Final research report

Title of the project: Cardiac electrophysiological changes and altered arrhythmia susceptibility after chronic heavy exercise in animal models

The project consists of two main research subtopics (ST):

1st subtask: Long-term endurance training-induced cardiac adaptation in several animal models (dog, rabbit, guinea pig and rat) of the human athlete's heart.

2nd subtask: Investigation of the cellular mechanism of arrhythmic, antiarrhythmic and proarrhythmic mechanism

As we already reported in the four *Period closing reports*, the project faced two unexpected problems, which affected especially the first subtask. The first problem occurred immediately at the beginning of the project in 2017, when our large animal (dog) treadmill setup has broken down. Due to the complicated administrative system (complicate and slow public tendering process) we could replace them only in 2019. After installing the new setups, in principle would have been able to recover the delay, but starting from March, 2020 the experimental work due to world affecting COVID 19 pandemic have been again delayed (second problem). Due to the strict restriction imposed by national and local actions, the PhD students were not allowed for several months (from March, 2020 to July, 2020) to enter into the laboratories, therefore, we should again postpone the minimum 12-16 weeks long physical training program of several dogs. Therefore, in 2020 summer, we decided to request the extension of the project for 6+6-month, request granted by the NKFIH officials. Despite of these difficulties, the delay was recovered in relative short time, and we could perform the intensive training experiments in least additional 20 dogs.

Finally, we were able to investigate the effect of the heavy exercise either in large (dog) or rodent (rabbit, rat and guinea pig) models. Some of the experiments from rodents were already published, the other are under submission and/or even revision, while the result of the experimental work from dogs are prepared for submission. The second subtask was not affected by the first problem (the beak-down of the dog treadmill), and fortunately neither so heavy by the pandemic, since we could perform the research work of the experiments which have been not so depending by experimental laboratory investigation, for example in silico computerized evaluations.

In conclusion we may report that we have successfully finished the research work designed in all subtasks. **We have published a substantial number of conference abstracts and altogether 17 full lengths papers having the cumulative impact factor of 106.985. Nine papers were published in D1, while 4-4 in Q1 and Q2 (Scimago rank) scientific journals. These papers have already received 70 citations from which 41 are independent citations. During implementing the project one colleague obtained his DSc degree, and several PhD students defended their PhD degree by presenting the research connected to this project.**

The following selected main results were obtained:

FIRST subtask: Long-term endurance training-induced cardiac adaptation in animal models of the human athlete's heart and in young competitive athletes

1. Experiments in dogs:

Introduction

Although there is no doubt that physical exercise and competitive sport are healthy, improving quality of life and life expectancy, several tragic sudden deaths involving young competitive athletes were reported in the press in recent years. Fortunately, sudden death among athletes is rare, approximately 1:50,000–1:100,000; however, it is likely that the real prevalence is underestimated by these statistics. Even when different statistics are considered, sudden cardiac death (SCD) has been shown to be two to four times more frequent in young athletes compared to their age-matched population with no sports activities. In general, in only few of the cases is the cause satisfactory established with proper autopsy findings and the majority of the rest is attributed to ventricular fibrillation due to ischemic origin. However, this latter explanation can be challenged, because very often SCD in top athletes does not happen during peak performance when oxygen demand is indeed very high in the myocardium, but it happens at warmup or after training or the game or even at home during rest, which means that the cause and mechanism of SCD due to heavy chronic exercise should be also sought elsewhere. There are only few studies in small rodents as rat investigating the effect of long-term endurance training on heart including different cardiac electrophysiological or morphological parameter. Until now there are no experimental results available on this subject in large animals which has more translational value than those of mice and rats having strikingly different heart rate. Therefore, the aim of the present study is to investigate the possible effect of 4 months' chronic regular heavy exercise on arrhythmia susceptibility and cardiac remodelling in canine experiments.

Methods

The aim of the present study is to investigate the possible effect of 4 months' chronic regular heavy exercise. Beagle dogs from either sex, weighing 9–15 kg, were randomized into sedentary ('Sed', n=12) and trained ('Tr', n=12) groups. All the animals were 12 months old at the beginning of the training. Running sessions were performed on a special dog treadmill system (Dogrunner K9 Racer Treadmill, Dendermonde, Belgium) with a controllable gradient and speed intensity. 'Tr' animals underwent a 16-week-long training session, while 'Sed' group did not participate in the training. The protocol started with a 2-week-long warm-up period, thereafter animals were trained for 5 days a week with 2×90 minutes at speed 12-18 km/h and with 2x50 minutes interval running at speed 4 and 22 km/h a day for 16 weeks. A rest period was regularly applied to maintain appropriate hydration, but the total interruption did not exceed 1.5 hours in total. The training intensity was maintained with the use of 5% to 12% inclination. The training protocol was tested in preliminary experiments and set to the maximum level which could be performed without distress yet. At their peak performance, 'Tr' dogs performed 82 km running per day, the cumulative distance travelled during 16 weeks was 3893 km, which indeed can be considered long-term heavy endurance training.

Investigated parameters

- Echocardiography - was performed at 0 and 16 weeks of the training protocol.

- Electrocardiography - In conscious dogs and ECGs were measured using precordial leads at 0 and at 16 weeks.
- Open chest arrhythmia provocation - were performed under pentobarbital (150 mg/kg i.v) anaesthesia. Four consecutive times of burst pacing (800/min bpm/equal to 13.3 Hz, 3× threshold in voltage, lasting for 1-3-6-9 seconds, were performed to induce ventricular fibrillation. The occurrence and manifestation time of VFs were compared.
- *In vitro* electrophysiology – action potential and transmembrane ion current measurements by applying conventional and patch-clamp technique.

