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Combined inhibition of key potassium currents has different effects on cardiac repolarization reserve and arrhythmia susceptibility in dogs and rabbits[1]

Zoltán Husti, Katalin Tábori, Viktor Juhász, Tibor Hornyik, András Varró, and István Baczkó

Abstract: A reliable assessment of the pro-arrhythmic potential for drugs in the development phase remains elusive. Rabbits and dogs are commonly used to create models of pro-arrhythmia, but the differences between them with respect to repolarizing potassium currents are poorly understood. We investigated the incidence of drug-induced torsades de pointes (TdP) and measured conventional ECG parameters and the short-term variability of the QT interval (STV_{QT}) following combined pharmacological inhibition of $I_{K1}+I_{Ks}$ and $I_{K1}+I_{Kr}$ in conscious dogs and anesthetized rabbits. A high incidence of TdP was observed following the combined inhibition of $I_{K1}+I_{Ks}$ in dogs (67% vs. 14% in rabbits). Rabbits exhibited higher TdP incidence after inhibition of $I_{K1}+I_{Kr}$ (72% vs. 14% in dogs). Increased TdP incidence was associated with significantly larger STV_{QT} in both models. The relatively different roles of I_{K1} and I_{Ks} in dog and rabbit repolarization reserve should be taken into account when extrapolating the results from animal models of pro-arrhythmia to humans. A stronger repolarization reserve in dogs (likely due to stronger I_{K1} and I_{Ks}), and the more human-like susceptibility to arrhythmia of rabbits argues for the preferred use of rabbits in the evaluation of adverse pro-arrhythmic effects.

Key words: cardiac electrophysiology, potassium channels, torsades de pointes, I_{K1}, I_{Ks}.

Résumé : L'évaluation fiable du potentiel arythmogène des médicaments en développement n'est pas encore à notre portée. On utilise fréquemment les lapins et les chiens dans les modèles d'arythmogénicité, mais on ne saisit pas bien les différences qui existent entre les espèces quant aux courants potassiques repolarisants. Nous avons étudié la fréquence de l'apparition de torsades de pointes et mesuré les paramètres conventionnels de l'ECG ainsi que la variabilité à court terme de la durée de l'intervalle QT (STV_{QT}) à la suite des associations d'inhibition pharmacologique d'$I_{K1}+I_{Ks}$ et d'$I_{K1}+I_{Kr}$ chez des chiens à l'état de conscience et des lapins sous anesthésie. La fréquence de l'apparition de torsades de pointes était élevée après l'inhibition associée d'$I_{K1}+I_{Ks}$ chez les chiens (67 % *vs.* 14 % chez les lapins). Par contre, l'apparition de torsades de pointes était plus fréquente chez les lapins après l'inhibition associée d'$I_{K1}+I_{Kr}$ (72 % *vs.* 14 % chez les chiens). L'augmentation de la fréquence de l'apparition de torsades de pointes était associée à une augmentation notable de la STV_{QT} dans les deux modèles. Les rôles relatifs distincts des courants I_{K1} et I_{Ks} sur la réserve de repolarisation observée chez le chien et le lapin doivent être pris en compte lorsque l'on extrapole l'interprétation des résultats obtenus avec des modèles animaux d'arythmogénicité en la faisant porter sur l'humain. Une réserve de repolarisation plus élevée chez le chien (probablement en raison de courants I_{K1} et I_{Ks} plus forts) et une susceptibilité aux arythmies chez le lapin plus ressemblante à celle de l'humain plaident pour l'utilisation préférentielle des lapins en vue d'évaluer les effets indésirables de type arythmogène. [Traduit par la Rédaction]

Mots-clés : électrophysiologie cardiaque, canaux potassiques, torsades de pointes, I_{K1}, I_{Ks}.

Introduction

The proper assessment of the pro-arrhythmic potential of candidate compounds is a major concern for drug development (Haverkamp et al. 2000) because drug-induced arrhythmias, including torsades de pointes (TdP), can lead to sudden cardiac death (Fenichel et al. 2004). Prediction of TdP in the clinical setting is very difficult because the incidence of drug-induced TdP is very low (1:100 000); however, drug-associated sudden cardiac death has led to the withdrawal of several compounds in the past. Therefore, the reliable preclinical assessment of pro-arrhythmic side-effects is essential. Importantly, current methods mostly concentrate on testing the hERG blocking and (or) ventricular repolarization prolonging effects of these compounds, primarily in healthy tis-sues and animals, and these cardiac safety tests are not sensitive enough (Food and Drug Administration, HHS 2005a, 2005b; Thomsen et al. 2006; Farkas and Nattel 2010).

The inhibition of cardiac potassium channels prolongs repolarization and refractoriness, leading to Class III antiarrhythmic effects (Singh and Vaughan Williams 1970). However, excessive prolongation of repolarization by Class III antiarrhythmic drugs, seen as marked QT interval prolongation on the electrocardiogram (ECG), can result in drug-induced TdP (Hondeghem and Snyders 1990; El-Sherif 1992). On the other hand, experimental and clinical studies have suggested that the degree of repolarization prolongation does not show a close correlation with subsequent ventricular arrhythmia development (van Opstal et al. 2001;

Z. Husti, K. Tábori, V. Juhász, T. Hornyik, and I. Baczkó. Department of Pharmacology and Pharmacotherapy, University of Szeged; H-6720, Dóm tér 12, P.O. Box 427, Szeged, Hungary.

A. Varró. Department of Pharmacology and Pharmacotherapy, University of Szeged; H-6720, Dóm tér 12, P.O. Box 427, Szeged, Hungary; MTA-SZTE Research Group of Cardiovascular Pharmacology, Hungarian Academy of Sciences, H-6720, Dóm tér 12, P.O. Box 427, Szeged, Hungary.

Corresponding author: Zoltan Husti (e-mail: husti.zoltan@gmail.com).

Thomsen et al. 2004; Lengyel et al. 2007; Hinterseer et al. 2009, 2010). In these cases, without marked prolongation of the QT interval, repolarization reserve may be reduced with a consequent increase in arrhythmia susceptibility. According to the concept of repolarization reserve, normal cardiac repolarization is controlled by different potassium currents in a redundant way, and congenital or acquired (e.g., mild potassium current inhibition by a non-cardiovascular drug) decrease in the function of a single repolarizing current does not always lead to marked prolongation of repolarization because other currents can compensate for the lost function (Roden 1998; Varró and Baczkó 2011). In the case of reduced repolarization reserve, additional inhibition of another repolarizing current can result in excessive prolongation of repolarization and provoke serious ventricular arrhythmias.

Experimental evidence from studies on both animal and human ventricular myocardium point to a critically important role for the slow component of the delayed rectifier potassium current (I_{Ks}) in ventricular repolarization reserve (Volders et al. 1999, 2003; Varró et al. 2000; Lengyel et al. 2001, 2007; Jost et al. 2005; Abi-Gerges et al. 2006). In addition to I_{Ks}, other ventricular repolarizing potassium currents may significantly contribute to repolarization reserve, including the transient outward current (I_{to}) (Virág et al. 2011) and the inward rectifier current (I_{K1}) (Biliczki et al. 2002). There is considerable variation in the expression of key repolarizing potassium channels in different mammalian species (Zicha et al. 2003). The dog and rabbit are frequently used in various in-vitro and in-vivo models of pro-arrhythmia. Therefore, it is reasonable to assume that species-specific ion channel expression profiles may result in species-dependent alterations in response to potassium channel blockers (Nerbonne and Kass 2005). Such differences may significantly influence the value of data obtained in these models for human extrapolation. Indeed, a recent study found similar densities for the rapid component of the delayed rectifier current (I_{Kr}) and significantly lower densities for I_{K1} and I_{Ks} in human ventricular myocytes compared with their canine counterparts, suggesting that humans may exhibit a reduced repolarization reserve and could be more susceptible to the adverse pro-arrhythmic effects of drugs that block I_{Kr} (Jost et al. 2013a). However, it is not clear how species-specific potassium channel expressions translate into differences in arrhythmia development in dogs and rabbits. We have previously shown that repolarization reserve impairment by pharmacological blocking of I_{Ks} increased susceptibility to arrhythmia to a similar degree during the subsequent I_{Kr} inhibition in dogs and rabbits (Lengyel et al. 2007).

