# SPECIES DIFFERENCES IN CARDIAC VENTRICULAR REPOLARIZATION, AND THEIR IMPLICATIONS FOR HUMAN CARDIAC ELECTROPHYSIOLOGY

**PhD thesis**

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2022

## LIST OF PUBLICATIONS RELATED

## TO THE SUBJECT OF THE THESIS

I **Árpádffy-Lovas T**, Baczkó I, Baláti B, Bitay M, Jost N, Lengyel C, Nagy N, Takács J, Varró A, Virág L. *Electrical Restitution and Its Modifications by Antiarrhythmic Drugs in Undiseased Human Ventricular Muscle.* Front Pharmacol. 2020 Apr 30;11:479. doi: 10.3389/fphar.2020.00479. PMID: 32425771
Journal impact factor: 5.811

II **Árpádffy-Lovas T**, Husti Z, Baczkó I, Varró A, Virág L. *Different effects of amiodarone and dofetilide on the dispersion of repolarization between well-coupled ventricular and Purkinje* fibers. Can J Physiol Pharmacol. 2021 Jan;99(1):48-55. doi: 10.1139/cjpp-2020-0234. Epub 2020 Jul 21. PMID: 32692935.
Journal impact factor: 2.273

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*In press.*

**Impact factor of publications related to the thesis: 8.084**

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**Impact factor of other publications: 36.425**
**Impact factor of all publications: 44.509**

## Introduction

### *Sudden cardiac death, ventricular fibrillation, and the repolarization reserve*

The annual incidence of sudden cardiac death (SCD) has been reported to approximate 50 to 100 per 100,000 in the general populations of Europe and North America, accounting for approximately half of the deaths in patients with cardiac conditions. The most common mechanism of SCD is cardiac ventricular fibrillation (VF), which is a complex and still poorly understood phenomenon in the heart.

*The mechanism of ventricular fibrillation due to enhanced dispersion of repolarization*

Under healthy conditions, a relatively long and consistent effective refractory period (ERP) is combined with fast impulse conduction in the heart, which ensures that the depolarization and repolarization waves are homogeneous. On the other hand, spatial changes in action potential duration (APD) and consequent changes in ERP and refractoriness lead to increased repolarization inhomogeneity, which may open otherwise inaccessible pathways to impulse conduction. Since this state of cardiac impulse conduction significantly increases the

chances of reentrant arrhythmias, it can be considered as a *substrate* to ventricular fibrillation.

*Substrate formation*

According to the concept of the repolarization reserve, all available repolarizing currents contribute to a total pool of available repolarization capacity. If the activation of one current is decreased, the activity of other currents may increase, at least partially taking over the function of the missing capacity of repolarization.

Local differences in APD (APD dispersion) can be changed by various agents and conditions that affect repolarization. Transmural APD dispersion can be derived from QT/QTc or JTc dispersion in the ECG *in* vivo. The dispersion between the ventricular myocardium and the Purkinje system can only be studied *ex vivo*. Increased dispersion in both areas may act as a substrate for reentrant arrhythmias. However, this vulnerable state of the heart is not sufficient to develop arrhythmias. A *trigger*, most likely an extra beat that can travel through the complex pathway of low-ERP areas of the heart is what starts the reentrant arrhythmia.

*Arrhythmia triggering events and electrical restitution*

Extra beats of any origin can be considered as triggering events, possibly leading to VF. The timing of these extra beats is critical in terms of arrhythmia risk. The APD/ERP of myocytes is determined by the diastolic interval (DI): the temporal proximity of the preceding beat. As the DIs increase, the APDs/ERPs of the extra beats also increase in human. This process is called electrical restitution. According to the restitution hypothesis, as DIs increase due to the propagation of an extrasystole, the next following possible extrasystole would encounter a prolonged APD/ERP, therefore a local conduction defect may occur. A steeper/faster restitution curve would favor such an effect, thus it would be considered proarrhythmic. A flattened/slower restitution curve would have the opposite effect.

***Cardiac electrophysiological experimentation***

Some mammalian hearts are more similar to the human heart than others. While rodents are relatively accessible and can even be genetically modified to explore genetic variability, their electrophysiological properties are deeply different from that of human.

The available spectrum for possible action potential shapes and durations of each species and each individual organism is primarily determined by the ion channel constitution of their hearts. Therefore, the suitability and translational value of each animal model is largely determined by its similarity in cardiac ion channel composition to that of human. In addition, the abundance of the same ion channels and the activation of the respective ion currents can also be different, leading to important differences in repolarization reserve between various species.

The aim of this work was o offer a systematic comparison between the repolarization of the human ventricle and that of the most commonly used model animals in cardiac electrophysiology. Comparisons were also made in terms of the characteristics of APD restitution in these species, and ion channel inhibitor effects on human restitution curves were also evaluated. The effects dofetilide and amiodarone were also assessed in preparations containing electrotonically well coupled Purkinje fibers and ventricular muscle in order to uncover additional features of these agents in the context of local dispersion of repolarization.

