Final research report

Establishment of reliable of human and canine atrial and ventricular action potential computer models

This project was an international co-operational grant performed together with the group of Dr. Blanca Rodriguez from the Department of Computer Science, University of Oxford, UK. Conform of the rules of the Hungarian National Research Fund (OTKA), the co-operational partner should also conduct a mirror project in her/his research facility funded by a national Grant Agency. The mirror grant conducted by Prof Blanca Rodriguez was funded by the Welcome Trust Senior Research Fellowship in Basic Biomedical Science (project number 100246/Z/12/Z) and had the title of: "Safety and efficacy of anti-arrhythmic drug therapy in acute myocardial ischaemia in human. An integrative, multiscale and mechanistic investigation".

The project initially was planned for three years (01.10.2013-30.09.2016), but in July, 2016 we need to opt for a one year extension. This extension applied on 7 July, 2016 was necessary because due to the significantly reduced number of available human donor hearts, we could not collect enough human data for developing the action potential in silico model. The extension request was granted by Prof. László Acsády the chairman of the Board for Medical and Biological Sciences Panel.

Based on the research plan the project consisted of several research subtopics (ST). During the four years long project the following selected main results were obtained:

Subtask 1. Development of a novel *in silico* assessment to determine the proarrhythmic effect of several K^+ currents to cardiac repolarization.

Causes and impact of inter-subject variability in cellular electrophysiological behaviour are unknown. Understanding the effects of this variability is important, particularly as it can modulate response to drug application. Differences between individuals in response to drug action may be due to ionic-level differences, but the effects of these differences may be masked under normal physiological conditions. Therefore, investigating these differences is important to understand the effects of drug action on cells from different individuals.

Beat-to-beat variability in repolarization (BVR) has been proposed as an arrhythmic risk marker for disease and pharmacological action. BVR changes in response to pharmacological inhibition have been linked to the pro-arrhythmic potential of drug compounds, and elevated levels of BVR have been shown to successfully identify individuals at high risk of arrhythmia. BVR quantified in isolated cardiomyocytes is substantially attenuated by gap junctional coupling in well-coupled tissue, and therefore its causal link with arrhythmic mechanisms may be limited in healthy tissue. However, BVR in isolated cells may represent a pro-arrhythmia indicator in conditions of reduced repolarization reserve caused by drugs, mutations or disease, and also impaired intercellular coupling, both known to enhance variability and pro-arrhythmic abnormalities in the heart. As a consequence, understanding the ionic mechanisms underlying BVR in isolated cells may therefore help to inform its use as

an arrhythmic risk biomarker (for example for drug testing), and also to better understand its causal relationship with arrhythmia in conjunction with other mechanisms.

In this study, we described the construction of an experimentally-calibrated set of stochastic cardiac cell models that captures both BVR and cell-to-cell differences in BVR displayed in isolated canine action potential measurements using pharmacological agents. Simulated and experimental ranges of BVR are compared in control and under pharmacological inhibition, and the key ionic currents determining BVR under physiological and pharmacological conditions are identified. We have analysed and quantified the effects of calcium insensitive $I_{to,1f}$, I_{Kr}, I_{Ks}. All these currents played a significant role in the beat to beat variability of the repolarization, while in contrast, I_{CaL} become the major contributors to BVR upon inhibition of the fast delayed rectifier potassium current, I_{Kr}. This observation could be concluded that both I_{Ks} and $I_{to,1f}$ as being key contributors to repolarization reserve. This study could be extended in a number of ways. Our study focuses on the contribution of four ionic currents to BVR. Additional sources of stochasticity could be implemented and investigated using our approach in further studies, provided the experimental measurements for model calibration and evaluation are available. This would be particularly important for currents, such as the persistent sodium current, which might contribute to the BVR, particularly under pharmacological condition. We focused on assessing the effects of I_{Kr}, I_{Ks}, I_{to1}, I_{CaL} because they have a large impact on repolarisation and we had a consistent experimental dataset including ionic current measurements, and AP recordings under control and pharmacological block of those currents. This allowed us to construct and calibrate a whole population of models rather than just considering a unique AP model, like the Decker model. The Decker model was, in fact, not included in our population because it did not lead to APD values in range with our experimental recordings in control as well as following ionic inhibitions.

