} inhibitor AVE0118 in rat. (**E**) Extended (5 h) application of dofetilide prolonged the AP in mice. Sotalol exerts significant AP lengthening effect in (**F**) humans, (**G**) dogs, and (**H**) rabbits. Panels **A, B** and **E** reproduced from Yang *et al*, 2014; panel **D** from Nagy *et al*, 2009; panel **F** from Jost *et al*, 2013; panel **G** from Varró *et al*, 2000, with permission. Panels **C** and **H** show unpublished results from our laboratory.

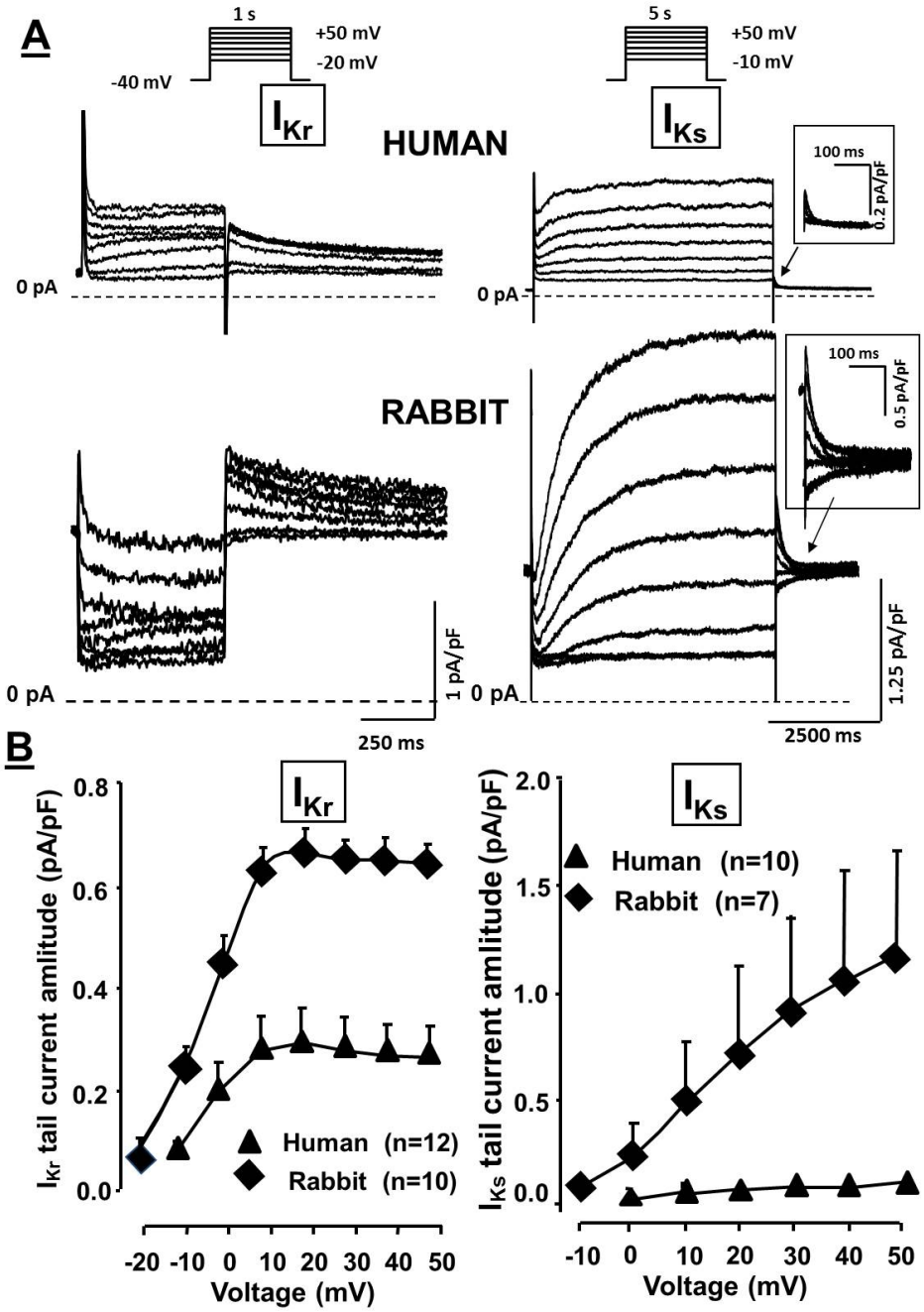

Figure 3. (**A**) Original recordings of E-4031 sensitive (I_{Kr}, left) and L-735.821 (I_{Ks}, right) rapid and slow delayed rectifier potassium currents in undiseased human (top), and rabbit (bottom) ventricular myocytes. Nisoldipine (1 μM) was used to block L-type inward calcium current (I_{CaL}) and L-735,821 (100 nM) or E-4031 (1 μM) to block I_{Kr} or I_{Ks} currents, respectively. (**B)** The peak I_{Kr} (left) and I_{Ks} (right) tail current-voltage (I-V) relationship in undiseased human (triangles) and rabbit (diamonds) ventricular myocytes. I_{Kr} and I_{Ks} currents were examined in isolated human or rabbit ventricular myocytes using test pulses of 1000 ms (I_{Kr}) or 5000 ms (I_{Ks}) in duration to between -20 mV and +50 mV from the holding potential of -40 mV. The pulse frequency was 0.05 Hz (I_{Kr}) or 0.1 Hz (I_{Ks}). Unpublished data from our laboratory.

