Final research report

In vitro (molecular, ion channel and cellular) and *in vivo* investigations for the elucidation of antiarrhythmic and proarrhythmic mechanisms: the role of the repolarization reserve

This was a consortia project performed together with the NK104397 grant (lead by dr. Zsuzsanna Bősze, Agricultural Biotechnology Institute, Gödöllő, Hungary). This is the fourth, and thereby the last and final research report. The project consisted of six research subtopics (ST). We have successfully finished the research work designed in all subtasks. We have published a substantial number of conference abstracts and altogether 45 full lengths papers having the cumulative impact factor of 145.

The following selected main results were obtained:

Subtask 1. Investigation of a hypothetical mechanisms underlying sudden cardiac death in competitive athletes

We have characterized the electrophysiological effects of the non-steroidal anti-inflammatory drug diclofenac and to study the possible proarrhythmic potency of the drug in ventricular muscle. Ion currents were recorded using voltage clamp technique in canine ventricular cells and action potentials were obtained from canine ventricular preparations using microelectrodes. The proarrhythmic potency of the drug was investigated in an anaesthetized rabbit proarrhythmia model. Action potentials were slightly lengthened in ventricular muscle but were shortened in Purkinje fibres by diclofenac (20 μM). The maximum upstroke velocity (Vmax) was decreased in both preparations. Larger repolarization prolongation was observed when repolarization reserve was impaired by previous $BaCl_2$ application. Diclofenac (3 mg/kg) did not prolong while dofetilide (25 microgram/kg) significantly lengthened the QTc interval in anaesthetized rabbits. The addition of diclofenac following reduction of repolarization reserve by dofetilide further prolonged QTc. Diclofenac alone did not induce Torsades de Pointes ventricular tachycardia (TdP) while TdP incidence following dofetilide was 20%. However, the combination of diclofenac and dofetilide significantly increased TdP incidence (62%). We have concluded that diclofenac at therapeutic concentrations and even at high dose does not prolong repolarization markedly and does not increase the risk of arrhythmia in normal heart. However, high dose diclofenac treatment may lengthen repolarization and enhance proarrhythmic risk in hearts with reduced repolarization reserve. The work was published in a paper accepted by PLoS One (Kristof et al, 2012).

We have developed and validated a novel rabbit proarrhythmia model that will be used later for testing the effect of several steroid based drugs use by athletes. Thus, we estimated the efficacy of proarrhythmic biomarkers in isolated hearts with attenuated repolarization reserve. Langendorff-perfused rabbit hearts were used. Repolarization reserve was reduced by concomitant inhibition of the I_{Kr} and I_{Ks} currents. Absolute QT variability parameters seem to be the most practical and sensitive biomarkers of proarrhythmic liability in rabbit hearts with reduced repolarization reserve. Absolute QT variability parameters could serve as surrogates for Torsades de Pointes in drug-safety investigations in isolated rabbit hearts with attenuated

repolarization reserve. The work was published in a paper accepted by J Cardiovasc Pharmacol (Orosz et al, 2014; this article serves as main paper for her PhD thesis).

We have developed a new method for identifying people suffering in diseases that make them more susceptible to sudden cardiac death. For that we investigated conventional electrocardiogram (ECG) repolarization parameters and the beat-to-beat short-term QT interval variability (QT-STV), a new parameter of proarrhythmic risk, in two diseases, in patients having hypertrophic cardiomyopathy (HCM, 37 patients) and in Acromegaly (30 patients). In summary, beat-to-beat QT-STV showed the most marked increase in patients with HCM and may represent a novel marker that merits further testing for increased SCD risk in HCM. Short-term beat-to-beat QT variability was elevated in patients with acromegaly in spite of unchanged conventional parameters of ventricular repolarization. This enhanced temporal QT variability may be an early indicator of increased liability to arrhythmia. These studies were published in Can J Physiol and Pharmacol (Orosz et al, 2015) and PLoS One (Orosz et al, 2015). These articles served the basis of Andrea Orosz's PhD thesis, which was successfully defended in October, 2015.