Results

Effect of the chronic endurance training on the heart muscle

The 16 weeks' endurance training in dogs (12 dogs) resulted significantly greater end diastolic and end systolic diameters in the left ventricle comparing it to those of the sedentary controls (12 dogs) or to those measured in the exercised animals before the training protocol started measured by in vivo by echocardiography. In addition, some degree of enhanced fibrosis was also present in the hearts of the exercised dogs (Figure 1).

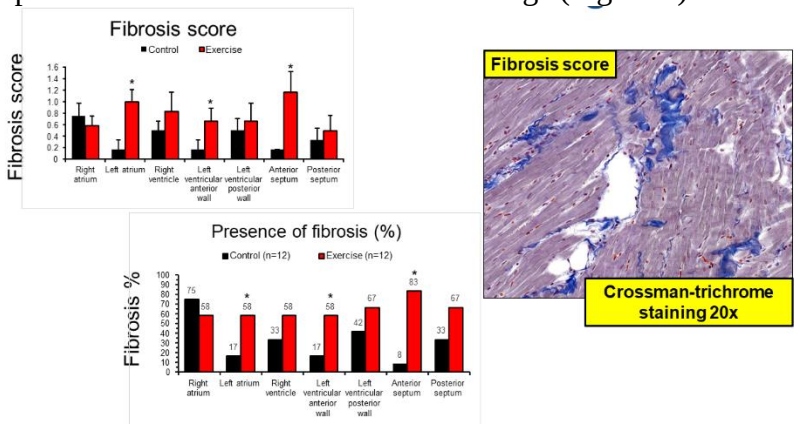


Figure 1. The fibrosis is more pronounced in Exercised (Ex) than in Sedentary (Sed) groups.

Effect of the chronic endurance training on the heart rate

The chronic endurance training caused significant bradycardia in exercised dogs and also slower heart rate was observed in these animals than those in the sedentary controls (Figure 2A).

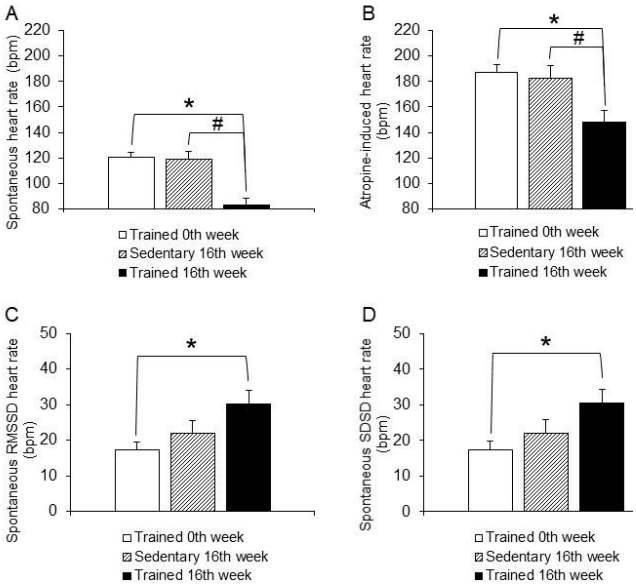


Figure 2. Effect of chronic endurance training on heart rate parameters

To study the heart rate changes independent of the possible alteration of the vagal tone heart rate was also studied after application of 0.04 mg/kg *i.v.* atropine. In these measurements the bradycardia of the exercised dogs was also observed in the exercised dogs but its degree was significantly less than similar measurements taken before atropine administration (Figure 2B). Spontaneously beating isolated right atrial tissue preparation obtained from chronically exercised dogs showed slower spontaneous frequency than those obtained from the sedentary controls (Figure 2C) suggesting that the bradycardia observed in the exercised dogs are not entirely due to possible enhancement of the vagal tone (Figures 2D and 2 E).

ECG changes and enhanced proarrhythmic response

The chronic 16 weeks heavy endurance training is lengthened RR, PQ, QT, QTc intervals and widened QRS significantly in conscious dogs (Figure 3A). The lengthened QT interval was also associated with significantly enhanced QT-STV and Tp-Te interval reflecting elevated level of dispersion of repolarization measured after the completion of training protocol in exercised animals and also comparing it to those measurements taken in the sedentary controls (Figures 3B-3G).

In open chest anaesthetized dogs TdP arrhythmias and consequent ventricular fibrillation (VF) was elicited in 6 out of the 10 exercised dogs while only 3 out of 10 of the sedentary control dogs.