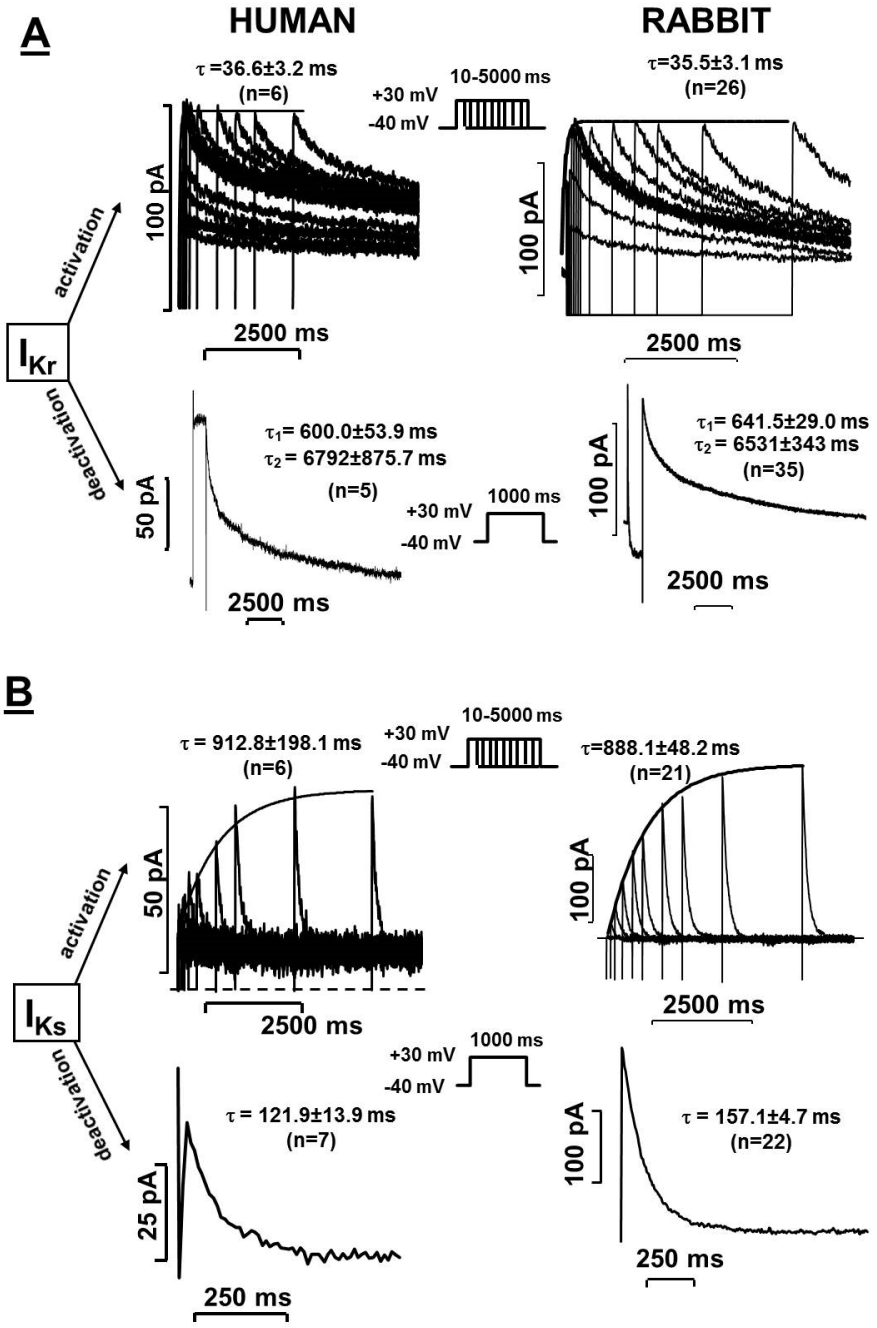

Figure 4. Activation and deactivation kinetics of I_{Kr} (**A**) and I_{Ks} (**B**), in undiseased human (left) and rabbit (right) ventricular myocytes. Activation kinetics of I_{Kr} and I_{Ks} were measured as tail currents at -40 mV, after test pulses to +30 mV with duration gradually increasing between 10 and 5000 ms. Deactivation kinetics of I_{Kr} and I_{Ks} outward tail currents were measured at -40 mV, after a 1000 ms (for I_{Ks}) or 5000 ms (for I_{Ks}), respectively, long test pulse to +30 mV. Average I_{Kr} and I_{Ks} activation and deactivation time constants (τ) values±SEM are given in insets. Adapted from Iost *et al*, 1998; Virag *et al*, 2001 and Lengyel *et al*, 2001, with permission.