Beat-to-beat variability of action potential duration (short-term variability, SV) is an intrinsic property of mammalian myocardium. Since the majority of agents and interventions affecting SV may modify also action potential duration (APD), we propose here the concept of relative SV (RSV), where changes in SV are normalized to changes in APD and these data are compared to the control SV-APD relationship obtained by lengthening or shortening of action potentials by inward and out-ward current injections. The results indicated that changes in beat-to-beat variability of APD must be evaluated exclusively in terms of RSV; furthermore, some experimental conditions, including the stimulation frequency, redox-state and temperature have to be controlled strictly when analyzing alterations in the short-term variability of APD. This study was published in Gen Physiol Biophys (Magyar et al 2016).

Subtask 2. Investigation of cardiac ventricular depolarization activated (Kv1.5 and Kv1.7) and calcium activated (SK2) currents

The inward rectifier K^+ current (I_{K1}) has crucial role in late repolarization and in maintaining the resting membrane potential. We aimed to elucidate the effect of non-adrenergic rise in $[Ca^{2+}]_i$ on the magnitude of I_{K1}, and its consequences in the ventricular repolarization. We conclude that the APD shortening effect of a $[Ca^{2+}]_i$ rise is partially mediated by the $[Ca^{2+}]_i$ -dependent augmentation of I_{K1}in both canine and human myocardium. Through its role in acceleration of repolarization and due to the subsequent increase in the repolarization reserve, the $[Ca^{2+}]_i$ -induced I_{K1} augmentation may serve as an endogenous defence mechanism against arrhythmia generation resulting from intracellular Ca^{2+}-overload. By lacking of selective blocker, in this study the I_{K1} was measured as Ba^{2+} sensitive current, dissected by 10 μM $BaCl_2$. Therefore, our definition of I_{K1} not only means the "classical" Kir2.x channels, rather the family of K^+ channels inhibited by 10 μM $BaCl_2$. The work was published in a paper accepted in the prestigious Pflügers Archiv-Eur J Physiol (Nagy et al, 2013).

Atrial fibrillation (AF) is the most common cardiac arrhythmia and is associated with an increased risk for stroke, heart failure and cardiovascular-related mortality. Candidate targets for anti-AF drugs include a potassium channel Kv1.5, and the ionic currents I_{KACh} and late I_{Na}. As pharmacological management of AF is currently suboptimal, we have designed and characterized a multifunctional small resveratrol derivative molecule, compound C1, to target these ion channels. C1 inhibited human peak and late Kv1.5 and the Nav1.5 sodium currents. C1 significantly delayed contractile and calcium dysfunction in rat ventricular myocytes. In a model of inducible AF in conscious dogs, C1 reduced the average and total AF

duration, therefore we concluded that C1 behaved as a promising multifunctional small molecule targeting a number of key pathways involved in AF. The work was published in a paper accepted by Br J Pharmacol, (Baczkó et al, 2014).

We have developed and validated a novel rabbit and dog proarrhythmia model in order to test the effects of combined K^+ currents. For that we investigated the incidence of drug-induced TdPs 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. The results indicated that 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. The work was published in Can J Physiol and Pharmacol (Husti et al, 2015), and served as a basis of the PhD thesis successfully defended by Zoltán Husti.

Understanding the role of ionic currents in shaping the cardiac action potential (AP) has great importance as channel malfunctions can lead to sudden cardiac death by inducing arrhythmias. Therefore, researchers frequently use inhibitors to selectively block a certain ion channel like 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS) and 9 anthracene carboxylic acid (9 AC) for calcium-activated chloride current (ICl(Ca)). This study aimed to explore to identify the proper blocker preferable to study ICl(Ca). The results showed that DIDS is equally useful to study ICl(Ca) during voltage-clamp but 9 AC is superior in AP measurements for studying the physiological role of ICl(Ca) due to the lack of sodium channel inhibition. 9 AC has also no action on other ion currents ($I_{Ca,L}$, I_{Kr}, I_{Ks}, I_{K1}), however, $I_{Ca,L}$ tracings can be contaminated with ICl(Ca) when measured in voltage-clamp condition. The work was published in a paper accepted by Current Pharm Design (Váczi et al, 2014), and served as a basis of the PhD thesis successfully defended by Krisztina Váczi.