In this study, we examined the effects of combined pharmacological inhibition of I_{K1} and I_{Ks} as well as I_{K1} and I_{Kr} on ECG parameters and the incidence of TdP in conscious dogs and anesthetized rabbits. We also investigated whether TdP development was paralleled by increased short-term variability of the QT interval; a novel ECG parameter that is recommended for a more reliable prediction of drug-induced ventricular arrhythmias.

Materials and methods

Ethical issues

All experiments were carried out in compliance with the *Guide for the Care and Use of Laboratory Animals* (ILAR 1996) and the protocol was approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Szeged, Hungary (approval number I-74-5-2012) and by the Department of Animal Health and Food Control of the Ministry of Agriculture (authority approval number XIII/1211/2012).

Conscious dogs

Beagles of either sex, weighing 10–15 kg, were used for the experiments. The animals were acclimated to the experiment's

personnel and equipment every day for a week before the start of the actual experiments. Baseline recordings were obtained after a 20 min equilibration period. The animals were then randomly assigned to 1 of 2 groups. The dogs in Group 1 ($n = 7$) were first intravenously (i.v.) administered the I_{K1} inhibitor BaCl$_2$ (3.0 mg/kg body mass) followed by the I_{Kr} inhibitor dofetilide (25.0 µg/kg; Gedeon Richter, Budapest, Hungary), after a 20 min equilibration period. The dogs in Group 2 ($n = 6$) first received the I_{Ks} inhibitor HMR 1556 (1.0 mg/kg, i.v.), followed by BaCl$_2$ (3.0 mg/kg, i.v.) after a 20 min equilibration period. The drugs were administered during a 5 min continuous i.v. infusion (Terufusion TE-3; Terumo Europe, Leuven, Belgium). The ECG was obtained using precordial leads and was digitized and stored on a computer for later analysis using National Instruments data acquisition hardware (National Instruments, Austin, Texas, USA) and SPEL Advanced Haemosys software (version 3.2; Experimetria, Budapest, Hungary; MDE Heidelberg GmbH, Heidelberg, Germany). The PQ, R–R, and QT intervals were measured from the mean of 30 consecutive beats (the minimum number of beats required for the calculation of beat-to-beat short-term variability of an interval; see below) and the frequency-corrected QT interval (QTc) was calculated using the formula recommended specifically for beagles: QTc = QT − [0.087(R–R − 1000)] (Van de Water et al. 1989; Tattersall et al. 2006). The intervals were measured at the following time points during the experiments: (*i*) 2 min before the start of drug infusion (baseline); (*ii*) 5 min after the end of the drug infusions; and (*iii*) at the ECG section directly preceding the arrhythmia still in sinus rhythm if TdP (Fig. 1) or any other ventricular arrhythmias occurred.

Anesthetized rabbits

Male New Zealand white rabbits (2–3 kg) were anesthetized with thiopentone (50 mg/kg, i.v.) delivered into the marginal vein of the right ear. A catheter filled with isotonic saline containing 500 IU/mL heparin was inserted into the left carotid artery for the measurement of arterial blood pressure. The right jugular vein was cannulated for subsequent i.v. drug administration. The animals were allowed to stabilize for 20 min and then baseline measurements were taken. Group 1 ($n = 7$) was administered the I_{K1} inhibitor BaCl$_2$ (0.3 mg/kg, i.v., in a volume of 2.0 mL/kg during a 5 min infusion) followed by the I_{Kr} inhibitor dofetilide (25.0 µg/kg, i.v.) 20 min after BaCl$_2$ administration. Group 2 ($n = 7$) received the I_{Ks} inhibitor HMR 1556 (0.1 mg/kg, i.v.; Aventis Pharma, Frankfurt am Main, Germany) followed by BaCl$_2$ (0.3 mg/kg, i.v.) 20 min after HMR 1556 administration.

Blood pressure and ECG (leads I, II, and III) were continuously recorded (at 200 Hz), digitized, and stored on a computer for analysis using National Instruments data acquisition hardware and SPEL Advanced Haemosys software. The PQ, R–R, and QT intervals were measured as the mean of 30 beats (the minimum number of beats required for the calculation of short-term variability of an interval; see below). During the measurement of the QT interval in anaesthetized rabbits, the guidelines described by Farkas et al. (2004) were followed. In rabbits with a significantly faster heart rate than that of humans, QTc calculated with Bazett's formula does not accurately reflect the heart-rate-dependent changes in the QT interval. Accordingly, QTc was calculated using the following formula specifically suggested for anaesthetized rabbits by Batey and Coker (2002): QTc = QT − [0.704(R–R − 250)].

Short-term beat-to-beat variability of the R–R (STV$_{R–R}$) and QT intervals (STV$_{QT}$)

Temporal instability of beat-to-beat heart rate and repolarization can be quantitatively characterized by the calculation of the beat-to-beat short-term variability (STV) of the R–R or QT intervals, respectively. The calculation of STV is based on previously detailed mathematical analysis (Brennan et al. 2001) and was calculated as follows: STV = $\Sigma|D_{n+1} - D_n|(30 \times \sqrt{2})^{-1}$, where D is the duration of the QT or R–R interval. The intervals used in the

Fig. 1. Representative torsades de pointes recordings from (A) a conscious dog following combined inhibition of $I_{Ks}+I_{K1}$, and (B) an anesthetized rabbit following the combined inhibition of $I_{K1}+I_{Kr}$.
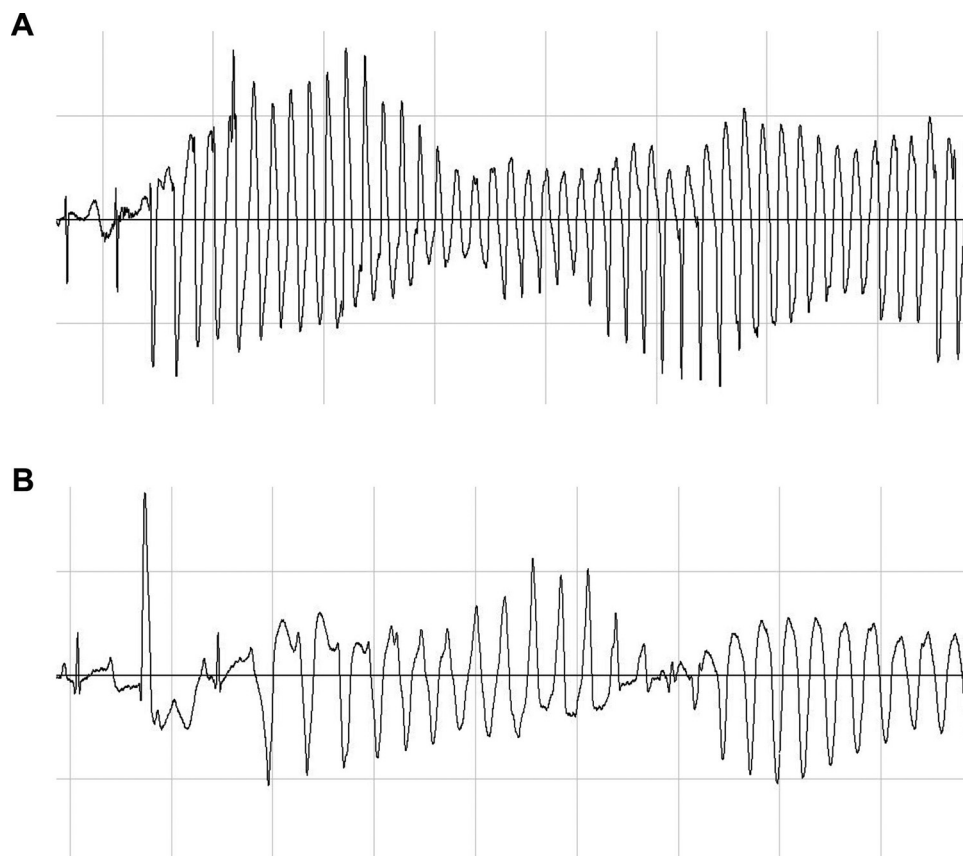
calculation were the result of 30 consecutive interval measurements in sinus rhythm at a given time point during the experiments. In the case of experiments where TdP occurred, the measurements were taken in sinus rhythm prior to the development of TdP. To illustrate temporal instability of the QT interval, Poincaré plots of the QT intervals were constructed in which each QT value was plotted against its former value (Figs. 3B and 5B). STV represents the mean orthogonal distance to the line-of-identity on the Poincaré plot.