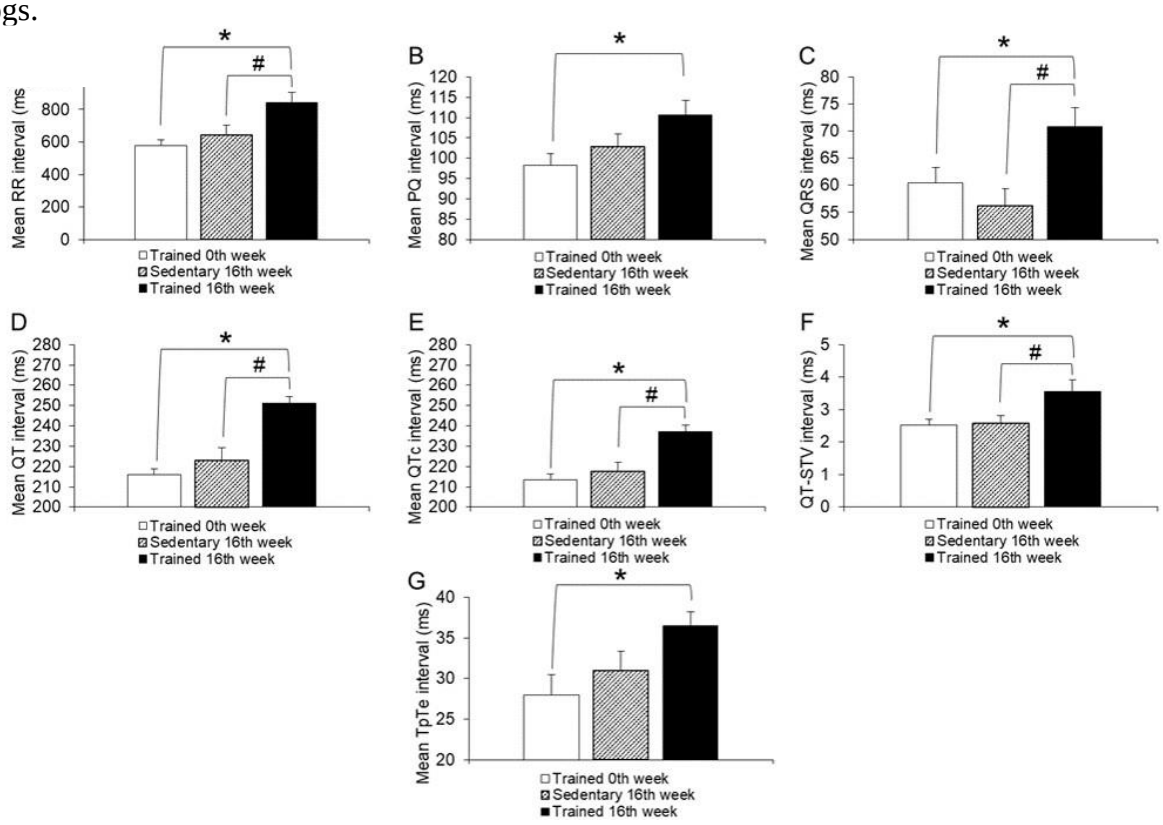


Figure 3. Effect of chronic endurance training on pharmacologically induced arrhythmias

The repolarization reflected as APD_{90} were significantly lengthened in the left ventricular myocytes isolated from the exercised dogs (437.9±29.5 ms, n=14) comparing it to those isolated from the sedentary animals (356±23.9 ms, n=13). The amplitude of the transient outward current (I_{to}) was significantly smaller in myocytes obtained from chronically trained dogs (7.6±0.6 pA/pF, n=54) comparing it to those measured in myocytes obtained from sedentary dogs (10.2±1.0 pA/pF, n=42) but no significant change was observed in the current magnitude of the I_{K1}, I_{Kr}, I_{Ks}, L-type I_{CaL}, I_{NaL} and NCX currents (Figure 4).

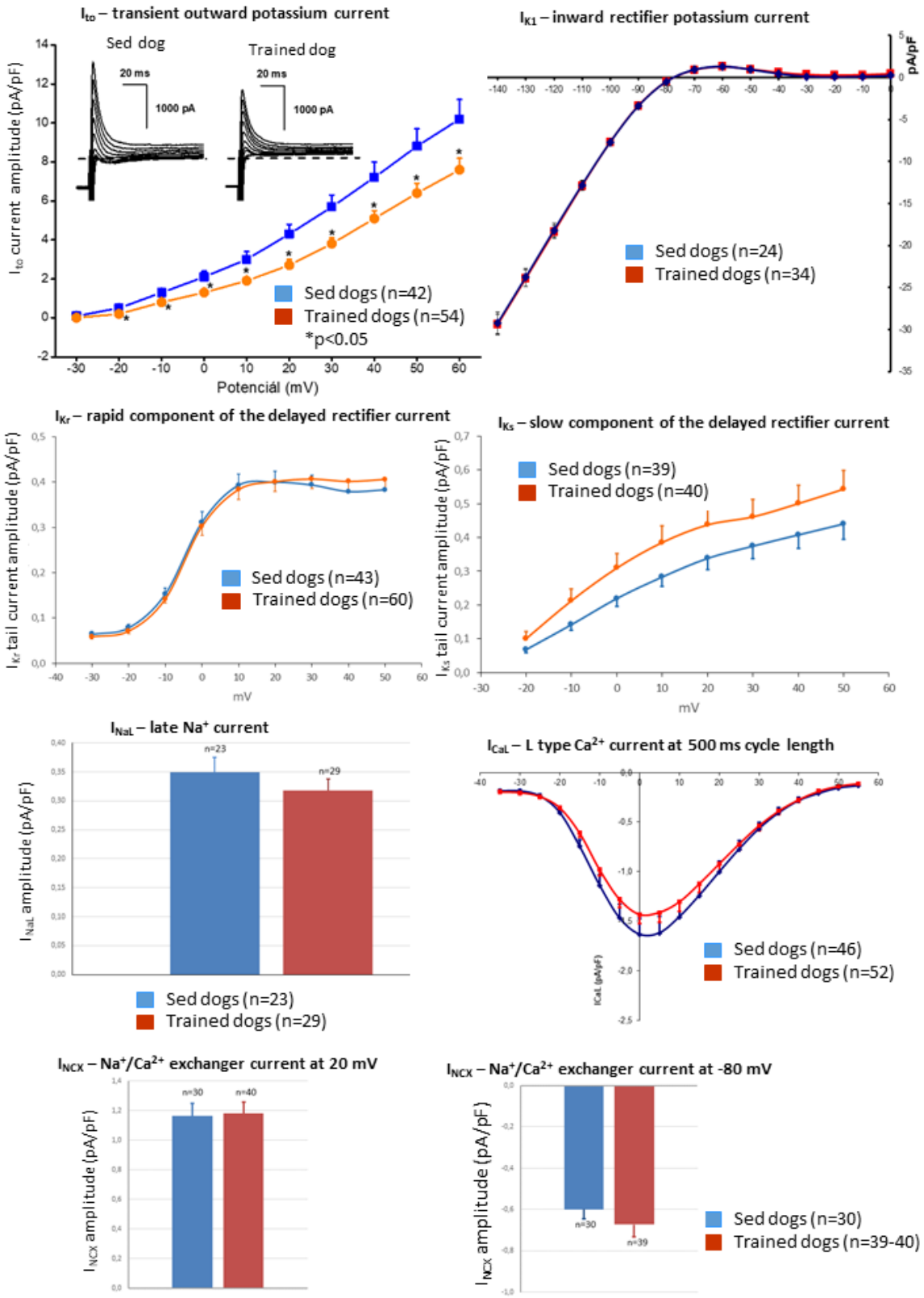


Figure 4. Effect of chronic endurance training on several transmembrane ionic currents (I_{to}, I_{K1}, I_{Kr}, I_{Ks}, L-type I_{CaL}, I_{NaL}, and on forward and reverse NCX currents).