The model population we developed allows investigating the relative importance of each of the analysed currents in contributing to BVR taking into consideration variability in ionic conductances and channel numbers. In the case of the persistent sodium current (I_{NaL}) we did not have available current traces and AP data measured under selective I_{NaL} inhibition that we could use to construct and calibrate the models following the same procedure that we used with all the other analysed currents. Future studies could assess the role of stochasticity in I_{NaL} in generating BVR using our proposed methodology as long as all necessary data are available, building on the methodology described in our study. Our experimentally-calibrated population of models was able to reproduce experimental observations regarding changes on BVR following sodium channel inhibition and enhancement, although widely varied responses could be quantified as a function of the analysed model (cell), degree of inhibition / enhancement as well as selectivity for both I_{NaL} and fast sodium current (I_{Na}) or for I_{NaL} only. The study was published in *PLoS One* (Pueyo *et al*. 11(3): e0151461. https://doi.org/10.1371/journal.pone.0151461, 2016).

In another study we aimed to quantitatively investigate the mechanisms underlying repolarization abnormalities in human cardiomyocytes, with a wide range of ionic profiles to consider variability in ionic properties. We specifically focus on investigating human cardiomyocytes yielding a normal AP under control conditions, using a population of human ventricular cardiomyocyte models calibrated with experimental electrophysiological recordings. Sixty two AP recordings from non-diseased human heart preparations were used to construct a population of human ventricular models with normal APs and a wide range of ion channel densities. Multichannel ionic block was applied to investigate susceptibility to

repolarization abnormalities. I_{Kr} block was necessary for the development of repolarization abnormalities. Models that developed repolarization abnormalities over the widest range of blocks possessed low Na^+/K^+ pump conductance (I_{NaK} below 50% of baseline, and I_{CaL} conductance above 70% of baseline. Furthermore, I_{NaK} made the second largest contribution to repolarizing current in control simulations and the largest contribution under 75% I_{Kr} block. Reversing intracellular Na^+ overload caused by reduced I_{NaK} was not sufficient to prevent abnormalities in models with low Na^+/K^+ pump conductance, while returning Na+/K+ pump conductance to normal substantially reduced abnormality occurrence, indicating I_{NaK} is an important repolarization current. Taken all of these in consideration we have concluded that I_{NaK} is an important determinant of repolarization abnormality susceptibility in human ventricular cardiomyocytes, through its contribution to repolarization current rather than homeostasis. While we found I_{Kr} block to be necessary for repolarization abnormalities to occur, I_{NaK} decrease, as in disease, may amplify the pro-arrhythmic risk of drug-induced I_{Kr} block in humans. The study was published recently in *Frontiers in Physiology* (Britton *et al,* Front Physiol, 8:278. doi: 10.3389/fphys.2017.00278)

Subtask 2. The investigation of the effect of selective NCX, K_{ATP} and HERG channel blocking effect in *in vitro*, *in vivo* and *in silico* model.

The sodium/calcium exchanger (NCX) is considered as the major transmembrane transport mechanism that controls Ca^{2+}-homeostasis. Its contribution to the cardiac repolarization has not yet been directly studied due to lack of specific inhibitors, so that an urgent need for more selective compounds. Therefore, in this study, the electrophysiological effects of two newly synthetized NCX inhibitors -GYKB-6635 and ORM-10962- on the NCX, L-type Ca and main repolarizing K currents as well as action potential (AP) parameters were investigated. Ion currents and AP recordings were investigated by applying the whole-cell patch clamp and standard microelectrode techniques in canine heart. Effects of the two compounds were also studied in ouabain induced arrhythmias in isolated guinea-pig hearts. Both GYKB-6635 and ORM-10962 significantly reduced either the inward or outward NCX currents at submicromolar concentration ranges. Even at high concentration (1-10 μM), neither GYKB-6635 nor ORM-10962 affected I_{CaL}, the maximum rate of depolarization (dV/dtmax), the main repolarizing K^+ currents and the main AP parameters. Drug pre-treatment with the two drugs significantly delayed the time to the development of cardiac arrhythmias as extrasystoles, ventricular tachycardia and of ventricular fibrillation. It is concluded that GYKB-6635 and especially ORM-10962 are potent and highly selective inhibitors of the cardiac NCX current, and in addition selective NCX inhibition may contribute to the prevention of DAD based arrhythmias (Geramipour *et al*, Can J Physiol Pharmacol, 94: 1090-1101, 2016; Kohajda *et al*, PLoS One, 11(11): e0166041. doi: 10.1371/journal.pone.0166041, 2016).