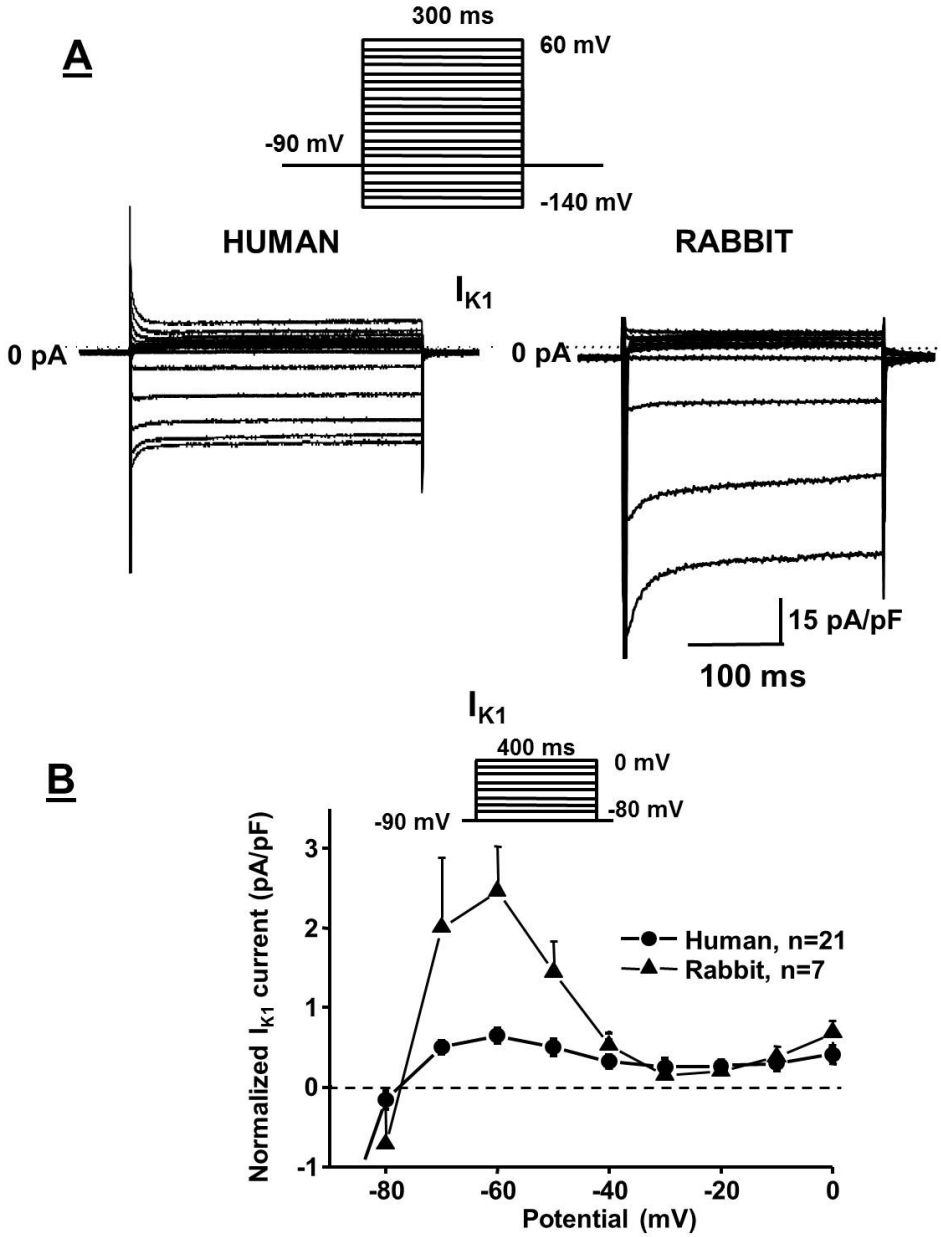

Figure 5. (**A**) Original recordings of inward rectifier potassium (I_{K1}) currents in undiseased human (left), and rabbit (right) ventricular myocytes. I_{K1} current was studied by measuring the steady-state current level at the end of the 400 ms long voltage pulse in the voltage range of -120 to 0 mV with a pulse frequency of 0.33 Hz. The holding potential was -90 mV. Nisoldipine (1 μM) was used to block L-type inward calcium current. (**B)** Current-voltage relationships of I_{K1} current in human (circle) and rabbit (triangle) ventricular myocytes measured after depolarizing voltage steps beetween -80 mV to 