Adrenergic activation of L-type Ca^{2+} and various K^+ currents is a crucial mechanism of cardiac adaptation; however, it may carry a substantial proarrhythmic risk as well. In order to elucidate this mechanism, we have investigated the timing of activation of Ca^{2+} and K^+ currents in isolated canine ventricular cells in response to exposure to isoproterenol (ISO). The results revealed that the effects of ISO were prevented by simultaneous blockade of both receptor types. Inhibition of phosphodiesterases decreased the differences observed in the turn on of the ISO induced plateau shift and APD shortening. ISO-induced activation of I_{Ca} is turned on faster than the stimulation of I_{Ks} and I_{Kr} in canine ventricular cells due to the involvement of different adrenergic pathways and compartmentalization. The work was published in a paper in Naunyn-Scmiedeberg's Arch Pharmacol (Ruzsnavszky et al, 2014; this article serves as main paper for his PhD thesis).

We have investigated the possible contributing role of Ca^{2+} activated Cl^- current [ICl(Ca)] to cardiac arrhythmias. The profile of ICl(Ca), identified as a 9-anthracene carboxylic acid-sensitive current under AP voltage-clamp conditions, contained an early fast outward and a late inward component, overlapping early and terminal repolarizations, respectively. Both components were moderately reduced by ryanodine, while fully abolished by BAPTA, but not EGTA. $[Ca^{2+}]_i$ was monitored using Fura-2-AM. Setting $[Ca^{2+}]_i$ to the systolic level measured in the bulk cytoplasm (1.1μM) decreased ICl(Ca), while application of Bay K8644, isoproterenol, and faster stimulation rates increased the amplitude of ICl(Ca). We concluded that activation of ICl(Ca) in canine ventricular cells requires Ca^{2+}-entry through neighbouring L-type Ca^{2+} channels and is only augmented by SR Ca^{2+}-release. Substantial activation of ICl(Ca) requires high Ca^{2+} concentration in the dyadic clefts which can be effectively buffered by BAPTA, but not EGTA. The work was published in a paper accepted by J Mol Cell Cardiol (Horváth et al, 2016).

Subtask 3. Investigation of constituting isoforms of the repolarizing (I_{Kr}, I_{Ks} and I_{K1} currents).

Dilated cardiomyopathy (DCM) is a multi-factorial disease characterized by left ventricular dilation that is associated with systolic dysfunction and increased action potential duration. The Kir2.x potassium channels (encoded by *KCNJ* genes) regulate the inward rectifier current (I_{K1}) contributing to the final repolarization in cardiac muscle. Here, we describe the transitions in the gene expression profiles of four *KCNJs*, from non-diseased to dilated cardiomyopathic human hearts. In undiseased adult ventricle, *KCNJ2, KCNJ12 and KCNJ4 (*Kir2.1-2.3) genes were expressed at high level; while the expression of *KCNJ14* (Kir2.4) gene was low. In DCM, the levels of Kir2.1 and Kir2.3 were upregulated but those of Kir2.2 channels were down-regulated. The work was published in a paper accepted in Can J Physiol and Pharmacol (Szűts et al, 2013).

Predicting lethal arrhythmia liability from beat-to-beat variability and instability (BVI) of the ECG intervals is a useful technique in drug assessment. Most investigators use only arrhythmia-free ECGs for this. The present study tested the broader applicability of this assessment by examining whether absolute BVI parameters predict another potential lethal arrhythmia, ischaemia-induced ventricular fibrillation (VF). Increased frequency of non-sinus beats and 'R on T' arrhythmic beats, shortened mean RR and electrical diastolic intervals, and increased BVI of cycle length and repolarization predicted VF occurrence. Absolute BVI parameters that quantify variability of repolarization had the best predictive power with high sensitivity and specificity. We concluded that the novel absolute BVI parameters that predicted TdP in rabbit also predict ischaemia-induced VF in rat, indicating a diagnostic and mechanistic congruence. The work was published in a paper accepted in Br J Pharmacol (Sarusi et al, 2014; this article serves as main paper for the PhD thesis of Annamária Sarusi).

