Investigations on different aspects of cardiac ventricular repolarization: repolarization reserve and adaptation to heart rate

Summary of Ph.D. thesis

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Introduction

1. The proper assessment of the pro-arrhythmic potential of candidate compounds is a major concern for drug development, since drug-induced arrhythmias, including Torsades de Pointes (TdP), can lead to sudden cardiac death. The prediction of TdP in clinical setting is very difficult since the incidence of drug-induced TdP is very low (1:100 000), however, drug associated sudden cardiac deaths have led to the withdrawal of a number, otherwise successful, compounds in the past. Importantly, current cardiac electrophysiological safety methods concentrate mostly on testing the hERG blocking and/or ventricular repolarization prolonging effects of candidate compounds and they use mostly healthy tissues and animals. It is, therefore, not entirely surprising that these cardiac safety tests are not sensitive enough. A large and still growing number of animal experimental and clinical studies suggest that the degree of repolarization prolongation does not show a close correlation with subsequent ventricular arrhythmia development. 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 repolarization prolongation, since other currents can compensate for the lost function. In the case of reduced repolarization reserve, additional inhibition of another repolarizing current can result in excessive prolongation of repolarization and can provoke serious ventricular arrhythmias. Evidence points to a critically important role for the slow component of the delayed rectifier potassium current (I_{Ks}) in ventricular repolarization reserve, however, other potassium currents may also significantly contribute to repolarization reserve. There is considerable variation in the

expression of key repolarizing potassium channels in different mammalian species, including dog and rabbit that are frequently used species in pro-arrhythmia models. Therefore, it is reasonable to assume that species specific ion channel expression profiles may result in species dependent alterations in responses to potassium channel blockers. Such differences may significantly influence the value of data obtained in these models for human extrapolation, however, it is unclear how species specific potassium channel expressions translate into differences in arrhythmia development in dogs and rabbits.

2. Disturbances in another important aspect of cardiac ventricular repolarization adaptation can also play a significant role in the development of serious cardiac arrhythmias and sudden cardiac death. Clinical, animal experimental and theoretical studies have shown that abrupt changes in heart rate result in a progressive adaptation of the QT interval measured on the ECG due to short-term cardiac memory effects. Patients exhibiting protracted QT interval heart rate adaptation dynamics have been identified to be at greater risk of developing cardiac arrhythmias and sudden cardiac death. Furthermore, clinical data also suggest that the extent of amiodarone-induced acceleration of QT interval heart rate adaptation could be used as a therapeutic marker of antiarrhythmic drug efficacy. However, despite strong evidence suggesting an important role of short-term cardiac memory in arrhythmogenesis, the underlying ionic mechanisms are still controversial.

Aims

1. It is not clear, how species specific potassium channel expressions translate into differences in arrhythmia development in dogs and rabbits, two species frequently used in pro-arrhythmia models. It has been shown previously that repolarization reserve impairment by inhibition of I_{Ks} increased arrhythmia susceptibility during subsequent I_{Kr} inhibition in dogs and rabbits in a similar degree. A possibly important role for I_{K1} has been suggested in repolarization reserve. In the first series of experiments we studied 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 suggested for more reliable prediction of drug-induced ventricular arrhythmias.

2. The aim of the second series of experiments was to investigate another important aspect of cardiac venricular repolarization adaptation: we performed studies on the ionic mechanisms of QT interval heart rate adaptation in ventricular tissue and their link to proarrhythmic mechanisms. Experiments were carried out to identify the specific mechanisms of ionic transport that determine QT interval rate adaptation and how alterations in those mechanisms might lead to arrhythmic events.

Materials and Methods

Ethical issues, experimental animals

All animal experiments were conducted in compliance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996), and the protocol was approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Hungary (I-74-125-2007 and I-74-5-2012) and by the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development (XIII/01031/000/2008 and XIII/1211/2012).

Beagle and mongrel dogs of either sex weighing 10-20 kg, and male New Zealand white rabbits (2-3 kg), anesthetized with thiopentone (50 mg/kg i.v.), were used for the experiments.