Compounds

HMR 1556 was dissolved in dimethylsulfoxide (0.1% *v/v*) to provide a stock solution of 10.0 μmol/L. Dofetilide was dissolved in saline to provide a stock solution of 5.0 μmol/L. BaCl$_2$ was dissolved in distilled water to provide a stock solution of 100.0 mmol/L. Each stock solution was diluted immediately before use.

Statistical analysis

The incidence of TdP (%) was compared using a χ^2 test with Yates' correction for continuity. All other data are the mean ± SD. Data within groups were compared after repeated-measures one-way analysis of variance (ANOVA) using the Bonferroni post-hoc test, and we used Student's *t* test for pair-wise comparisons of the groups. Values for $p < 0.05$ were considered to be statistically significant.

Results

Effect of combined inhibition of I_{K1} and I_{Kr} on R-R and QTc intervals in conscious dogs and anesthetized rabbits

For both dogs and rabbits, administration of the I_{K1} inhibitor BaCl$_2$ alone did not change heart rate or the R–R intervals (Fig. 2A). However, infusion of the I_{Kr} inhibitor dofetilide significantly in-

creased R–R intervals (Fig. 2A) and decreased heart rate (dogs: 66.9 ± 16.96 beats/min vs. 80.5 ± 12.12 beats/min in the controls; rabbits: 213.9 ± 1.92 beats/min vs. 258.6 ± 1.26 beats/min in the controls; $p < 0.05$). In a previous study by our group, the I_{Kr} inhibitor dofetilide administered alone did not alter the R–R interval but, as expected, significantly prolonged the QTc interval in conscious dogs and anesthetized rabbits (Lengyel et al. 2007).

In conscious dogs, inhibition of I_{K1} significantly prolonged the QTc interval, as calculated with the Van de Water formula (Van de Water et al. 1989), and dofetilide infusion caused a further, significant prolongation of the QTc interval (Fig. 3A, left panel). The uncorrected QT intervals yielded similar prolongation: 317.4 ± 47.9 ms after I_{K1} blockade and 373.5 ± 37.8 ms after combined inhibition of $I_{K1}+I_{Kr}$, vs. 221.4 ± 11.7 ms in the controls ($p < 0.05$). In anesthetized rabbits, only the combined inhibition of $I_{K1}+I_{Kr}$ resulted in significant QTc interval prolongation (Fig. 3A, right panel). Again, the uncorrected QT intervals showed a similar prolongation, but only after combined inhibition of $I_{K1}+I_{Kr}$ (150.6 ± 5.88 ms after inhibition of I_{K1}, and 167.8 ± 6.27 ms after combination treatment with BaCl$_2$+dofetilide vs. 147.2 ± 5.46 ms in the controls).

Effect of combined inhibition of I_{K1} and I_{Kr} on the short-term variability of R–R (STV$_{R–R}$) and QT intervals (STV$_{QT}$) in conscious dogs and anesthetized rabbits

Because heart rate affects the duration of repolarization, the short-term variability in the R–R intervals was also calculated in addition to STV$_{QT}$, which is the ECG parameter recently suggested for more reliable prediction of ventricular arrhythmias. In conscious dogs, STV$_{R–R}$ did not change significantly following I_{K1} and combined inhibition of $I_{K1}+I_{Kr}$ (Fig. 2B, left panel). In anesthetized rabbits, the combination of BaCl$_2$+dofetilide slightly, but signifi-

Fig. 2. Effects from the inhibition of I_{K1} (i.v. $BaCl_2$) and combined inhibition of $I_{K1}+I_{Kr}$ (i.v. $BaCl_2$+dofetilide) on (A) R–R interval, and (B) short-term variability of the R–R interval (STV_{R-R}) in conscious dogs and anesthetized rabbits; n = 7 dogs or 7 rabbits/group; *, $p < 0.05$ compared with the control values; #, $p < 0.05$ compared with inhibition of I_{K1}.
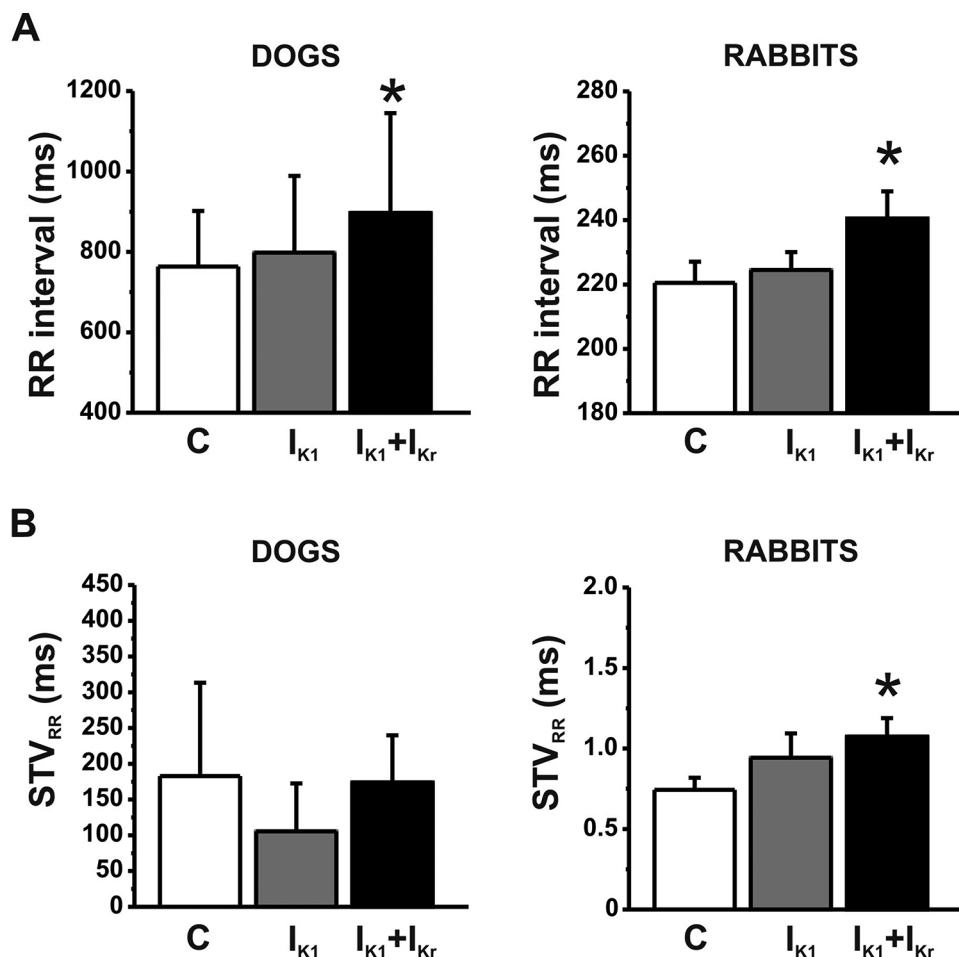
cantly, increased STV_{R-R}; however, the magnitude of these changes (< 0.5 ms) makes it very unlikely that this STV_{R-R} change markedly influenced repolarization variability (Fig. 2B, right panel). The I_{Kr} inhibitor dofetilide administered alone did not alter STV_{R-R} in conscious dogs and anesthetized rabbits in our previous study (Lengyel et al. 2007).

The Poincaré plots in Fig. 3B illustrate the temporal instability of repolarization in 2 individual animals: a conscious dog (left panel), and an anesthetized rabbit (right panel), following inhibition of I_{K1} and combined inhibition of $I_{K1}+I_{Kr}$. Both animals exhibited TdP arrhythmia as a result of the combined treatment with $BaCl_2$+dofetilide, and the scatter of data points covering a large area on the plot following this combination treatment represents large STV_{QT} values in these animals (15.8 ms in the dog and 4.9 ms in the rabbit). Grouped STV_{QT} data showed a significant increase in both species following combined inhibition of $I_{K1}+I_{Kr}$ (Fig. 3C). Dofetilide alone did not increase STV_{QT} in conscious dogs; however, in our previous study, it significantly increased STV_{QT} in anesthetized rabbits (Lengyel et al. 2007).