Conclusions

The cardiac morphological changes and the increased parasympathetic tone are characteristics of the athlete's heart. Under certain conditions, repolarization changes (I_{to} and I_{Ks}

downregulation) with elevated fibrotic tissue level may indicate a higher risk of the development of life-threating arrhythmias in athletes. However, further animal experimental investigations are warranted, and it is necessary to improve the in vivo screening methods (electrophysiology, echocardiography, etc.) in top athletes.
These experiments were published as preliminary results in a paper by Polyak et al (Reviews in Cardiovasc Medicine, 2018) and after finishing the second and third set of experiments in 2020/21 a complete new manuscript has been prepared and is ready for submission to a Q1 or D1 Scimago ranked journal.

2. Experiments in rabbits:

Introduction
In the present study, the effect of long-term exercise training was investigated on myocardial morphological and functional remodelling and on proarrhythmic sensitivity in a rabbit athlete's heart model.
Methods
New-Zealand white rabbits ('Ex' group) were trained during a 12-week long treadmill-running protocol and compared to a 'Sedentary' ('Sed') group. At the end of the training protocol, echocardiography, *in vivo* and *in vitro* ECG recordings, proarrhythmic sensitivity studies in isolated hearts, and APD measurements were performed at different potassium concentrations. Expression levels of the slow component of delayed rectifier K^+ current (I_{Ks}) and fibrotic biomarkers were quantified.

Results
ECG PQ and RR intervals were significantly longer and heart rate variability was higher in the 'Ex' group *in vivo* (Figures 5 and 6).

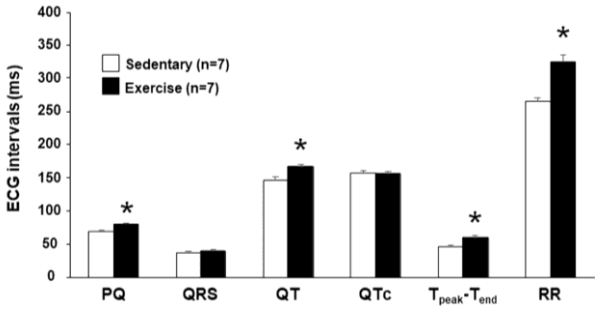


Figure 5. The mean *in vivo* ECG intervals at 12th week. Values were derived from 40 consecutive ventricular complexes during sinus rhythm.

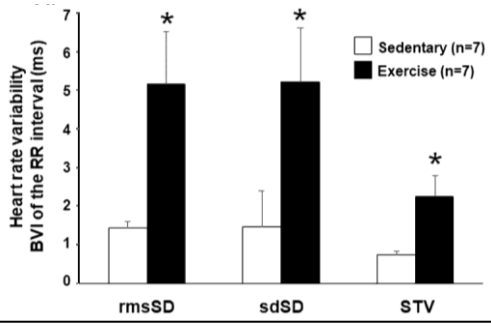


Figure 6. *In vivo* beat-to-beat variability parameters (BVI) of the RR interval at 12th week. rmsSD, Root mean square of successive differences; sdSD, standard deviation of successive differences; STV, Short-term variability.

Echocardiography showed significantly dilated left ventricle in the running rabbits (Figure 7), while RT-qPCR showed a slight decrease in I_{Ks} and significantly greater mRNA expression of fibrotic biomarkers in the 'Ex' group (Figure 8).

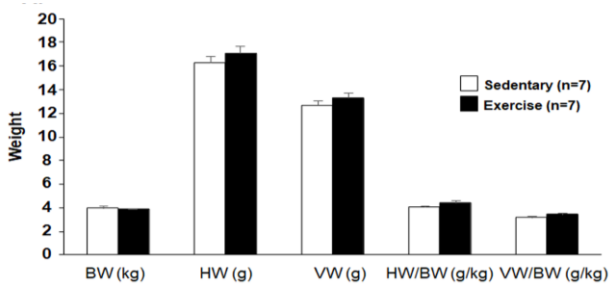


Figure 7. The mean values for body weight (BW), heart weight (HW), ventricular weight (VW), HW/BW and VW/BW ratio at 12th week. Heart weight and ventricular weight are in g and body weight in kg.

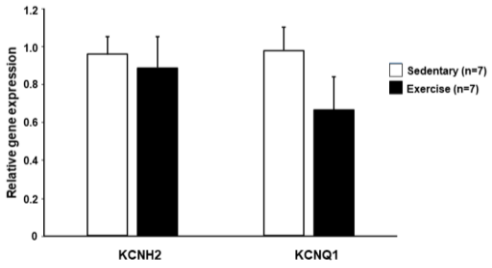


Figure 8. The relative expression levels of I_{Kr}- and I_{Ks}-related genes (B) determined by real-time quantitative RT-PCR.

Dofetilide tended to increase the QTc interval in a greater extent and significantly increased the arrhythmic beats in the 'Ex' group *in vitro* (Figure 9). APD was longer in 'Ex' group at low potassium level (Figure 10).