ECG studies in conscious dogs and anesthetized rabbits

Following a 20 min equilibration period, baseline recordings were obtained. In preliminary experiments, we determined the $BaCl_2$ doses used in our studies. The first group of dogs (n=7) were first administered the I_{K1} inhibitor $BaCl_2$ (3 mg/kg) followed by the I_{Kr} inhibitor dofetilide (25 μg/kg) i.v. after a 20 min equilibration period and in the second group (n=6) the animals received the I_{Ks} inhibitor HMR 1556 (1 mg/kg) first, followed by the I_{K1} inhibitor $BaCl_2$ (3 mg/kg) during a 5 min continuous i.v. infusion after a 20 min equilibration period. In rabbits, the first group (n=7) was administered the I_{K1} inhibitor $BaCl_2$ (0.3 mg/kg) followed by the I_{Kr} inhibitor dofetilide (25 μg/kg), 20 min after $BaCl_2$ administration. The second group (n=7) received the I_{Ks} inhibitor HMR 1556 (0.1 mg/kg) followed by the I_{K1} inhibitor $BaCl_2$ (0.3 mg/kg) i.v. 20 min after HMR 1556 administration. The obtained electrocardiogram was digitized and stored for later analysis using National Instruments data acquisition hardware SPEL Advanced Haemosys software. The PQ, RR, QT intervals were measured as the average of 30

consecutive beats (the minimum number of beats required for the calculation of beat-to-beat short-term variability of an interval) and in dogs the frequency corrected QT interval (QTc) was calculated using a formula recommended for Beagle dogs: QTc = QT – (0.087 * (RR-1000)), while in rabbits QTc was calculated by a formula specifically suggested for anaesthetized rabbits: QTc = QT – (0.704 * (RR-250)). Temporal instability of beat-to-beat heart rate and repolarization was characterized by the beat-to-beat short-term variability (STV) of RR or QT intervals, respectively. The calculation of STV is based on previous detailed mathematical analysis and was calculated as follows: $STV = \sum|D_{n+1}-D_n| \ (30x\sqrt{2})^{-1}$, where D is the duration of the QT or RR interval.

Action potential recordings using the conventional microelectrode technique

After intravenous euthanasia with thiopentone dog hearts were rapidly removed through right lateral thoracotomy and placed in oxygenated modified Locke's solution containing (in mM): NaCl 120, KCl 4, $CaCl_2$ 1.0, $MgCl_2$ 1, $NaHCO_3$ 22, and glucose 11. The pH of this solution was set between 7.35 and 7.4 when saturated with the mixture of 95% O_2 and 5% CO_2 at 37^{o}C. Right ventricular papillary muscles were paced at cycle length (CL) of 1000 ms until steady-state through bipolar platinum electrodes. Transmembrane potentials were recorded using conventional glass microelectrodes filled with 3 M KCL with a tip resistance of 5-20 MΩ. Recordings were made using a high impedance amplifier connected to a dual beam oscilloscope. Electrophysiological parameters were measured applying the Action Potential Evaluation Software (APES) and data for action potentials were evaluated using EvokeWave by measurement of the amplitude of the action potential (AMP), the maximum upstroke velocity (V_{max}), resting potential and the action potential duration at 10%, 25%, 50%, 75% and 90% repolarisation (APD_{10}, APD_{25}, APD_{50}, APD_{75} and APD_{90}). After control measurements, selective I_{K1} (30 μM $BaCl_2$), I_{Kr} (100 nM dofetilide),

and I_{Ks} (1μM HMR 1556) blocker drugs were added to the preparations alone and in combination to reduce repolarisation reserve. The rate adaptation of the action potential duration at 90% repolarisation (APD_{90}) was evaluated using the following protocol: pacing at cycle length (CL) of 1000 ms was applied until steady state, then CL was changed stepwise to 600 ms for 10 minutes (acceleration) and back to 1000 ms for an additional 10 min (deceleration). Two phases of APD adaptation dynamics were identified: APD_{90} heart rate adaptation following step CL changes consisted of two phases with time constants τ_{fast} and τ_{slow}, calculated from fitting the corresponding portion of APD time course with an exponential function: $f(t) = a + b\, e^{-(t-c)/t}$. Time constants τ_{fast} and τ_{slow} were obtained both after CL increase and decrease. APD HR adaptation was evaluated both in control and in the presence of $BaCl_2$, HMR 1556, dofetilide, $I_{Ca,L}$ (1μM nisoldipin) blocker, and I_{NaK} blocker (600 nM strophantin).

Statistical analysis

The incidence of TdP (%) was compared by using the χ^2 test with Yates' correction. All other data are expressed as means + SD. Data within groups were compared after analysis of variance (repeated measures one-way ANOVA) by Bonferroni's post test and the groups were compared in pairs by means of Student's "t" test. A level of $p<0.05$ was considered to be statistically significant.