Effect from combined inhibition of $I_{Ks}+I_{K1}$ on R–R and QTc intervals in conscious dogs and anesthetized rabbits

The I_{Ks} inhibitor HMR 1556 did not change the R–R interval significantly in conscious dogs or anesthetized rabbits, and this result was in agreement with our previous observations (Lengyel et al. 2007). However, the combined inhibition of $I_{Ks}+I_{K1}$ caused a

significant increase in R–R intervals and a decrease in heart rate in rabbits only (Fig. 4A, right panel).

In conscious dogs, inhibition of I_{Ks} significantly increased the QTc interval, similar to our previous observations in conscious dogs (Lengyel et al. 2007). In these animals, subsequent infusion of $BaCl_2$ caused a further, significant QTc prolongation (Fig. 5A, left panel). The uncorrected QT intervals showed similar prolongation: 317.5 ± 35.2 ms after I_{Ks} blockade and 387.4 ± 45.4 ms after combined inhibition of $I_{Ks}+I_{K1}$, vs. 230.5 ± 7.6 ms in the controls ($p < 0.05$). In anesthetized rabbits, inhibition of I_{Ks} did not alter QTc, and combined inhibition of $I_{Ks}+I_{K1}$ only had a slight tendency to increase QTc, but this change did not prove to be statistically significant (Fig. 5A, right panel).

Effect from combined inhibition of I_{Ks} and I_{K1} on the short-term variability in R–R (STV_{R-R}) and QT (STV_{QT}) intervals in conscious dogs and anesthetized rabbits

HMR 1556 did not alter STV_{R-R} in conscious dogs or anesthetized rabbits. The combined inhibition of $I_{Ks}+I_{K1}$ again caused a significant, but very small (<1 ms) increase in STV_{R-R} in anesthetized rabbits (Fig. 4B, right panel).

The Poincaré plots in Fig. 5B illustrate repolarization temporal instability in 2 individual animals, a conscious dog (left panel) and an anesthetized rabbit (right panel), following inhibition of I_{Ks} and combined inhibition of $I_{Ks}+I_{K1}$. The shift in QT interval data, pointing to the right and upward direction in the conscious dog,

Fig. 3. Effect from the inhibition of I_{K1} (i.v. BaCl$_2$) and combined inhibition of $I_{K1}+I_{Kr}$ (i.v. BaCl$_2$+dofetilide) inhibition on (A) frequency-corrected QT interval (QTc) and (B and C) short-term variability of the QT interval (STV$_{QT}$) in conscious dogs and anesthetized rabbits. For details on the Poincaré plot (B) see text; $n = 7$ dogs or 7 rabbits/group; *, $p < 0.05$ compared with the control values; #, $p < 0.05$ compared with inhibition of I_{K1}.
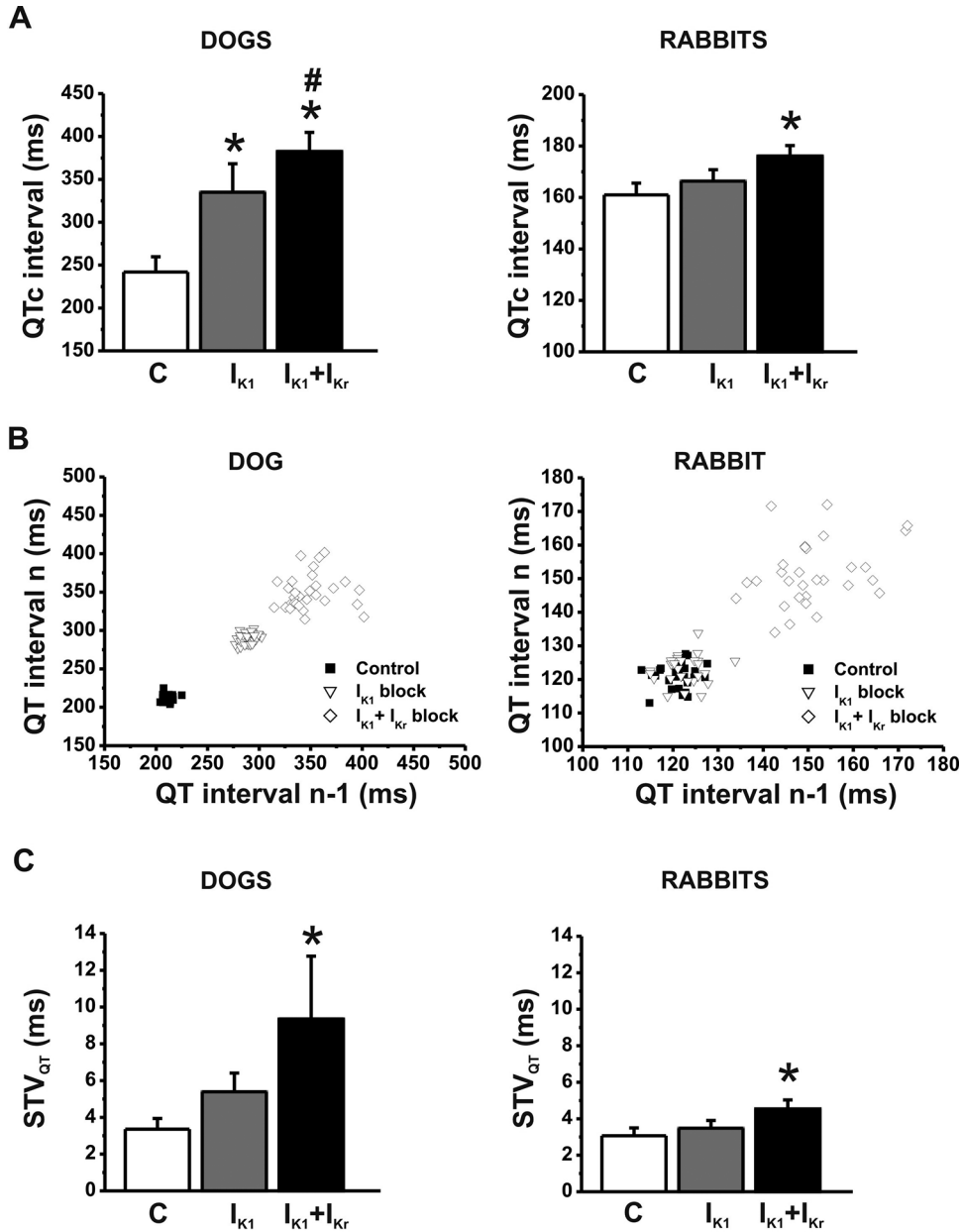
represent the previously described QT prolongation following HMR 1556 administration. Careful observation of the plot reveals that in this animal (that later developed TdP after combined inhibition of $I_{Ks}+I_{K1}$), the QT variability only increased after combination treatment with HMR 1556+BaCl$_2$ (Fig. 5B, left panel). In accordance with the grouped STV$_{QT}$ data represented in Fig. 5C (right panel) for the representative rabbit in Fig. 5B, STV$_{QT}$ did not increase significantly after treatment with HMR 1556 or combination treatment with HMR 1556+BaCl$_2$ in anesthetized rabbits. In contrast, in conscious dogs this combination treatment led to a significant increase in STV$_{QT}$ (Fig. 2E, left panel). Importantly, in parallel to this STV$_{QT}$ increase, conscious dogs responded to combined inhibition of $I_{Ks}+I_{K1}$ with significantly increased TdP incidence, whereas only 1 of 7 rabbits developed TdP following this combined inhibition, and the grouped data for the rabbits did not show elevated STV$_{QT}$ (Fig. 5C).

Effect from combined inhibition of $I_{K1}+I_{Kr}$, and $I_{Ks}+I_{K1}$ on the incidence of TdP in conscious dogs and anesthetized rabbits

As shown in Figs. 6A and 6B, inhibition of I_{K1} or I_{Ks} alone did not provoke TdP in any of the animals. We have previously shown that the I_{Kr} inhibitor dofetilide alone did not cause TdP in conscious dogs but did cause TdP in 25% of the anesthetized rabbits (Lengyel et al. 2007). Combined inhibition of repolarizing currents, however, led to a significant number of TdP episodes in both species, albeit in a different manner.