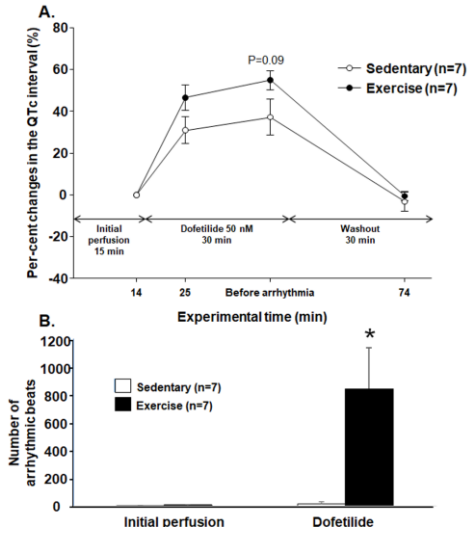


Figure 9. The per-cent change in the mean heart rate-corrected QT (QTc) interval under dofetilide challenge in vitro (A). The total number of arrhythmic beats under dofetilide challenge in vitro (B).

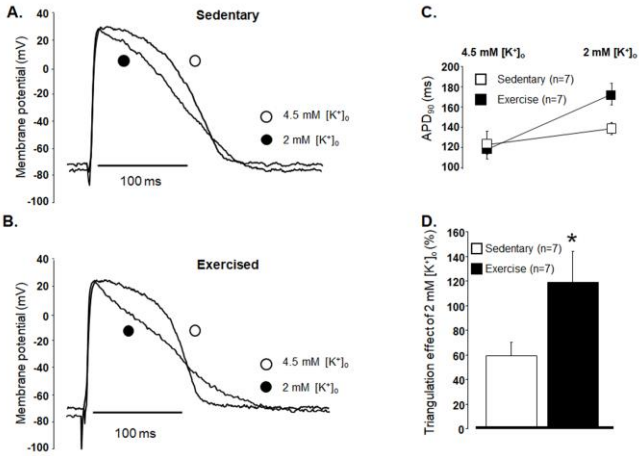


Figure 10. action potentials in papillary muscles obtained from hearts excised from sedentary (A) and exercised (B) animals under normal and low potassium levels. APD_{90} in papillary muscles from sedentary or exercised hearts excised from sedentary and exercised animals under normal and low K^+ levels (C).

Conclusions

Increased repolarization variability with decreased repolarization reserve and increased APD at low potassium level, elevated fibrotic biomarker gene expressions may indicate higher sensitivity of the rabbit "athlete's heart" to life-threatening arrhythmias. The manuscript prepared from this study is under promising revision in Frontiers of Physiology. (Kui P, Polyák A, Morvai N, Tiszlavicz L, Nagy N, Ördög B, Takács H, Leprán I, Farkas A, Papp JG, Jost N, Varró A, Baczkó I, Farkas AS. Long-term physical endurance training alters repolarization in a new rabbit athlete's heart model. Frontiers Physiol, under revision, 2021).

3. Experiments in rats.

This study has been performed in a co-operation together with colleagues from Department of Cardiology, Semmelweis University Budapest (lead by Dr. Tamás Radovits). 12 weeks long swimming exercise-trained and sedentary control Wistar rats were used. The ECG and left ventricular pressure were measured by Langendorff-apparatus, while the ion currents were monitored by the whole cell configuration of the patch clamp technique. The Ca^{2+} transients were measured by fluorescent optical technique and cell contractions were detected by video-edge detector. The exercised group exerted significantly lower resting heart rate, higher incidence of extra beats which could be underlined by enhanced expression level of calsequestrin (Casq), and ryanodine receptors (RyR). In parallel, the phosphorylated PLN oligomer form increased. In line with this, the Ca^{2+} content of the sarcoplasmic reticulum (SR) as well as the Ca^{2+} release and cell shortening showed considerable larger magnitude in the exercised group. Our results indicate that the intensive physical training could be associated with elevated SR Ca^{2+} content as a consequence of modification of the RyR, Casq and PLN complex. However, this remodelling could be an important part of physiological cardiac adaptation mechanism in response of the training it may markedly sensitize the heart for the development of spontaneous Ca^{2+} releases and extra beats.
These investigations were published in paper submitted to Scientific Reports (Gazdag et al, Sci Rep, 2020), and will serve as main paper for the PhD thesis of P. Gazdag.

4. Evaluation of ECG repolarization parameter in young competitive athletes.

In this study performed in co-operation with the colleagues of University of Kragujevac (Serbia) and we investigated ECG repolarization parameters, including QT variability in young athletes. 105 professional athletes (age: 24±5 years) and 105 age-matched healthy volunteers (age: 26±10 years) were enrolled in the study (Figure 11A).

A

Investigated QT variability parameters

Short-term QT interval variability	$STV\text{-}QT = \sum ID_{n+1}\text{-}D_n I/(N\times\sqrt{2})$
Long-term QT interval variability	$LTV\text{-}QT = \sum ID_{n+1}\text{+}D_n\text{-}2D_{mean} I/(N\times\sqrt{2})$
Normalized QT interval variance	$QTVN = SDQT^2/QT_{mean}^2$
QT variability index	QTVI = log (QTVN/RRVN)
Variability ratio	VR = STV-QT/STV-RR

Athletes population

	Controls	Athletes
n	105	105
Sex (male/female)	104/1	104/1
Age (years)	25.5 ± 10.1	23.5 ± 5.4
Body weight (kg)	77.4 ± 14.6	77.4 ± 9.0
Height (cm)	180 ± 7.7	183 ± 7.7*
BMI (kg/m²)	23.9 ± 4.1	23.2 ± 1.8

Mean ± SD
*p < 0.05

- 102 professional football players, 1 judo olympic champion, 1 athletic sport, 1 leisure sport
- All football players are from the first league, training twice daily around 2 hours 5-6 days/week
- Judo olympic champion and athletic sportman have 10+ hours training daily.
- The leisure sport means training 2-3 times weekly up to hour and a half.