Ischemia and heart failure-related cardiac arrhythmias, both atrial (e.g., atrial fibrillation) and ventricular (e.g., malignant tachyarrhythmias) represent a leading cause of morbidity and mortality worldwide. Despite the progress made in the last decade in understanding their pathophysiological mechanisms there is still an unmet need for safer and more efficacious pharmacological treatment, especially when considering the drawbacks and complications of implantable devices. Cardiac ATP-sensitive potassium channels located in

the sarcolemmal membrane (sarcKATP) and the inner mitochondrial membrane (mitoKATP) have emerged as crucial controllers of several key cellular functions. In the past three decades a tremendous amount of research led to their structural and functional characterization unveiling both a protective role in cardiac adaptive responses to metabolic stress and a seemingly paradoxical role in promoting as well as protecting against atrial and ventricular arrhythmias. On the other hand, several KATP inhibitors have emerged as potential ischemia selective antiarrhythmic drugs. In this respect, cardioselective, chamber specific and combined sarcKATP and mitoKATP modulators currently represent a promising field for drug development. Therefore we have designed , synthetized and assessed the effects of 7 novel pharmacological modulators of the ATP-sensitive potassium channels (K_{ATP}): KL-1487, KL-1488, KL-1491, KL-1492, KL-1495, KL-1507 and KL-1509, respectively on mitochondrial respiratory function. Our results show that a concentration of 150 μM of KL-1487, KL-1492, KL-1495, and KL-1509 significantly increased respiratory rates in State 2 and 4, and decreased State 3 respiration, respectively. Concentrations of 100 μM, 75 μM and 50 μM still elicited the uncoupling effect whereas the inhibitory effect of the active respiration diminished progressively. KL-1488, KL-1491 and KL-1507 did not influence mitochondrial respiration. In conclusion, we report a persistent uncoupling effect for KL-1487, KL-1492, KL-1495, and KL-509 in rat heart isolated mitochondria for both CI and CII-supported respiration (Muntean *et al*, Curr Pharm Design, 21: 1091-1102, 2015).

Chelidonium majus or greater celandine is spread throughout the world, and it is a very common and frequent component of modern phytotherapy. Although C. majus contains alkaloids with remarkable physiological effect, moreover, safety pharmacology properties of this plant are not widely clarified, medications prepared from this plant are often used internally. In our study the inhibitory effects of C. majus herb extracts and alkaloids on hERG channel as well as on cardiac action potential were investigated. These extracts and alkaloids also prolong the cardiac action potential in dog ventricular muscle. Therefore these compounds may consequently delay cardiac repolarization, which may result in the prolongation of the QT interval and increase the risk of potentially fatal ventricular arrhythmias (Orvos *et al*, Fitoterapia 100:156-165, 2015).

<u>Subtask 3.</u> The investigation and characterisation of diseased related mutations

Data obtained in canine cardiac electrophysiology studies are often extrapolated to the human heart. However, as we in several previous studies have demonstrated, human ventricular action potential, due to the lower density of its K^+ currents, has less powerful repolarization reserve. Since the relevance of canine data to the human heart has not yet been fully clarified, the aim of the present study was to determine for the first time the parameter of the action potentials of undiseased human Purkinje fibres (PFs) and to compare them directly with those of dog PFs. Current, action potential and simulation data suggests that largely differing protein expression profiles of the two species may underlie these important disparities. Therefore, caution is advised when extrapolating canine PF data to human, and further experiments are required to investigate the characteristics of human PF repolarization and its possible role in arrhythmogenesis. This investigation was published in Canadian Journal of Physiology and Pharmacology (Nagy *et al*, Can J Physiol Pharmacol, 93, 803-810 2015).