Interestingly, conscious dogs and anesthetized rabbits exhibited different TdP incidence following the combined inhibition of key potassium currents: a significant number of conscious dogs developed TdP following combined inhibition of $I_{Ks}+I_{K1}$, whereas only one rabbit developed TdP. On the other hand, TdP incidence increased significantly following combined inhibition of $I_{K1}+I_{Kr}$ in

Fig. 4. Effect from the inhibition of I_{Ks} (i.v. HMR 1556) and combined inhibition of I_{Ks}+I_{K1} (i.v. HMR 1556+BaCl$_2$) inhibition on (A) R–R interval and (B) short-term variability of the R–R interval (STV$_{R-R}$) in conscious dogs and anesthetized rabbits; $n = 6$ dogs or 7 rabbits/group; *, $p < 0.05$ compared with the control values; #, $p < 0.05$ compared with inhibition of I_{Ks}.
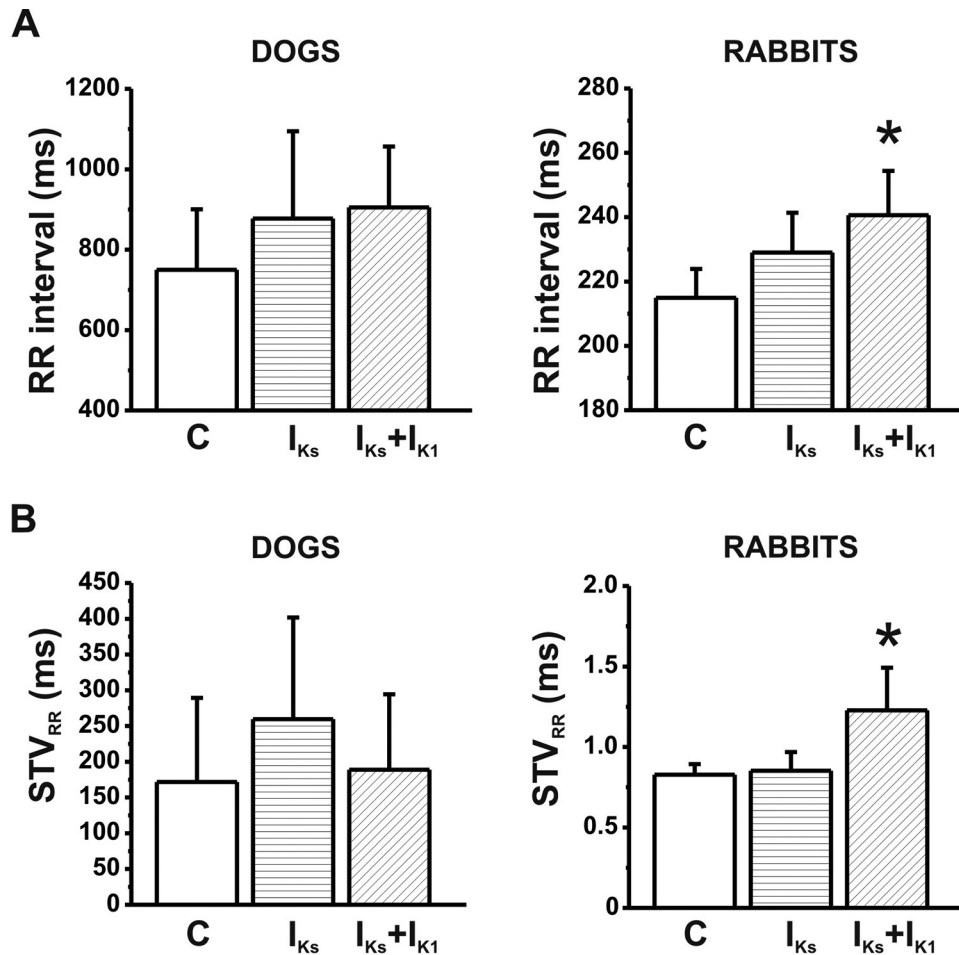
rabbits, whereas only one dog exhibited TdP following combination treatment with BaCl$_2$+dofetilide (Figs. 6A and 6B).

Discussion

Dog and rabbit are animals frequently used for studying proarrhythmia, and we have previously established a model of proarrhythmia based on repolarization reserve impairment achieved by pharmacological inhibition of I_{Ks}, a key potassium current in repolarization reserve (Lengyel et al. 2007). Recently, other potassium channels were also implicated in repolarization reserve, including I_{K1} (for a recent review see Varró and Baczkó 2011). Species differences exist in the expression of important ion channels carrying repolarizing current (Zicha et al. 2003), most likely leading to species-specific differences in repolarization reserve and in responses to compounds with even mild potassium channel inhibitory properties (Nerbonne and Kass 2005). Therefore, further characterization of the roles different potassium currents play in canine and rabbit pro-arrhythmia models are needed to assist in the proper extrapolation of data obtained in these models to human subjects.

In this study, we investigated the effects of repolarization reserve impairment by pharmacological inhibition of I_{K1} in combination with I_{Ks} and I_{Kr} on the incidence of the typical drug-induced arrhythmia, TdP, and different ECG parameters in an experimental setup similar to that used in our previous study in which the effects of the combined pharmacological inhibition of I_{Ks} and I_{Kr} were characterized in conscious dogs and anesthetized

rabbits (Lengyel et al. 2007). We have shown that combined pharmacological inhibition of other potassium currents leads to repolarization reserve impairment and a high incidence of TdP in conscious dogs and anesthetized rabbits. However, dogs and rabbits exhibited markedly different patterns of TdP development in response to combined inhibition of I_{K1}+I_{Kr} and I_{Ks}+I_{K1}, suggesting that at least some of these currents may play relatively different roles in repolarization reserve in these animals. In contrast, we have previously shown that both animals responded with a high incidence of TdP, paralleled by significant increases in short-term variability of the QT interval, irrespective of the sequence in which the inhibitors of I_{Ks} and I_{Kr} were administered (Lengyel et al. 2007).

The key role of I_{Ks} in ventricular repolarization reserve is well-established in different animals/species and in humans (Volders et al. 1999, 2003; Varró et al. 2000; Lengyel et al. 2001, 2007; Jost et al. 2005, 2007; Abi-Gerges et al. 2006; Johnson et al. 2010). However, a recent study highlighted that because of lower I_{Ks} densities in human hearts, repolarization reserve may be reduced in humans by comparison with dogs (Jost et al. 2013a). Attributed to low-level I_{Ks} beta-subunit minK expression, a low I_{Ks} current density and increased TdP susceptibility were described in rabbits (Lu et al. 2001; Zicha et al. 2003). In a recent comparative study, the kinetics of rabbit I_{Ks} were found to be more human-like compared with I_{Ks} in guinea pigs and dogs (Jost et al. 2013b). These data indicate that despite the relatively well-characterized role of I_{Ks} in repolarization reserve, the significant differences described in I_{Ks}