## Materials and Methods

Action potentials were recorded from ventricular muscle preparations and Purkinje fibers using the conventional microelectrode technique, and the APD prolonging effect of various ion channel inhibitors was tested. These effects were compared in the following species: the dog, the rabbit, the guinea pig, and the rat. The electrophysiological characteristics of these species were compared to those of human undiseased heart preparations. The investigations conformed to the principles of the Declaration of Helsinki. Experimental protocols were approved by the National Scientific and Research Ethical Review Boards (4991-0/2010-1018EKU [339/PI/010]). All experiments involving animals were carried out in compliance with the Guide for the Care and Use of Laboratory Animals (USA NIH publication NO 85-23, revised 1996) and conformed to the Directive 2010/63/EU of the European Parliament. The protocols have been approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Szeged, Hungary (approval number: I-74-24-2017) and by the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development (authority approval number XIII/3331/2017).

The APD prolonging effect of the drugs was evaluated by comparing the changes in $APD_{90}$ values (action potential duration at 90% of repolarization) for each preparation. Species-wise comparisons were made using ANOVA, based on the extent of $APD_{90}$ prolongation.

Data points of restitution curves were fitted by a mono-exponential function in order to calculate the kinetic time constant of the $APD_{90}$ restitution process:

$$APD = APD_{ss} - A^{-DI/\tau}$$

where $APD_{ss}$ is the maximal APD (measured as $APD_{90}$), $A$ is the amplitude of the exponential function, $DI$ is the diastolic interval, and $\tau$ is the time constant.

**Results**

***Selective inhibition of ion currents at constant 1000 ms pacing***

Rapid delayed-rectifying potassium current ($I_{Kr}$) inhibitor dofetilide (50 nM) did not affect rat repolarization, but markedly prolonged APD in human and rabbit preparations. Inhibition of the slow delayed-rectifying potassium current ($I_{Ks}$) by HMR-1556 (500 nM) elicited slight prolongation in the

repolarization of guinea pig, rabbit and rat, but did not alter it in human and dog. The transient outward current ($I_{to}$) was inhibited by administering chromanol-293B (100 μM) after HMR-1556. This elicited slight APD prolongation in the repolarization of guinea pig, and considerable prolongation in dog, rabbit and rat, but slightly abbreviated APD in human. $BaCl_2$ (10 μM), inhibitor of the inward rectifier potassium current ($I_{K1}$) significantly prolonged rat repolarization in, but elicited only slight changes in human and guinea pig, and moderate prolongation dog and rabbit. We applied XEN-D0101 (1 μM) to inhibit the ultrarapid delayed rectifier current ($I_{Kur}$). XEN-D0101 significantly prolonged rat repolarization, but elicited only slight to no change in human, dog, rabbit and guinea pig.

***Frequency-dependent changes in APD (variable cycle length pacing)***

The frequency-dependent changes in APD were compared in human, dog, rabbit, guinea pig and rat preparations in the range of 300–5000 ms cycle lengths, the cycle length was dynamically increased in this protocol. In human and dog, APD consistently increased with the cycle length. Rabbit and guinea pig preparations showed a different pattern: APD slightly

increased up to 1000 ms cycle length, but showed a declining slope at longer cycle lengths. The opposite was found in rat preparations, as APD decreased up to 1000 ms cycle length, and showed a consistent increase with the increase of the cycle length afterwards.

***Ion channel inhibitor effects on human APD restitution***

In further experiments, the effects of several antiarrhythmic drugs were studied on the electrical restitution curves in human undiseased ventricular muscle preparations. Selective $I_{Kr}$ inhibitors E-4031 and sotalol increased overall APD and slowed the kinetics of the restitution curve (from $\tau$=82.6 ms to $\tau$=160.3 ms, n=5; and from $\tau$=95.8 ms to $\tau$=152.7 ms, n=5, respectively). L-735,821, a specific inhibitor of the $I_{Ks}$ current did not influence APD and electrical restitution curves ($\tau$=113.1 ms vs. $\tau$=111.9 ms, n=7). The effects of the $I_{Na}$ inhibitor mexiletine and the $I_{Ca\text{-}L}$ inhibitor nisoldipine were also studied on human ventricular electrical restitution curves. Both mexiletine and nisoldipine shortened APD but only mexiletine slowed restitution kinetics in human ventricular muscle preparations (from $\tau$=98 ms to $\tau$ = 133.2 ms, n=6; from $\tau$ = 111.1 ms to $\tau$ = 113.1 ms, n=6, respectively).