Predicting lethal arrhythmia liability from beat-to-beat variability and instability (BVI) of the ECG intervals is a useful technique in drug assessment. We have investigated the effects of oxidative stress and of the cardiac action potential duration (APD) and two new drugs (chelodinium majus extract and desethylamiodarone-DEA) on the properties of the action potential in different in *in vivo* (conscious rat) and in vitro models (multicellular and single channel models). We found that: i) changes in beat-to-beat variability of APD must be evaluated exclusively in terms of short term variability, furthermore, some experimental conditions, including the stimulation frequency, redox-state and temperature have to be controlled strictly when analyzing alterations in the short term variability of APD; ii) hydroalcoholic extracts of greater celandine and its alkaloids, especially berberine, chelidonine and sanguinarine have a significant hERG potassium channel blocking effect. 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; iii) chronic oral treatment with DEA resulted in similar cardiac tissue levels to that of chronic AMIO treatment, and offered an equivalent degree of antiarrhythmic effect against acute coronary artery ligation induced ventricular arrhythmias in conscious rats. These investigations were published in three papers accepted in Fitoterapia (Orvos et al, 2015) and Can J Physiol and Pharmacol (Kistamás et al, 2015 and Morvai et al, 2015). The CJPP articles served as main papers for the PhD thesis of Kornél Kistamás and Nikolett Morvai.

Preclinical in vivo QT measurement as a proarrhythmia essay is expensive and not reliable enough. In another study we developed a sensitive, cost-effective, Langendorff perfused guinea pig heart model for proarrhythmia safety screening. Dofetilide and HMR-1556 alone or co-perfused, prolonged the QTc interval. Similarly, cisapride and HMR-1556

alone or co-perfused, prolonged the QTc interval. Catecholamine-induced fast heart rate abolished the QTc prolonging effects of the I_{Kr} inhibitors, but augmented the QTc prolongation during I_{Ks} inhibition. The effect of catecholamines on the QTc facilitated differentiation between I_{Kr} and I_{Ks} inhibitors. Thus, QTc measurement in Langendorff perfused guinea pig hearts with pharmacologically attenuated repolarization reserve and periodic catecholamine perfusion seems to be suitable for preclinical proarrhythmia screening. This investigation was published in J Pharmacol Toxicol Methods (Kui et al, 2016). The paper serves as main paper for the PhD thesis of Péter Kui.

ST4. Establishment of reliable human and canine atrial and ventricular action potential computer models

The species-specific determinants of repolarization are poorly understood. This study compared the contribution of various currents to cardiac repolarization in canine and human ventricle. Conventional microelectrode, whole-cell patch-clamp, molecular biological and mathematical-modelling techniques were used. Selective I_{Kr}-block by dofetilide lengthened APD_{90}>3-fold more in human than dog, suggesting smaller repolarization-reserve in humans. Selective I_{K1}-block and I_{Ks} block increased APD_{90} more in canine than human right-ventricular papillary muscle. Ion-current measurements in isolated cardiomyocytes showed that I_{K1}- and I_{Ks}-density were 3- and 4.5-fold larger in dogs than humans respectively. I_{Kr}-density and kinetics were similar in human versus dog. Cardiac mRNA and protein level measurements supported these data. A dog and human action potential based mathematical model incorporating observed human-canine ion-current differences confirmed the role of I_{K1} and I_{Ks} in repolarization-reserve differences. Thus, humans show greater repolarization-delaying effects of I_{Kr}-block than dogs, because of lower repolarization-reserve contributions from I_{K1} and I_{Ks}, emphasizing species-specific determinants of repolarization and the limitations of animal models for human disease. The work was published in the prestigious Journal of Physiology (Jost et al, 2013). The significance of the paper was highlighted by an editorial (Paterson, J Physiol, 591:4065, 2013).