Fig. 5. Effect from inhibition of I_{Ks} (i.v. HMR 1556) and combined inhibition of $I_{Ks}+I_{K1}$ (i.v. HMR 1556+BaCl$_2$) on (A) frequency-corrected QT interval (QTc) and (B and C) short-term variability of the QT interval (STV$_{QT}$) in conscious dogs and anesthetized rabbits. For details on the Poincaré plot (B) see text; n = 6 dogs or 7 rabbits/group; *, $p < 0.05$ compared with the control values; #, $p < 0.05$ compared with the inhibition of I_{Ks}.
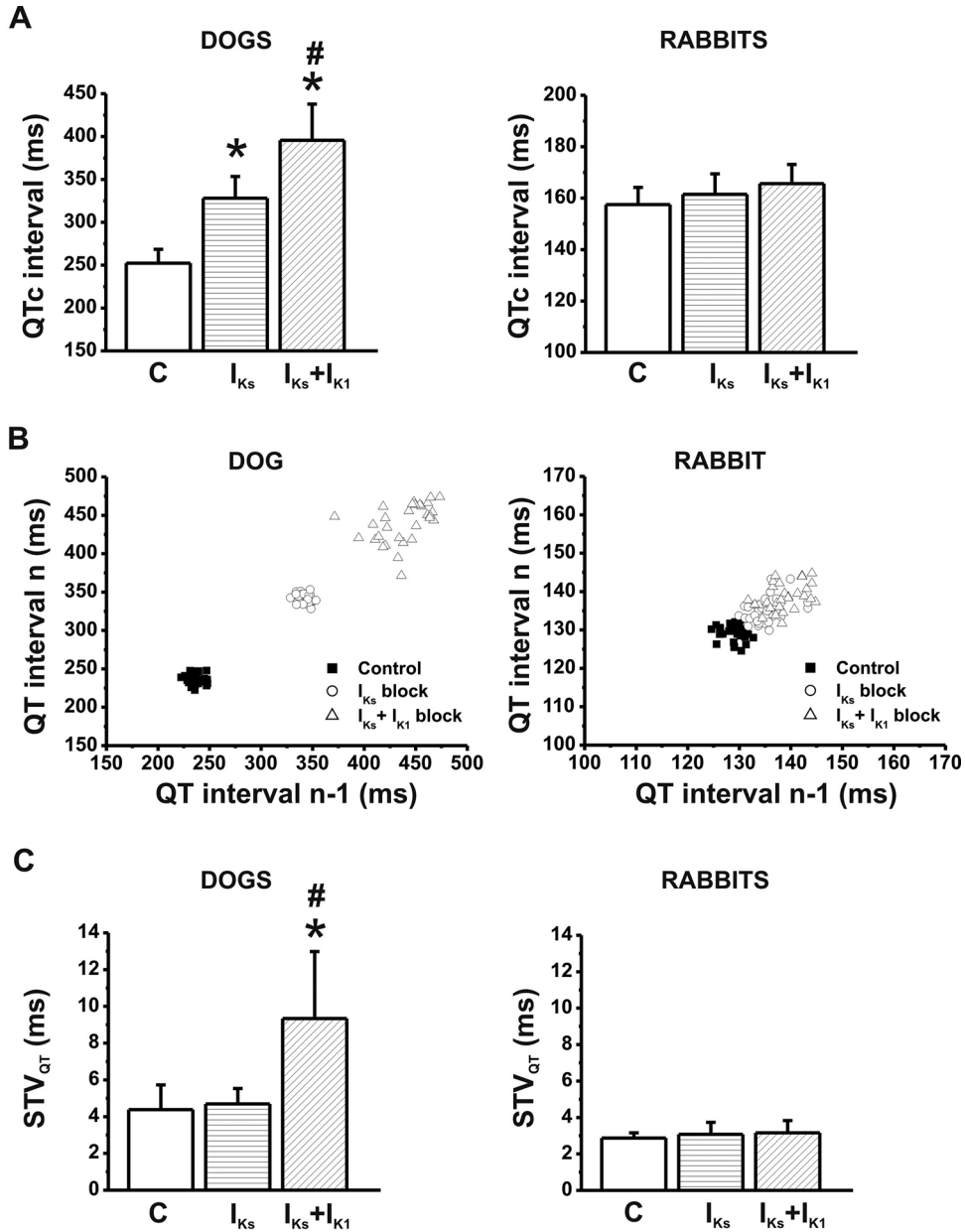

subunit expression, current densities, and kinetics in rabbits and dogs make extrapolation of the results to humans somewhat difficult.

The roles other repolarizing potassium currents may play in repolarization reserve are poorly understood. Among these repolarizing potassium currents, possible roles for I_{to} (Virág et al. 2011) and I_{K1} (Biliczki et al. 2002; Varró and Baczkó 2011) in repolarization reserve have been suggested. In LQT7 (Andersen–Tawil syndrome), loss of function mutations in Kir2.1 channels may significantly decrease I_{K1} current; however, the substantially increased pro-arrhythmic risk in these patients is not accompanied by marked QTc prolongation on the ECG (Zhang et al. 2005). Computer modelling studies also support a key role for I_{K1} in ventricles: in these models, reduction of I_{K1} in addition to I_{Kr} blockade led to early after-depolarizations (Ishihara et al. 2009). A variety of channel subtypes can be responsible for the I_{K1} current, including

alpha-subunits Kir2.1, Kir2.2, Kir2.3, and Kir2.4 (for a recent review see Anumonwo and Lopatin 2010). Significant species-specific differences in the expression of Kir2.x proteins have been previously reported (Wang et al. 1998; Melnyk et al. 2002; Dhamoon and Jalife 2005). Different heteromeric assembly of Kir2.x proteins leads to different I_{K1} characteristics (Dhamoon et al. 2004) and, most likely, to altered overall cardiac electrophysiological responses to drugs. A heteromeric assembly of Kir2.1 and Kir2.2 was reported in rabbit cardiomyocytes (Zobel et al. 2003). In dogs, Kir2.2 and Kir2.4 levels were minimal, whereas in humans, Kir2.3 mRNA expression was on a similar level to Kir2.1, and Kir2.1 mRNA expression was 3 times higher in dogs compared with humans (Jost et al. 2013a).

Based on the abovementioned studies, and also supported by the results from this study, because of stronger I_{K1} and I_{Ks} in dogs by comparison with rabbits and humans, dogs may exhibit larger

Fig. 6. Effect of (A) inhibition of I_{K1} (i.v. BaCl$_2$) and combined inhibition of $I_{K1}+I_{Kr}$ (i.v. BaCl$_2$+dofetilide) and (B) inhibition of I_{Ks} (i.v. HMR 1556) and combined inhibition of $I_{Ks}+I_{K1}$ (i.v. HMR 1556+BaCl$_2$) inhibition on incidence of torsades de pointes (TdP) chaotic ventricular arrhythmia in conscious dogs (left panels) and anesthetized rabbits (right panels); n = dogs/group: 7 (A), 6 (B); rabbits/group: 7 (A and B); *, $p < 0.05$ compared with the control values.
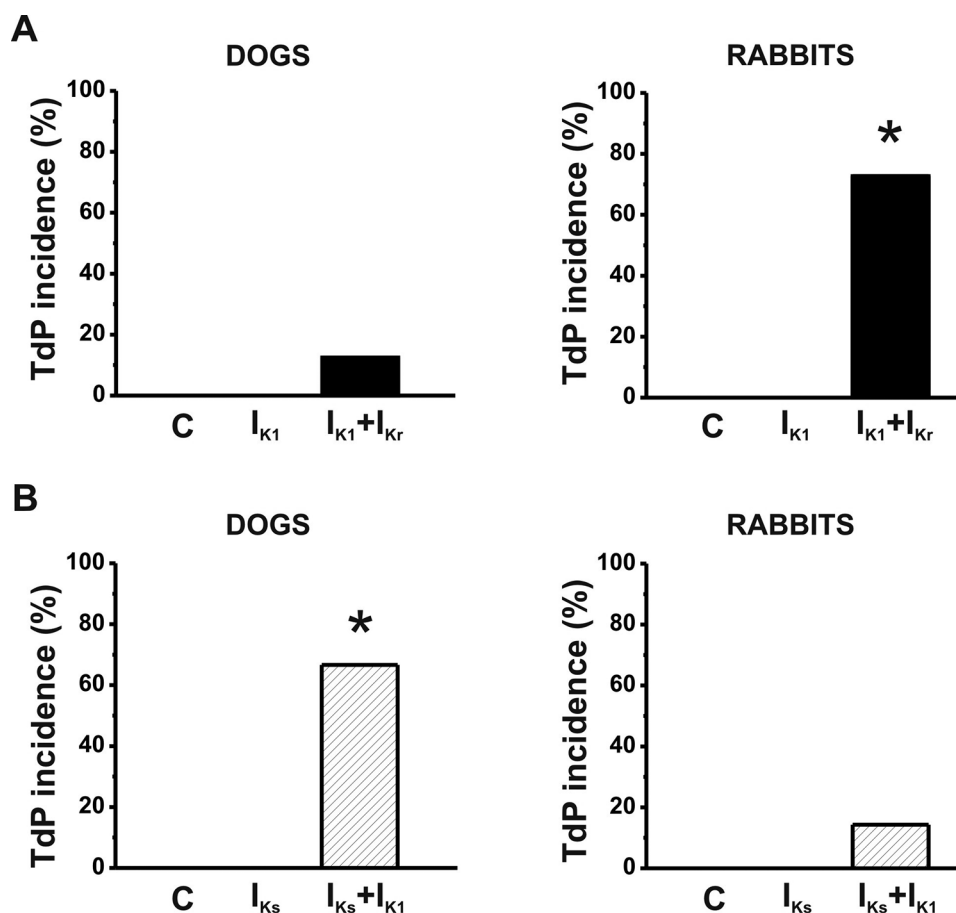
repolarization reserve. Therefore, rabbit pro-arrhythmia models based on pharmacologically impaired repolarization reserve may represent greater arrhythmia susceptibility and may be more useful than canine models in predicting human electrophysiological responses to drugs affecting cardiac ventricular repolarization.