Loss-of-function mutations of the KCNJ2 gene encoding for the inward rectifier potassium channel subunit Kir2.1 cause Andersen-Tawill Syndrome (ATS), a rare genetic disorder characterised by periodic paralysis, ventricular arrhythmias and dysmorphic features. In this study we describe the identification and functional characterisation of a novel KCNJ2 mutation Val302del, isolated from an ATS patient. Confocal imaging, current measurements and simulations data indicated that the wild type and the Val302del mutant subunits co-assemble in the cell membrane and that the mutation affects potassium conductivity and/or gating of the heteromeric Kir2.1 channels. Our results strongly suggest the Val302del mutation to be the causative variant in the ATS patient (Ördög *et al*, Can J Physiol Pharmacol, 93: 569-579, 2015).

Data obtained in canine cardiac electrophysiology studies are often extrapolated to the human. The reliable assessment of proarrhythmic risk of compounds under development remains an elusive goal. Current safety guidelines focus on the effects of blocking the KCNH2/HERG ion channel-in tissues and animals with intact repolarization. Novel models with better predictive value are needed that more closely reflect the conditions in patients with cardiac remodelling and reduced repolarization reserve. Therefore, we have developed a model for the long QT syndrome type-5 in rabbits (LQT5) with cardiac-specific overexpression of a mutant (G52R) KCNE1 β-subunit of the channel that carries the slow delayed-rectifier $K(^{+})$ -current (I_{Ks}). ECG parameters, including short-term variability of the QT interval (STVQT), a biomarker for proarrhythmic risk, and arrhythmia development were recorded. *In vivo*, arrhythmia susceptibility was evaluated by *i.v.* administration of the I_{Kr} blocker dofetilide. $K(^{+})$ currents were measured with the patch-clamp technique. Patch-clamp studies in ventricular myocytes isolated from LQT5 rabbits revealed accelerated I_{Ks} and I_{Kr} deactivation kinetics. At baseline, LQT5 animals exhibited slightly but significantly prolonged heart-rate corrected QT index (QT_i) and increased STVQT. Dofetilide provoked Torsade-de-Pointes arrhythmia in a greater proportion of LQT5 rabbits, paralleled by a further increase in STVQT .We may concluded that we have created a novel transgenic LQT5 rabbit model with increased susceptibility to drug-induced arrhythmias that may represent a useful model for testing proarrhythmic potential and for investigations of the mechanisms underlying arrhythmias and sudden cardiac death due to repolarization disturbances (Major et al, *Br J Pharmacol*, 173: 2046–2061, 2016 and Baczkó et al; *Prog Biophys Mol Biol*, 121:157-168, 2016).

Science educating, management and publishing activities

The results obtained by implementing the present project have been published in 12 full lengths research papers having the cumulative impact factor of 36.29. In addition we have published two abstracts, 3 other conference related papers and two book chapters.

In October 8-11, 2014 and October 1-4, 2016 we have organized in Balatongyörök (Hungary) and Marseille (France), the First and the Third European Section Meetings of the International Academy of Cardiovascular Sciences (IACS), respectively. Both conferences were attended by the presence of to about 150-150 scientists from all over the world. At the end we were invited by the chief-editor of the Canadian Journal of Physiology and Pharmacology to edit a special Symposia related volumes. As a main congress organizer, the principal investigator (PI) was one who was assigned to be Guest associate editor for these

Special Issues. We have edited 3 special issues with 12-12 papers each other introduced by special editorials.

The PI was invited by Springer Publishing to contribute with two chapters for a Cardiac Electrophysiology book entitled: Pathophysiology and Pharmacotherapy of Cardiovascular Disease. Eds. Jagadeesh G, Balakumar P, Maungh-U K, 2015, ISBN: 978-3-319-15960-7.

The investigations presented in this report serve as basis of the following three PhD theses:

Orvos Péter: Role of automated patch-clamp systems in drug research and development. Supervisor: Dr. László Virág; PhD thesis defended at 31.05.2016.

Nagy (Kohajda) Zsófia: Characterization of different aspects of selective NCX inhibition in the heart: from inotropy to arrhythmias. Supervisor: Dr. Norbert Jost, PhD thesis defended at 17.05.2017.

Geramipour Amir: Electrophysiological properties and pharmacological modulation of several transmembrane ion currents in mammalian hearts. Supervisor: Dr. Norbert Jost, PhD thesis defended at 25.05.2017.