Although beat-to-beat variability (short-term variability, SV) of action potential duration (APD) is considered as a predictor of imminent cardiac arrhythmias, the underlying mechanisms are still not clear. Therefore, we aimed to determine the role of the major cardiac ion currents, APD, stimulation frequency, and changes in the intracellular Ca^{2+} concentration on the magnitude of SV. These results serve as a basis for designing novel cardiac action potential models. Action potentials were recorded from isolated canine ventricular cardiomyocytes using conventional microelectrode techniques. SV was an exponential function of APD, when APD was modified by current injections. Drug effects were characterized as relative SV changes by comparing the drug-induced changes in SV to those in APD according to the exponential function obtained with current pulses. Relative SV was increased by dofetilide, HMR 1556, nisoldipine, and veratridine, while it was reduced by BAY K8644, tetrodotoxin, lidocaine, and isoproterenol. We conclude that drug-induced effects on SV should be evaluated in relation with the concomitant changes in APD. Blockade of these currents, or the beta-adrenergic pathway, may carry also some additional proarrhythmic risk in addition to their well-known antiarrhythmic action. These investigations were published in Pflugers Archiv Eur J Physiol (Szentandrássy et al, 2014). Can J Physiol and Pharmacol (Nagy et al, 2015 and Horváth et al, 2015), Journal of Physiology and Pharmacology (Kistamás et al, 2015).

In another 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. Results show that the 4-aminopyridine-sensitive transient outward potassium current, I_{to1}, is a fundamental driver of BVR in control and upon complete inhibition of the slow delayed rectifier potassium current, I_{Ks}. In contrast, I_{Ks} and the L-type calcium current, I_{CaL}, become the major contributors to BVR upon inhibition of the fast delayed rectifier potassium current, I_{Kr}. This highlights both I_{Ks} and I_{to1} as key contributors to repolarization reserve. Partial correlation analysis identifies the distribution of Ito1 channel numbers as an important independent determinant of the magnitude of BVR and drug-induced change in BVR in control and under pharmacological inhibition of ionic currents. Distributions in the number of I_{Ks} and I_{CaL} channels only become independent determinants of the magnitude of BVR upon complete inhibition of I_{Kr}. These findings provide quantitative insights into the ionic causes of BVR as a marker for repolarization reserve, both under control condition and pharmacological inhibition. Study published recently in PloS One in an international co-operation (Pueyo et al, 2016).

ST5. The cellular level investigation of the Na^+/Ca^{2+} exchanger (NCX)

The sodium/calcium exchanger (NCX) is considered to be a major regulator maintaining the Ca^{2+} homeostasis in the myocardium. The aim of the study was to investigate the selectivity of the ORM-10103 ORM 10962 and GYKB-6635 in mammalian (canine, guinea pig and rabbit) heart muscle preparations. We tested the compounds on different repolarizing potassium currents such as the transient outward, inward rectifier, the slow and fast components of the delayed rectifier potassium current and on the L-type calcium current and on experimentally induced early (EAD) and delayed (DAD) after depolarizations. Both drugs (ORM-10103, ORM-10962 and GYKB-6635) significantly reduced both the inward and outward NCX currents with estimated submicromolar EC_{50} values. The drugs were all selective, since did not significantly decrease the: i) amplitude of I_{Ca} in canine myocytes. ii) the amplitude of the V_{max} of the slow response action potentials recorded from guinea-pig papillary muscles; iii) the amplitudes of main repolarizing potassium currents (I_{K1}, I_{to}, I_{Kr} and I_{Ks}). The amplitudes of pharmacologically induced early and delayed afterdepolarizations were significantly decreased by ORM-10103, ORM 10-962, while ORM-10962 and GYKB-6635 delayed the formation of DADs in ouabain induced model of guinea pig hearts. We have concluded that the present study provides evidences for the strong NCX-inhibitory activity of these compounds, and specific inhibition of the NCX current can abolish triggered arrhythmias. These works were published in Br J Pharmacol (Jost et al, 2013), Plos One (Kohajda et al, 2016) and Can J Physiol Pharmacol (Geramipour et al, 2016). They will serve as main papers for the PhD thesis of Zsófia Kohajda and Amir Geramipour, which will be hopefully defended in May, 2017. The significance of the paper from Jost et al (2013) was highlighted by an editorial (Terraciano, Br J Pharmacol, 2013).