In this study, the short-term variability in the QT interval was calculated to assess the temporal instability of repolarization following pharmacological inhibition of repolarizing potassium currents. The STV$_{QT}$ characterizes differences in the QT intervals of consecutive heart beats, and has been suggested as a more reliable measure of pro-arrhythmic risk associated with impaired repolarization reserve compared with conventional ECG parameters of repolarization (Berger 2003; Varkevisser et al. 2012). In this regard, a number of previous in vivo and in vitro experimental and clinical animal studies (van Opstal et al. 2001; Thomsen et al. 2004; Lengyel et al. 2007; Hinterseer et al. 2009, 2010) found that STV$_{QT}$ was increased and showed a better correlation with subsequent arrhythmias than QT prolongation in animals or individuals with decreased repolarization reserve later exhibiting serious ventricular arrhythmias. In this study we found that combined inhibition of $I_{K1}+I_{Kr}$ and $I_{Ks}+I_{K1}$ led to increased STV$_{QT}$ in parallel with increased incidence of TdP in most cases, further supporting the role of STV$_{QT}$ determination in ventricular arrhythmia risk estimation. It should be noted that we found a significant increase in STV$_{QT}$ in the dogs after combined inhibition of $I_{K1}+I_{Kr}$ inhibition, with only one animal showing TdP; however, very short episodes of nonsustained monomorphic ventricular tachycardia were observed in 3 additional animals.

In conclusion, both rabbits and dogs are susceptible to pharmacological impairment of repolarization reserve and concomitant ventricular arrhythmia induction by compounds that inhibit repolarizing currents. This study has further confirmed that STV$_{QT}$ may be a better predictor of subsequent drug-induced TdP development than conventional ECG parameters characterizing repolarization prolongation. Importantly, however, rabbits are more susceptible to combined inhibition of $I_{K1}+I_{Kr}$ than dogs, and dogs are more susceptible to combined inhibition of $I_{K1}+I_{Ks}$ than rabbits, suggesting different relative roles for I_{K1} and I_{Ks} in repolarization reserve for these animals. These results warrant cautious evaluation of the potentially adverse pro-arrhythmic effects and cardiovascular safety of candidate compounds in rabbit and dog models. The different relative roles of repolarizing potassium currents in these species need to be considered when extrapolating rabbit and canine pro-arrhythmia study results to humans.

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References

Abi-Gerges, N., Small, B.G., Lawrence, C.L., Hammond, T.G., Valentin, J.P., and Pollard, C.E. 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}, β-adrenoceptor stimulation, pacing rate and gender. Br. J. Pharmacol. **147**: 653–660. doi:10.1038/sj.bjp.0706491. PMID:16314855.

Anumonwo, J.M., and Lopatin, A.N. 2010. Cardiac strong inward rectifier potassium channels. J. Mol. Cell. Cardiol. **48**: 45–54. doi:10.1016/j.yjmcc.2009.08.013. PMID:19703462.

Batey, A.J., and Coker, S.J. 2002. Proarrhythmic potential of halofantrine, terfenadine and clofilium in a modified in vivo model of torsade de pointes. Br. J. Pharmacol. **135**: 1003–1012. doi:10.1038/sj.bjp.0704550. PMID:11861329.

Berger, R.D. 2003. QT variability. J. Electrocardiol. **36**(Suppl.): 83–87. doi:10.1016/j.jelectrocard.2003.09.019. PMID:14716597.

Biliczki, P., Virág, L., Iost, N., Papp, J.Gy., and Varró, A. 2002. Interaction of different potassium channels in cardiac repolarization in dog ventricular preparations: role of the repolarization reserve. Br. J. Pharmacol. **137**: 361–368. doi:10.1038/sj.bjp.0704881. PMID:12237256.

Brennan, M., Palaniswami, M., and Kamen, P. 2001. Do existing measures of Poincaré plot geometry reflect nonlinear features of heart rate variability? IEEE Trans. Biomed. Eng. **48**: 1342–1347. doi:10.1109/10.959330. PMID:11686633.

Dhamoon, A.S., and Jalife, J. 2005. The inward rectifier current (I_{K1}) controls cardiac excitability and is involved in arrhythmogenesis. Heart Rhythm, **2**: 316–324. doi:10.1016/j.hrthm.2004.11.012. PMID:15851327.

Dhamoon, A.S., Pandit, S.V., Sarmast, F., Parisian, K.R., Guha, P., Li, Y., et al. 2004. Unique Kir2.x properties determine regional and species differences in the cardiac inward rectifier K⁺ current. Circ. Res. **94**: 1332–1339. doi:10.1161/01.RES.0000128408.66946.67. PMID:15087421.

El-Sherif, N. 1992. The proarrhythmic mechanism of drugs that prolong repolarisation. Role of early afterdepolarisation. New Trends in Arrhythmias, **8**: 617–626.

Farkas, A.S., and Nattel, S. 2010. Minimizing repolarization-related proarrhythmic risk in drug development and clinical practice. Drugs, **70**: 573–603. doi:10.2165/11535230-000000000-00000. PMID:20329805.

Farkas, A., Batey, A.J., and Coker, S.J. 2004. How to measure electrocardiographic QT interval in the anaesthetized rabbit. J. Pharmacol. Toxicol. Methods, **50**: 175–185. doi:10.1016/j.vascn.2004.05.002. PMID:15519904.

Fenichel, R.R., Malik, M., Antzelevitch, C., Sanguinetti, M., Roden, D.M., Priori, S.G., et al; Independent Academic Task Force. 2004. Drug-induced torsades de pointes and implications for drug development. J. Cardiovasc. Electrophysiol. **15**: 475–495. doi:10.1046/j.1540-8167.2004.03534.x. PMID:15090000.

Food and Drug Administration, HHS. 2005a. 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 Reg. **70**: 61133–61134. PMID:16237859.

Food and Drug Administration, HHS. 2005b. 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. PMID:16237860.

Haverkamp, W., Breithardt, G., Camm, A.J., Janse, M.J., Rosen, M.R., Antzelevitch, C., et al. 2000. The potential for QT prolongation and pro-arrhythmia by non-anti-arrhythmic drugs: clinical and regulatory implications. Report on a Policy Conference of the European Society of Cardiology. Cardiovasc. Res. **47**: 219–233. doi:10.1016/S0008-6363(00)00119-X. PMID:10947683.

Hinterseer, M., Beckmann, B.M., Thomsen, M.B., Pfeufer, A., Dalla Pozza, R., Loeff, M., et al. 2009. Relation of increased short-term variability of QT interval to congenital long-QT syndrome. Am. J. Cardiol. **103**: 1244–1248. doi:10.1016/j.amjcard.2009.01.011. PMID:19406266.

Hinterseer, M., Beckmann, B.M., Thomsen, M.B., Pfeufer, A., Ulbrich, M., Sinner, M.F., 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. doi:10.1016/j.amjcard.2010.02.033. PMID:20599006.

Hondeghem, L.M., and Snyders, D.J. 1990. Class III antiarrhythmic agents have a lot of potential but a long way to go. Reduced effectiveness and dangers of reverse use dependence. Circulation, **81**: 686–690. doi:10.1161/01.CIR.81.2.686. PMID:2153477.

ILAR (Institute of Laboratory Animal Resources). 1996. Guide for the Care and Use of Laboratory Animals. NIH Publication No. 85-23 (revised 1996). National Academy Press, Washington, D.C. [Available from www.nap.edu/openbook.php?record_id=5140].