***Comparison of amiodarone and dofetilide effects on Purkinje-muscle dispersion of repolarization***

In further experiments, the APD prolonging effects of amiodarone (dogs were orally treated with 50 mg/kg/day amiodarone for 4 weeks) and dofetilide (50 nM, administered in the tissue chamber during the experiment) were compared in electrotonically coupled and uncoupled ventricular muscle and Purkinje fiber preparations. Electrotonically coupled preparations were 25–35 mm in diameter, containing free-running Purkinje strands coupled with the surrounding myocardium. Uncoupled preparations were individual papillary muscles and individual Purkinje fibers not connected with each other.

We found that amiodarone increased APD both in coupled and in uncoupled Purkinje fibers in a similar extent. The APD prolonging effect in coupled ventricular muscles was more pronounced compared to that of the uncoupled ventricular muscle preparations.

Therefore, amiodarone increased APD in uncoupled preparations without altering the local dispersion of repolarization between Purkinje fibers and ventricular muscle. On the contrary, in coupled preparations, amiodarone increased APD in such a manner that resulted in a significant decrease in the local dispersion of repolarization in the Purkinje-muscle junction.

Dofetilide, on the other hand, had a much more pronounced APD prolonging effect on Purkinje fibers compared to its effect on the ventricular muscle, thus the APD dispersion was significantly increased. The change in $APD_{90}$ in uncoupled ventricular muscle preparations was comparable to that of the coupled preparations, but the APD of uncoupled Purkinje fibers showed a more pronounced prolongation compared to the coupled ones. Therefore, dofetilide elicited a marked increase of local dispersion both in coupled and uncoupled preparations, but the effect was more pronounced in the latter.

These findings suggest that electrotonic coupling or the lack thereof in preparations may affect the perceived drug effects on dispersion in the Purkinje–muscle-junction: dispersion may be

over or underestimated if it is only estimated from the findings in uncoupled preparations.

**Discussion**

The formation of potentially life-threatening arrhythmias requires a trigger event that occurs during a vulnerable period in the heart. Such vulnerable periods may form due to the prolongation of APD, caused by intrinsic or extrinsic defects in repolarization. In order to gather relevant data on the APD prolonging effects of drugs or drug candidates, our choice of models should appropriately represent human repolarization, and when interpreting the results obtained from model animals, the limitations of the given model, such as the degree of electrotonic coupling or the differences in ion currents compared to human, should be taken into account. The dog has been considered a reasonably satisfactory model of human cardiac electrophysiology, regarding its ion channel constitution. Our findings are in accordance with this notion. The guinea pig and the rabbit are more accessible models compared to the dog, and also seem more similar to human repolarization when compared to that of the rat. In addition, we also found that testing drug effects on electrotonically uncoupled Purkinje fibers and ventricular muscle may lead to

the overestimation of APD prolonging effects in the former, but underestimation in the latter compared to drug effects on the same tissue types in their interconnected state.

## Conclusion

Ideally, testing novel agents on at least two models at the same time may significantly increase the translational value of basic research in cardiac electrophysiology, and taking the species differences presented in this work into account may provide further insight in expected drug effects on human APD.

**Acknowledgments**

I would like to express my gratitude for the invaluable support and continuous professional guidance I received over the years from my supervisors, Prof. András Varró, and Dr. László Virág. I am thankful to Dr. István Baczkó, Head of the Department, for his suggestions and for providing me the opportunity to conduct my PhD work at the Department of Pharmacology and Pharmacotherapy. I am grateful for the insightful advice I received from researchers Prof. Norbert Jost, Dr. Norbert Nagy, Dr. Zoltán Husti, Dr. István Koncz, Dr. Péter Orvos, Dr. Zsófia Kohajda, and Dr. Andrea Orosz. I am also thankful for the constructive comments and criticism I received from Dr. Balázs Horváth and Dr. Norbert Szentandrássy at various conferences. I would like to thank fellow PhD students Dr. Noémi Tóth, Dr. Leila Topal, Aiman Mohammed, Muhammad Naveed, Dr. Bence Pászti, and Dr. Tibor Magyar for all their help and companionship. Furthermore, I would like to thank Zsuzsa Molnárné for the technical advice during my early years with the conventional microelectrode technique.

**Funding**

This work was funded by the National Research Development and Innovation Office (NKFIH K-119992 and GINOP-2.3.2-15-2016-00048-STAY ALIVE), the Ministry of Human Capacities Hungary (20391-3/2018/FEKUSTRAT, EFOP-3.6.2-16-2017-00006-LIVE LONGER and EFOP 3.6.3-VEKOP-16-2017-00009), the New National Excellence Program of the Ministry for Innovation and Technology (UNKP-19-3-SZTE, UNKP-20-3-SZTE, and UNKP-21-3-SZTE), and by the Hungarian Academy of Sciences. The GINOP and EFOP projects are co-financed by the European Union and the European Regional Development Fund.