B

Conventional ECG repolarization parameters in study subjects

	Controls	Athletes before exercise	Athletes after exercise
RR (ms)	869 ± 148	1014 ± 166***	690 ± 94*** ###
HR (1/min)	71 ± 12	61 ± 10***	87 ± 11*** ###
QT (ms)	390 ± 33	423 ± 32***	370 ± 25*** ###
QTc (ms) Bazett	421 ± 21	422 ± 21	447 ± 18*** ###
QTc (ms) Fridericia	410 ± 20	422 ± 19***	419 ± 18***
QTc (ms) Framingham	410 ± 20	421 ± 20***	418 ± 16***
QTc (ms) Hodges	410 ± 20	425 ± 21***	420 ± 15***
Tpeak-Tend (ms)	99 ± 24	96 ± 13	82 ± 12*** ###
QTd (ms)	35 ± 11	38 ± 13	36 ± 11

Mean ± SD, n = 105 in each group; ***p < 0.001 vs. control; ###p < 0.0001 vs. before exercise

Figure 11. Analysis of ECG repolarization parameters in young competitive athletes. Personal data (A panel) and ECG parameters (B panel).

Twelve-lead electrocardiograms were recorded before and following treadmill exercise testing, the ECGs were digitized and evaluated off-line. We determined the frequency corrected QT interval (QTc), QT dispersion (QTd), Tpeak-Tend distance (Figure 11B), and among QT variability parameters: QT variance normalized for QT mean (QTVN), QT variability index (QTVI), variability ratio (VR), the long-term and the short-term beat-to-beat

QT interval variability (LTV-QT and STV-QT) parameters based on constructed Poincaré plots (Figure 12).

QT variability parameters in athletes and in controls

	Controls	Athletes before exercise	Athletes after exercise
STV-RR (ms)	26.4 ± 18.7	43.2 ± 33.3***	6.2 ± 8.6*** ###
STV-QT (ms)	3.41 ± 0.8	4.29 ± 1.2***	3.77 ± 1.1* ##
LTV-QT (ms)	4.09 ± 1.1	5.12 ± 1.7***	4.45 ± 1.7#
QTVN	-3.84 ± 0.2	-3.71 ± 0.2**	-3.72 ± 0.2**
QTVI	-1.18 ± 0.6	-1.27 ± 0.4	-0.21 ± 0.6*** ###
VR	0.24 ± 0.4	0.16 ± 0.2*	1.19 ± 0.9*** ###

Mean ± SD, n = 105 in each group;
*p < 0.05 vs. control; **p < 0.001 vs. control ; ***p < 0.0001 vs. control;
#p < 0.05 vs. before exercise; ##p < 0.001 vs. before exercise; ###p < 0.0001 vs. before exercise

Figure 12. QT variability parameters in athletes and controls.

Heart rate was significantly lower in professional athletes at rest (62±11 vs. 71±12/min in controls, $p<0.0001$). The QT interval was prolonged in athletes at rest (419±34 vs. 390±32 ms in controls, $p<0.0001$). QTc was significantly longer in athletes before and after treadmill exercise testing compared to controls calculated with Framingham, Fridericia and Hodges correction formulas. However, the QTd and Tpeak-Tend intervals at rest did not differ significantly. Importantly, the STV-QT was significantly higher in athletes both at rest and following treadmill exercise testing compared to controls (4.24±1.1 and 3.68±1.1 vs. 3.36±0.8 ms, both $p<0.001$, respectively). LTV-QT was significantly higher in athletes at rest; QTVI and VR were significantly higher in athletes after exercise compared to controls and compared to athletes at rest.

Some of the alterations in repolarization parameters and the significant increase in STV-QT may indicate increased repolarization instability in competitive athletes compared to age-matched controls, however, further studies are needed to relate this finding to increased arrhythmia propensity in this population.

This project was presented at several domestic and international congresses and is prepared to be published in a Q1 ranked sport medicine journal (Vagvolgyi A, Nikolic I, Stojmenovic T, Dikic N, Nedeljkovic I, Szabó LA, NemesA, Varro A, Lengyel C, Baczko I, Jakovljevic V, Orosz A, prepared for submission, Sports Health, 2021.

Second subtask: Investigation of the cellular mechanism of arrhythmic, antiarrhythmic and proarrhythmic mechanism

1. Evaluation of possible proarrhythmic potency: comparison of the effect of several cardiac and seemingly harmless non-cardiac drugs on native I_{Kr} and hERG currents and in cardiac action potential.

It is known that otherwise seemingly harmless medications can block the fast component of the delayed rectifier potassium (I_{Kr}) current, conducted by hERG channels, that leads to repolarization prolongation and can sometimes result in proarrhythmic side effects and very rarely in sudden cardiac death. Therefore, we have investigated, compared and evaluated the