0 mV (outward range of I_{K1} current). Values represent mean±SEM. Insets show applied voltage protocols on both panels. Adapted from Jost *et al*, 2013, with permission and unpublished data from our laboratory.

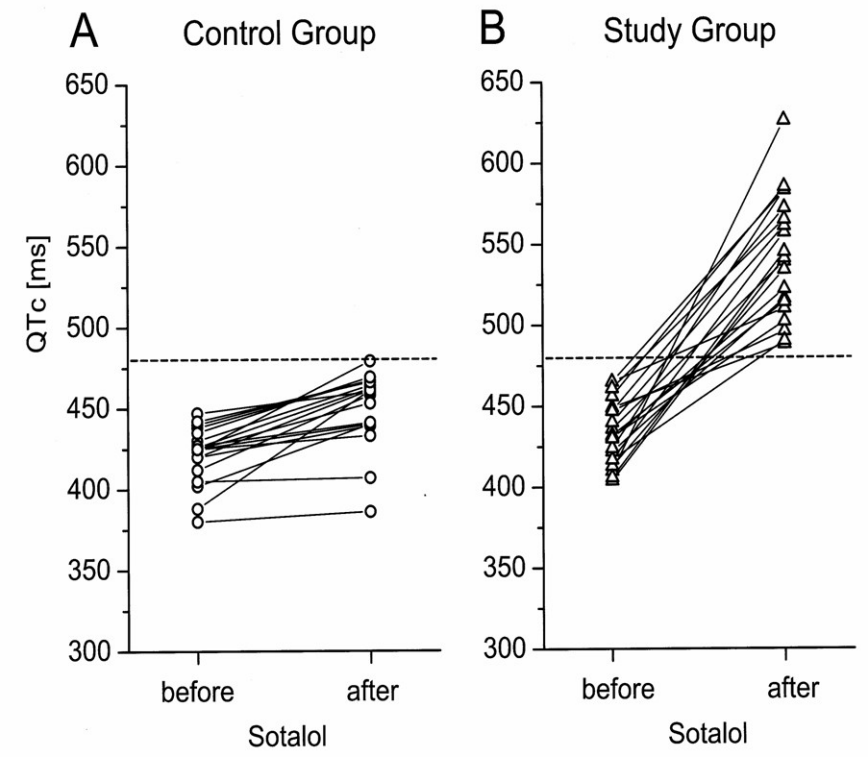

Figure 6. Clinical significance of repolarization reserve. Changes in individual QTc intervals in control patients (**A**) and in patients with suspected acquired long QT syndrome (**B**, Study Group) following the i.v. administration of sotalol. The dotted line represents the cut-off value of 480 ms that differentiated between the study group and the control group. From Kääb *et al*, 2003, with permission.