Results and Discussion

Combined inhibition of I_{K1}+I_{Kr} and I_{K1}+I_{Ks} differently affects cardiac repolarization reserve and arrhythmia susceptibility in dogs and rabbits

In the first series of experiments we investigated the effects of repolarization reserve impairment by pharmacological block 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. Heart rates were significantly decreased by combined I_{K1}+I_{Kr} block in both species, while I_{K1}+I_{Ks} inhibition reduced heart rate only in rabbits. Inhibition of I_{Ks} alone as well as I_{K1} alone significantly prolonged the QTc interval in dogs but did not do so in rabbits. Increased QTc intervals by combined potassium channel inhibitions did not appear to be informative on subsequent TdP development in either species.

We found that combined pharmacological inhibition of I_{K1}+I_{Kr} and I_{K1}+I_{Ks} led to repolarization reserve impairment and high incidence of TdP in conscious dogs and anesthetized rabbits. However, dogs and rabbits exhibited markedly different patterns of TdP suggesting that at least some of these currents may play different relative roles in repolarization reserve in the two species. In contrast, our laboratory showed in previously published experiments that both species responded with a high incidence of TdP paralleled by significant increases of short-term variability of the QT interval (STV_{QT}) following I_{Ks}+I_{Kr} inhibitor administration. In this study, a high TdP incidence was observed following inhibition of I_{K1}+I_{Ks} in dogs (67% vs 14% in rabbits). Rabbits exhibited higher TdP incidence after I_{K1}+I_{Kr} block (72% vs 14% in dogs). Increased TdP incidence was associated with significantly larger STV_{QT} in both models.

The key role of I_{Ks} in ventricular repolarization reserve is well established in animals as well as in humans. However, some studies highlighted that due to lower I_{Ks} densities in human hearts,

repolarization reserve may be reduced in humans compared to dogs. A small I_{Ks} attributed to low-level I_{Ks} beta-subunit minK expression and increased TdP susceptibility were described in rabbits in the literature, indicating that despite the relatively well characterized role of I_{Ks} in repolarization reserve, the significant differences described in I_{Ks} expression and current densities in rabbits and dogs make human extrapolation of results difficult. A role for I_{K1}, another significant repolarizing current, in repolarization reserve has been suggested. Different channel subtypes can be responsible for the I_{K1} current (alpha-subunits Kir2.1, Kir2.2, Kir2.3, Kir2.4). Significant species-specific differences in the expression of Kir2.x proteins have been reported previously. In rabbit cardiomyocytes, a heteromeric assembly of Kir2.1 and Kir2.2 was reported, while in dogs, Kir2.2 and Kir2.4 levels were minimal, and in humans, Kir2.3 mRNA expression was on a similar level to Kir2.1, and Kir2.1 mRNA expression was three times higher in dogs compared to human. The literature and the present study suggests that due to stronger I_{K1} and I_{Ks} in dogs compared to rabbits and humans, dogs may exhibit larger repolarization reserve compared to the other two species. Therefore, rabbit pro-arrhythmia models based on pharmacologically impaired repolarization reserve may present greater arrhythmia susceptibility and may be more useful than canine models in predicting human electrophysiological responses to drugs affecting cardiac ventricular repolarization. These results also warrant cautious evaluation of the potential pro-arrhythmic adverse effects and cardiovascular safety of candidate compounds in rabbit and dog models.

Ionic mechanisms responsible for adaptation of repolarization following abrupt heart rate changes

Clinically, the slow adaptation of QT interval to abrupt changes in heart rate, also termed short term cardiac memory, has been proposed as an indicator of arrhythmic risk and sudden cardiac death. In the second set of experiments, we investigated the ionic basis of action potential duration rate adaptation (at 90%

repolarisation; APD_{90}), wich is the cellular manifestation of QT heart rate adaptation dynamics. These studies were part of a larger work utilizing synergistic combination of theoretical (computer modeling) and experimental methods to investigate the ionic basis of QT interval and APD heart rate adaptation and their link to proarrhythmic mechanisms. Our results show that the APD heart rate adaptation consists of two phases: in dogs a fast initial phase was observed with a time constant t_{fast}: 12.95±2.25/15.81±3.87 seconds and a second slow phase with a time constant t_{slow}: 176.10±43.41/226.07±51.78 seconds. We investigated the roles of different important currents in APD heart rate adaptation by application of selective pharmacological blockers during our rate adaptation protocol: 250 nM HMR 1556 for I_{Ks} block, 100 nM dofetilide for I_{Kr} block, 30 μM $BaCl_2$ for I_{K1} block, 1 μM nisoldipine for $I_{Ca,L}$ block, 600 nM strophantin for I_{NaK} block.