NCX inhibitors and/or the dependence of the experimental model on the degree of $[Ca^{2+}]_i$ overload. Hence, we used NCX inhibitors SEA0400 and the more selective ORM-10103 to evaluate the efficacy of NCX inhibition against arrhythmogenic [Ca2+]i rise in conditions when $[Ca^{2+}]$i was augmented via activation of the late sodium current (I_{NaL}) or inhibition of the Na+/K+ pump. Also we investigated and compared the efficacy of individual or the simultaneous inhibition of the NHE and NCX against reperfusion-induced arrhythmias in Langendorff-perfused rat hearts by applying a commonly used regional ischaemia-reperfusion protocol. We found that: i) selective NCX inhibition -presumably by blocking reverse mode of NCX current- is effective against arrhythmogenesis caused by $[Na^+]_i$ -induced $[Ca^{2+}]_i$ elevation, without influencing the AP waveform. Therefore, selective NCX

current inhibition, by significantly reducing the arrhythmogenic trigger activity caused by the perturbed $[Ca^{2+}]_i$ handling, should be considered as a promising anti-arrhythmic therapeutic strategy. ii) although principal simultaneous NHE+NCX inhibition should be highly effective against all types of the recorded reperfusion-induced arrhythmias, NCX inhibitors, alone or in combination with cariporide, seem to be moderately suitable to provide satisfactory cardioprotection - at least in the present arrhythmia model. These investigations were published in Br J Pharmacol (Nagy et al, 2014), Journal of Physiology and Pharmacology (Szepesi et al, 2015; this article serves as main paper for her PhD thesis).

We evaluated the effects of the selective NCX inhibition by ORM-10103 on the $[Ca^{2+}]_i$ transient (CaT), AP, and cell viability in isolated canine ventricular cardiomyocytes exposed to a simulated ischemia/reperfusion protocol performed either alone (modelling moderate low-flow ischemia) or with simultaneous strophantidine challenge (modelling more severe low-flow ischemia). CaTs were monitored using a Ca^{2+}-sensitive fluorescent dye, APs were recorded by intracellular microelectrodes. APs moderately shortened, CaTs prolonged. Diastolic $[Ca^{2+}]$ level increased significantly during ischemia and further elevated following strophantidine application. Reperfusion normalized the NADH level, the amplitude of the AP and duration of the $[Ca^{2+}]_i$ transient, but only partially restored action potential triangulation and the amplitude of the CaT. During reperfusion ORM-10103 decreased $[Ca^{2+}]_i$ and eliminated its diastolic elevation in untreated and strophantidine-treated cardiomyocytes. In conclusion, the selective NCX inhibition is highly effective against ischemia/reperfusion induced pathologic alterations in $[Ca^{2+}]_i$ homeostasis, however, it fails to normalize untoward arrhythmogenic changes in AP morphology. The work was published in a paper accepted by Eur J Pharmacol (Kormos et al, 2014; this article served as main paper for her PhD thesis).

Subtask 6. Investigation of the proarrhythmic effects of different cardiovascular and non-cardiovascular drugs in transgenic rabbit model of LQT.

This subtask was performed together with the consortia partner lead by Dr. Zsuzsanna Bősze, Agricultural Biotechnology Institute, Gödöllő, Hungary, Gödöllő (consortia partner grant number NK-104397).

Our aim was to characterize the LQT5 (long QT5) transgenic rabbit model, which is the third non-mouse, LQT syndrome rabbit model of cardiac arrhythmias worldwide. The first two LQT transgenic rabbit models, LQT1 and LQT2 (Brunner, Peng et al. 2008) were found to be extremely useful, illustrated by the fact, that studying drug induced heart arrhythmia in those rabbits resulted fifteen important publications in less than ten years. The LQT 5 rabbit which was created in our laboratory and characterized together with the consortium leader Prof Andras Varró's research group added to the existing rabbit models. The LQT5 rabbits were created by expressing human KCNE1, carrying a missense mutation identified in a Chinese LQT syndrome family (Ma, Lin et al. 2003). The mutation leads to an amino acid substitution of arginine for glicine at position 52. In cardiac myocytes the I_{Ks} channel is composed of a pore-forming α (KCNQ1) and the modulatory β subunits (KCNE1). Although KCNQ1 alone assembles to form voltage-gated potassium channel, the presence of KCNE1 is required to reproduce the kinetic properties of the native I_{Ks} current (Sanguinetti, Curran et al. 1996). I_{Ks}, the slowly activating cardiac potassium current, is an important determinant of myocardial repolarization. Using high resolution isoelectric focusing followed by immunoprobing the mutant human protein was separated from the wild type rabbit KCNE1 protein in the transgenic rabbit heart samples and could be quantitatively determined. The quantity of the mutant human mink protein was 2:1 that of the endogenous rabbit's. The G52R-KCNE1 mutation causes significant changes in I_{Ks} currents characteristics, e.g., severe reductions of steady- and tail-I_{Ks} and a shift of the voltage-dependent activation towards more