possible proarrhythmic potency of several drugs known to possess proarrhythmic side effects by applying of both automated and manual patch-clamp methods on HEK293 cells. The following drugs were tested in native I_{Kr} currents and HERG currents and on cardiac action potential: dofetilide, cisapride, sotalol, terfenadine, and verapamil. Dofetilide, cisapride, sotalol, terfenadine, and verapamil blocked hERG channels at 37C with an IC_{50} of 7 nM, 18 nM, 343 nM, 165 nM, and 214 nM, respectively. Using manual patch-clamp, the IC_{50} values of sotalol and terfenadine were 78 mM and 31 nM, respectively. The IC_{50} values calculated from I_{Kr} measurements at 37 C were 13 nM, 26 nM, 52 nM, 54 nM, and 268 nM, respectively. Cisapride, dofetilide, and sotalol excessively lengthened, terfenadine, and verapamil did not influence the action potential duration. We concluded that: *i)* several known even non cardiac drugs may possess potential proarrhythmic side effects. Therefore, individuals taking even seemingly harmless medications under proper medical control should not be concerned about proarrhythmic side effects, however, their administration may add to increased risk for serious arrhythmia development in persons associated with subsidiary risk factors including certain diseases or genetic defects that impair repolarization, as well as in individuals taking part in top competitive sports activities; *ii)* Relative variability is increased by ion channel blockers that decrease the negative feedback control of APD (i.e. blockers of I_{Ca}, I_{Kr} and I_{Ks}) and also by elevation of cytosolic Ca^{2+}. Cardiac arrhythmias are also often categorized according to the characteristic heart rate (tachy- and bradyarrhythmias). Tachycardia is proarrhythmic primarily due to the concomitant Ca^{2+} overload causing delayed afterdepolarizations. Early afterdepolarizations (EADs) are complications of the bradycardic heart. What is common in the reverse rate-dependent nature of drug action on APD, increased SV and EAD incidence associated with bradycardia.
These investigations were published in a research paper accepted in Toxicol Sci (D1 ranked; Orvos P, et al, 2019), and were discussed in a review paper in Progress Biophys Mol Biol (Q1 ranked; Nánási et al, 2020). These papers served as main publications for the PhD thesis of Péter Orvos.

2. Cardiac electrophysiological effects of ibuprofen in dog and rabbit ventricular preparations

We studied the effects of ibuprofen on action potential characteristics and several transmembrane ionic currents. In dog papillary muscles, ibuprofen moderately but significantly prolonged repolarization at 1 Hz stimulation frequency. In dog Purkinje fibres, repolarization was abbreviated, and maximal rate of depolarization was depressed in a frequency-dependent manner. In dog myocytes, ibuprofen (250 μM) did not significantly influence I_{K1}, but decreased the amplitude of I_{to} and I_{Kr}, I_{NaL} and I_{Ca} currents. We conclude that ibuprofen seems to be free from effects on AP parameters at lower concentrations. However, at higher concentrations it may alter repolarization reserve, contributing to the observed proarrhythmic risk in patients. This investigation was published in Can J Physiol and Pharmacol (Q2 ranked; Pászti et al, 2021) and will serve as main paper for the PhD thesis of Bence Pászti in last trimester of 2021.

3. Investigation of positive inotropic effect of a novel NCX blocker ORM-11372

A distinctively different NCX 1.1 inhibitor, ORM-11372, was discovered and investigated in an international co-operation. Its potency against human and rat NCX 1.1 and selectivity against other ion channels was assessed. The cardiovascular effects of ORM-11372 were studied in normal and infarcted rats and rabbits. ORM-11372 inhibited human NCX 1.1 reverse and forward currents; IC50 values were 5 and 6 nM respectively. ORM-11372 inhibited human cardiac sodium 1.5 (I_{Na}) and hERG KV11.1 currents (I_{hERG}) in a

concentration-dependent manner. ORM-11372 caused no changes in action potential duration; short-term variability and triangulation were also observed. ORM-11372 induced positive inotropic effects in anaesthetized rats with myocardial infarctions and in healthy rabbits respectively; no other haemodynamic effects were observed, except improved relaxation at the lowest dose. These investigations published in Br J Pharmacol (D1 ranked journal; Otsomaa, et al, 2020).

4. Investigation of the electrical restitution modifications by antiarrhythmic drugs in canine and undiseased ventricular muscle

The action potential duration (APD) of an extrasystole depends on the proximity of the preceding beat, and the relation between its timing and its APD is called electrical restitution. The aim of the present work was to study and compare the effect of several antiarrhythmic drugs on restitution in preparations from canine and undiseased human ventricular muscle. In human ventricle, restitution kinetics were slower in preparations with large phase 1 repolarization with shorter APDs at 1000 ms BCL compared to preparations with small phase 1. Preparations having APD longer than 300 ms at 1000 ms BCL had slower restitution kinetics than those having APD shorter than 250 ms. The selective I_{Kr} inhibitors E-4031 and sotalol increased overall APD and slowed the restitution kinetics, while I_{Ks} inhibition did not influence APD and electrical restitution. In coupled preparations amiodarone decreased the difference in APDs between PF and VM, thus decreasing dispersion. In the same preparations dofetilide increased the dispersion by causing a more pronounced prolongation in PF. Mexiletine and nisoldipine shortened APD, but only mexiletine slowed restitution kinetics. These results indicate that although basic APD has an important role in restitution, other transmembrane currents, such as I_{Na} or I_{to}, can also affect restitution kinetics. These investigations were published in Frontiers Pharmacol (Q1 ranked journal; Árpádffy et al, 2020) and Can J Physiol Pharmacol (Árpádffy et, 2021), and will serve as main paper for the PhD thesis of Tamás Árpádffy-Lovas in 2022.

5. The electrophysiological effect of cannabidiol on hERG current and in guinea-pig and rabbit cardiac preparations

Cannabis use is associated with cardiovascular adverse effects ranging from arrhythmias to sudden cardiac death. The exact mechanism of action behind these activities is unknown. The aim of our work was to study the effect of cannabidiol (CBD), tetrahydrocannabinol and 11-nor-9-carboxy-tetrahydrocannabinol on cellular cardiac electrophysiological properties including ECG parameters, action potentials, hERG and I_{Kr} ion channels in HEK cell line and in rabbit and guinea pig cardiac preparations. CBD increased action potential duration in rabbit and guinea pig right ventricular papillary muscle at lower concentrations but did not significantly change it at 10 μM. CBD at high concentration (10 μM) decreased inward late sodium and L-type calcium currents as well. CBD inhibited hERG potassium channels with an IC_{50} value of 2.07 μM at room temperature and delayed rectifier potassium current with 6.5 μM at 37 °C, respectively. The frequency corrected QT interval (QT_c) was significantly lengthened in anaesthetized guinea pig without significantly changing other ECG parameters. Although the IC_{50} value of CBD was higher than literary C_{max} values after CBD smoking and oral intake, our results raise the possibility that hERG and potassium channel inhibition might have a role in the possible proarrhythmic adverse effects of cannabinoids in situations where metabolism of CBD impaired and/or the repolarization reserve is weakened. This investigation was published in Sci Reports (D1 ranked journal; Orvos et al, 2020).