Ishihara, K., Sarai, N., Asakura, K., Noma, A., and Matsuoka, S. 2009. Role of Mg²⁺ block of the inward rectifier K⁺ current in cardiac repolarization reserve: A quantitative simulation. J. Mol. Cell. Cardiol. **47**: 76–84. doi:10.1016/j.yjmcc.2009.03.008. PMID:19303883.

Johnson, D.M., Heijman, J., Pollard, C.E., Valentin, J.P., Crijns, H.J., Abi-Gerges, N., 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. doi:10.1016/j.yjmcc.2009.08.033. PMID:19744496.

Jost, N., Virág, L., Bitay, M., Takács, J., Lengyel, C., Biliczki, P., et al. 2005. Restricting excessive cardiac action potential and QT prolongation: a vital role for I_{Ks} in human ventricular muscle. Circulation, **112**: 1392–1399. doi:10.1161/CIRCULATIONAHA.105.550111. PMID:16129791.

Jost, N., Papp, J., and Varró, G.A. 2007. Slow delayed rectifier potassium current (I_{Ks}) and the repolarization reserve. Ann. Noninvasive Electrocardiol. **12**: 64–78. doi:10.1111/j.1542-474X.2007.00140.x. PMID:17286653.

Jost, N., Virág, L., Comtois, P., Ördög, Szűts, B., Seprényi, V.G., et al. 2013a. Ionic mechanisms limiting cardiac repolarization reserve in humans compared to dogs. J. Physiol. **591**: 4189–4206. doi:10.1113/jphysiol.2013.261198. PMID:23878377.

Jost, N., Kohajda, Z.S., Corici, C., Horvath, A., Bitay, M., Bogats, G., et al. 2013b. 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. doi:10.1093/europace/eut170.

Lengyel, C., Iost, N., Virág, L., Varró, A., Lathrop, A.D., and Papp, J.G. 2001. Pharmacological block of the slow component of the outward delayed rectifier current (I_{Ks}) fails to lengthen rabbit ventricular muscle QTc and action potential duration. Br. J. Pharmacol. **132**: 101–110. doi:10.1038/sj.bjp.0703777. PMID:11156566.

Lengyel, C., Varró, A., Tábori, K., Papp, J.G., and Baczkó, I. 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. doi:10.1038/sj.bjp.0707297. PMID:17533421.

Lu, Z., Kamiya, K., Opthof, T., Yasui, K., and Kodama, I. 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. doi:10.1161/hc3401.093151. PMID:11514385.

Melnyk, P., Zhang, L., Shrier, A., and Nattel, S. 2002. Differential distribution of Kir2.1 and Kir2.3 subunits in canine atrium and ventricle. Am. J. Physiol. Heart Circ. Physiol. **283**: H1123–H1133. doi:10.1152/ajpheart.00934.2001. PMID:12181143.

Nerbonne, M., and Kass, R.S. 2005. Molecular physiology of cardiac repolarization. Physiol. Rev. **85**: 1205–1253. doi:10.1152/physrev.00002.2005. PMID:16183911.

Roden, D.M. 1998. Taking the idio out of idiosyncratic — predicting torsades de pointes. Pacing Clin. Electrophysiol. **21**: 1029–1034. doi:10.1111/j.1540-8159.1998.tb00148.x. PMID:9604234.

Singh, B.N., and Vaughan Williams, E.M. 1970. A third class of anti-arrhythmic action. Effects on atrial and ventricular intracellular potentials, and other pharmacological actions on cardiac muscle, of MJ 1999 and AH 3474. Br. J. Pharmacol. **39**: 675–687. doi:10.1111/j.1476-5381.1970.tb09893.x. PMID:5485144.

Tattersall, M.L., Dymond, M., Hammond, T., and Valentin, J.P. 2006. Correction of QT values to allow for increases in heart rate in conscious Beagle dogs in toxicology assessment. J. Pharmacol. Toxicol. Methods, **53**: 11–19. doi:10.1016/j.vascn.2005.02.005. PMID:15886026.

Thomsen, M.B., Verduyn, S.C., Stengl, M., Beekman, J.D., de Pater, G, van Opstal, J., et al. 2004. Increased short-term variability of repolarization predicts D-sotalol-induced torsades de pointes in dogs. Circulation, **110**: 2453–2459. doi:10.1161/01.CIR.0000145162.64183.C8. PMID:15477402.

Thomsen, M.B., Matz, J., Volders, P.G., and Vos, M.A. 2006. Assessing the proarrhythmic potential of drugs: current status of models and surrogate parameters of torsades de pointes arrhythmias. Pharmacol. Ther. **112**: 150–170. doi:10.1016/j.pharmthera.2005.04.009. PMID:16714061.

Van de Water, A., Verheyen, J., Xhonneux, R., and Reneman, R.S. 1989. An improved method to correct the QT interval of the electrocardiogram for changes in heart rate. J. Pharmacol. Methods, **22**: 207–217. doi:10.1016/0160-5402(89)90015-6. PMID:2586115.

van Opstal, J.M., Schoenmakers, M., Verduyn, S.C., de Groot, S.H., Leunissen, J.D., van Der Hulst, F.F., 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. doi:10.1161/hc4701.099579. PMID:11723026.

Varkevisser, R., Wijers, S.C., van der Heyden, M.A., Beekman, J.D., Meine, M., and Vos, M.A. 2012. Beat-to-beat variability of repolarization as a new biomarker for proarrhythmia in vivo. Heart Rhythm, **9**: 1718–1726. doi:10.1016/j.hrthm.2012.05.016. PMID:22609158.

Varró, A., and Baczkó, I. 2011. Cardiac ventricular repolarization reserve: a principle for understanding drug-related proarrhythmic risk. Br. J. Pharmacol. **164**: 14–36. doi:10.1111/j.1476-5381.2011.01367.x. PMID:21545574.

Varró, A., Baláti, B., Iost, N., Takács, J., Virág, L., Lathrop, D.A., et al. 2000. The role of the delayed rectifier component I_{Ks} in dog ventricular muscle and Purkinje fibre repolarization. J. Physiol. **523**: 67–81. doi:10.1111/j.1469-7793.2000.00067.x. PMID:10675203.

Virág, L., Jost, N., Papp, R., Koncz, I., Kristóf, A., Kohajda, Z., et al. 2011. Analysis of the contribution of I_{to} to repolarization in canine ventricular myocardium. Br. J. Pharmacol. **164**: 93–105. doi:10.1111/j.1476-5381.2011.01331.x. PMID:21410683.

Volders, P.G., Sipido, K.R., Vos, M.A., Spatjens, R.L., Leunissen, J.D., Carmeliet, E., 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. doi:10.1161/01.CIR.100.24.2455. PMID:10595960.

Volders, P.G., Stengl, M., van Opstal, J.M., Gerlach, U., Spatjens, R.L., Beekman, J.D., et al. 2003. Probing the contribution of I_{Ks} to canine ventricular repolarization: key role for β-adrenergic receptor stimulation. Circulation, **107**: 2753–2760. doi:10.1161/01.CIR.0000068344.54010.B3. PMID:12756150.

Wang, Z., Yue, L., White, M., Pelletier, G., and Nattel, S. 1998. Differential distribution of inward rectifier potassium channel transcripts in human atrium versus ventricle. Circulation, **98**: 2422–2428. doi:10.1161/01.CIR.98.22.2422. PMID:9832487.

Zhang, L., Benson, D.W., Tristani-Firouzi, M., Ptacek, L.J., Tawil, R., Schwartz, P.J.,

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. doi:10.1161/CIRCULATIONAHA.104.472498. PMID:15911703.

Zicha, S., Moss, I., Allen, B., Varró, A., Papp, J.G., Dumaine, R., et al. 2003. Molecular basis of species-specific expression of repolarizing K$^+$ currents in the heart. Am. J. Physiol. Heart Circ. Physiol. **285**: H1641–H1649. doi:10.1152/ajpheart.00346.2003. PMID:12816752.

Zobel, C., Cho, H.C., Nguyen, T.T., Pekhletski, R., Diaz, R.J., Wilson, G.J., et al. 2003. Molecular dissection of the inward rectifier potassium current (I_{K1}) in rabbit cardiomyocytes: evidence for heteromeric co-assembly of Kir2.1 and Kir2.2. J. Physiol. **550**: 365–372. doi:10.1113/jphysiol.2002.036400. PMID:12794173.