negative potentials. The inability of the mutant G52R-KCNE to modulate the gating properties of I_{Ks} in a normal fashion, leads to a current that resembles the characteristics of currents from KCNQ1 channels not co-assembled with KCNE1 (Harmer, Wilson et al. 2010). It was published earlier that the more chaotic the ventricular rhythm, the greater the probability of TdP initiation (Farkas, Rudas et al. 2010). Following administration of dofetilide, the incidence of typically drug induced Torsades de Pointes (TdP) arrhythmia was significantly higher in LQT5 rabbits compared to wild type littermates. These results strongly suggest that KCNE1 mutant rabbits has higher susceptibility to drug induced arrhythmias and may represent a useful model for testing the proarrhythmic potential of new drugs under development (*Major et al. Br J Pharmacol. 2016*). In the frame of the NK104397 grant as consortia partners, another aim was to create for the first time a transgenic rabbit model of LQT3 syndrome.

Contrary to the existing LQT transgenic rabbit models, which were created by plasmid based pronuclear microinjection method, this model was planned to be created with so called Sleeping Beauty (SB) transgenesis. The first transgenic rabbits by SB transposon mediated transgenesis were created by the participation of our research group (*Katter et al Faseb J. 2012*) in collaboration with the inventors of the SB transgenic method. The SB100 sleeping beauty transposon resulted with highly efficiency, germline transgenic rabbit lines, which were expressing the indicator gene without epigenetic silencing through six generations to date. The advantage of this novel method beyond its efficiency is that due to the transposon mediated mechanism always one transgene copy integrates at one site *(Ivics et al, Nature Protocol 2014*), contrary to the earlier exclusively applied plasmid based microinjection method, when tandem copies were integrated in random numbers, which in some reported cases resulted rearrangements and/or transgene silencing through generations. As we reported earlier to OTKA agencies, the transgene construct carrying the I1768V mutant human SCN5 cDNA under the myosin promoter was cloned in the pT2/BH plasmid and coinjected with the in vitro synthetized SB100x transposase mRNA into one cell rabbit embryos. In spite of our promising pilot experiments, to date we were unable to produce a transgenic rabbit line with the mutant SCN5 (among the 36 born none was transgenic). Experiments are still ongoing to reach the ultimate aim to create the first LQT3 model rabbit. We suppose that the size of the inserted transgene (14.5kb) could be the restricting factor, literature data earlier, reported successful transgenesis with up to 12 kb insert with the SB transposon method, albeit with significantly reduced efficiency (da Silva et al. Gene Ther. 2010*)*. In order to examine the effect of a double transgenic LQT mutation in rabbit - in collaboration with Prof Katja Odening (Univ. of Freiburg) -the LQT2-5 rabbit model was generated by overexpressing the loss-of-function mutants of human HERG (HERG-G628S, loss of I_{Kr}, LQT2) and KCNE1 (KCNE1-G52R, decreased I_{Ks}, LQT5) in the heart. Our preliminary data revealed that the LQT2 and LQT2-5 rabbits exhibit reduced repolarization reserve and are particularly sensitive to ion channel blocking drugs that prolong repolarization. LQT2-5 rabbits may also provide further insights in mechanisms of reduced repolarisation reserve associated with inherited LQTS. Main conclusions on the potential impact of transgenic rabbit models (*Bősze et al, Prog Biophys Mol Biol. 2016*) and perspectives of rabbit models as tools for preclinical cardiac electrophysiology testing (*Baczkó et al. Prog Biophys Mol Biol. 2016*) were published in two reviews.