6. Investigation of the cardiac late sodium current in human, guinea pig and doc cardiac preparations.

The late sodium current ($I_{Na\text{-}late}$) has long been known to contribute to plateau formation of mammalian cardiac action potentials. Lately it was considered as possible target for antiarrhythmic drugs. However, many aspects of this current are still poorly understood. The present work was designed to study the true profile of $I_{Na\text{-}late}$ in canine and guinea pig ventricular cells and compare them to $I_{Na\text{-}late}$ recorded in undiseased human hearts. $I_{Na\text{-}late}$ was defined as a tetrodotoxin-sensitive current, recorded under action potential voltage clamp conditions using either canonic- or self-action potentials as command signals. Conventional voltage clamp experiments revealed that the crescendo $I_{Na\text{-}late}$ profile in guinea pig was due to the slower decay of $I_{Na\text{-}late}$ in this species. Sharp microelectrode experiments showed that the action potentials were shortened by tetrodotoxin, which effect was the largest in human, while smaller in canine, and the smallest in guinea pig preparations.
In another study the present study, effects of GS967 (an agent considered as a selective blocker of I_{NaL}.) on I_{NaL} and action potential (AP) morphology were studied in canine ventricular myocytes by using conventional voltage clamp, action potential voltage clamp and sharp microelectrode techniques. The effects of GS967 (1 μM) were compared to those of the class I/B antiarrhythmic compound mexiletine (40 μM). Under conventional voltage clamp conditions, I_{NaL} was significantly suppressed by GS967 and mexiletine, causing 80.4 ± 2.2% and 59.1 ± 1.8% reduction of the densities of I_{NaL} measured at 50 ms of depolarization, and 79.0 ± 3.1% and 63.3 ± 2.7% reduction of the corresponding current integrals, respectively. Both drugs shifted the voltage dependence of the steady-state inactivation curve of I_{NaL} towards negative potentials. GS967 and mexiletine dissected inward I_{NaL} profiles under AP voltage clamp conditions having densities, measured at 50% of AP duration (APD), of -0.37 ± 0.07 and -0.28 ± 0.03 A/F, and current integrals of -56.7 ± 9.1 and -46.6 ± 5.5 mC/F, respectively. Drug effects on peak Na^+ current (I_{NaP}) were assessed by recording the maximum velocity of AP upstroke (V_{max}) in multicellular preparations. Based on these investigations it was concluded that: *i)* At present canine myocytes seem to represent the best model of human ventricular cells regarding the properties of $I_{Na\text{-}late}$. These results should be taken into account when pharmacological studies with $I_{Na\text{-}late}$ are interpreted and extrapolated to human; *ii)* It is concluded that the electrophysiological effects of GS967 are similar to those of mexiletine, but with somewhat faster offset kinetics of V_{max} block. However, since GS967 depressed V_{max} and I_{NaL} at the same concentration, the current view that GS967 represents a new class of drugs that selectively block I_{NaL} has to be questioned and it is suggested that GS967 should be classified as a class I/B antiarrhythmic agent.
These investigations have been performed in co-operation with the colleagues from the Department of Physiology, University of Debrecen lead by Prof. Nánási Péter, and have been published in two Scimago D1 ranked papers J Mol Cell Cardiol (Horváth et al, 2020) and Sci Reports (Hézső et al, 2021).

7. Investigation of the pathogenic role of the p.Glu293Lys variant in Andersen-Tawil syndrome type 1 (ATS1).

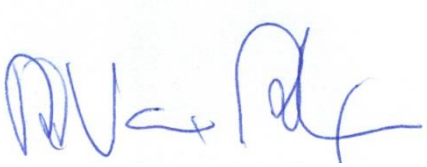
This study aimed to assess the involvement of Glu293 in CD-I subunit interactions and to establish the pathogenic role of the p.Glu293Lys variant in ATS1. The results indicated that the loss of function and dominant-negative effect confirm the causative role of p.Glu293Lys in ATS1. Co-assembly of Kir2.1 subunits is impaired in homomeric channels consisting of p.Glu293Lys subunits and is partially rescued in heteromeric complexes of WT and

p.Glu293Lys Kir2.1 variants. These data point to an important role of Glu293 in mediating subunit assembly, as well as in gating of Kir2.1 channels.
The results of this investigations performed in co-operation with the colleagues from and 2nd Department of Medicine and Cardiology Centre, University of Szeged (lead by Prof. Róbert Sepp) have been published in the prestigious Cardiovascular Research (D1 ranked journal, Deri et al, 2021).

8. Cardiac transmembrane ion channels and action potentials: cellular physiology and arrhythmogenic behaviour

Cardiac arrhythmias are among the leading causes of mortality. They often arise from alterations in the electrophysiological properties of cardiac cells and their underlying ionic mechanisms. It is therefore critical to further unravel the pathophysiology of the ionic basis of human cardiac electrophysiology in health and disease. We have discussed in a detailed review the current knowledge on the differences in ion channel expression and properties of the ionic processes that determine the morphology and properties of cardiac action potentials and calcium dynamics from cardiomyocytes in different regions of the heart. Special attention has been paid on the understanding of the diverse pathophysiology of human cellular electrophysiology, which may help in in developing novel and effective antiarrhythmic strategies for specific subpopulations and disease conditions. The review has been published in Physiology Reviews (the World Top 1 physiology journal, D1 ranked, impact factor 37.312; (Varro et al, Physiol Reviews, 2021).

Szeged, 6th September, 2021



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