Our experiments show (confirmed by computer simulations) that $I_{Ca,L}$ and I_{Ks} currents determine the fast phase of heart rate adaptation while I_{NaK} dynamics are critical in the slow and final phase. The results also demonstrate that I_{NaK} inhibition, as it occurs in myocardial ischemia and heart failure patients, results in decreased APD adaptation, and might be a pro-arrhythmic risk factor. Large t_{slow} due to I_{NaK} inhibition is associated with an increase in action potential triangulation and may promote $I_{Ca,L}$ reactivation, resulting in an increased risk of delayed afterdepolarization formation. Different outcomes were observed in the case of I_{Ks} and $I_{Ca,L}$. Experiments demonstrate that reduced I_{Ks} is associated with delayed APD adaptation in the fast phase while $I_{Ca,L}$ block correlated with increased fast phase APD adaptation kinetics. It should be noted that experimental results following $I_{Ca,L}$ block were in conflict with computer modeling results, highlighting some existing imperfections in the human action potential computer model. Interestingly, I_{Kr} inhibition had no effect on APD heart rate adaptation kinetics and these results were in agreement with computer simulation studies.

These results provide new insights into the mechanisms of ventricular rate adaptation and its connection to proarrhythmic risk.

Appendix

Publications related to the subject of the thesis:

1. **Husti Z**, Tábori K, Juhász V, Hornyik T, Varró A, Baczkó I. Combined inhibition of key potassium currents differently affects cardiac repolarization reserve and arrhythmia susceptibility in dogs and rabbits. *Can J Physiol Pharmacol*, 2014, accepted for publication.
   IF (2013) = 1.546

2. Pueyo E, **Husti Z**, Hornyik T, Baczkó I, Laguna P., Varró A., Rodríguez B. Mechanisms of ventricular rate adaptation as a predictor of arrhythmic risk. *Am J Physiol – Heart Circ Physiol*, 2010, 298(5): H1577-1587.
   DOI: 10.1152/ajpheart.00936.2009
   IF (2010) = 3.88

Impact factor of publications related to the thesis: 5.426

List of other publications:

1. Baczkó I, Liknes D, Yang W, Hamming KC, Searle G, Jaeger K, **Husti Z**, Juhász V, Klausz G, Pap R, Sághy L, Varró A, Dolinsky V, Wang S, Hall D, Dyck JR, Light PE. Characterization of a novel multi-functional resveratrol derivative for the treatment of atrial fibrillation. *British Journal of Pharmacology*, 2014, 171(1): 92-106.
   DOI: 10.1111/bph.12409
   IF (2013) = 4.99

2. Kristóf A, **Husti Z**, Koncz I, Kohajda Zs, Szél T, Juhász V, Biliczki P, Jost N, Baczkó I, Papp JGy, Varró A, Virág L. Diclofenac prolongs repolarization in ventricular muscle with

impaired repolarization reserve. *PLoS ONE*, 2012, 7(12): e53255. DOI:10.1371/journal.pone.0053255
IF (2012) = 3.73

3. Jost N, Kohajda Zs, Kristóf A, Kovács PP, **Husti Z**, Juhász V, Kiss L, Varró A, Virág L, Baczkó I. Atrial remodeling and novel pharmacological strategies for antiarrhythmic therapy in atrial fibrillation. *Curr Med Chem*, 2011, 18(24): 3675-3694.
DOI: 10.2174/092986711796642373
IF (2011) = 4.859

4. Baczkó I, **Husti Z**, Lang V, Leprán I, Light PE. Sarcolemmal K_{ATP} channel modulators and cardiac arrhythmias. *Curr Med Chem*, 2011, 18(24): 3640-3661.
DOI: 10.2174/092986711796642472
IF (2011) = 4.859

5. Szél T, Koncz I, Jost N, Baczkó I, **Husti Z**, Virág L, Bussek A, Wettwer E, Ravens U, Papp JGy, Varró A. Class I/B antiarrhythmic property of ranolazine, a novel antianginal agent, in dog and human cardiac preparations. *Eur J Pharmacol*, 2011, 662: 31-39.
DOI: 10.1016/j.ejphar.2011.04.042
IF (2011) = 2.516

Impact factor of other publications: 20.954

Impact factor of all publications: 26.38