THE POSSIBLE PROARRHYTHMIC EFFECTS OF SOME NON–ANTIARRHYTHMIC DRUGS

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Summary of PhD thesis

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LIST OF PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

I. **Paszti B**, Prorok J, Magyar T, Arpadffy-Lovas T, Gyore B, Topal L, Gazdag P, Szlovak J, Naveed M, Jost N, Nagy N, Varro A, Virag L, Koncz I. Cardiac electrophysiological effects of ibuprofen in dog and rabbit ventricular preparations: possible implication to enhanced proarrhythmic risk. *Can J Physiol Pharmacol*. 2021;99(1):102-9.
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II. Orvos P, **Paszti B**, Topal L, Gazdag P, Prorok J, Polyak A, Kiss T, Toth-Molnar E, Csupor-Loffler B, Bajtel A, Varro A, Hohmann J, Virag L, Csupor D. The electrophysiological effect of cannabidiol on hERG current and in guinea-pig and rabbit cardiac preparations. *Sci Rep*. 2020;10(1):16079.
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LIST OF OTHER PUBLICATIONS AND ABSTRACTS

I. Magyar T, Arpadffy-Lovas T, **Paszti B**, Toth N, Szlovak J, Gazdag P, Kohajda Z, Gyokeres A, Gyore B, Gurabi Z, Jost N, Virag L, Papp JG, Nagy N, Koncz I. Muscarinic agonists inhibit the ATP-dependent potassium current and suppress the ventricle-Purkinje action potential dispersion. *Can J Physiol Pharmacol*. 2021;99(2):247-53.
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II. Tibor Magyar, **Bence Pászti**, Tamás Árpádffy-Lovas, András Gyökeres, Zsolt Gurabi, Norbert Jost, András Varró, László Virág, Charles Antzelevitch, István Koncz: Acetylcholine attenuates pinacidil-induced abbreviation of the action potential in canine cardiac Purkinje fibers and papillary muscles. EHRA Internation Congress, Lisbon, Portugal, 17-19 March 2019

III. Árpádffy-Lovas Tamás, Magyar Tibor, **Pászti Bence**, Gurabi Zsolt, Jost Norbert, Charles Antzelevitch, Varró András, Virág László, Koncz István: Az acetilkolin mérsékli a pinacidil akciós potenciál időtartamot rövidítő hatását kutya Purkinje-rostokon és papillaris izmokon. Magyar Élettani Társaság Vándorgyűlése, Szeged, 2018. 06. 27-30.

IV. Zsolt Gurabi, Bence Patocskai, **Bence Pászti**, Balázs Györe, László Virág, Péter Mátyus, Gyula Papp, András Varró, István Koncz: Different electrophysiological effects of the levo- and dextrorotatory isomers of mexiletine in isolated rabbir cardiac muscle. IACS 3rd European Section Meeting, Marseille, France, 1-4 Octobre 2016

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1. Introduction

1.1. Epidemiology of cardiovascular diseases and dispersion of repolarization

According to the World Health Organization, cardiovascular diseases (CVDs) are the first cause of deaths worldwide. Among other factors, arrhythmias are one of the most common precipitating causes of sudden deaths in CVDs. It was postulated that antiarrhythmic drugs would be valuable therapeutic options to prevent sudden death due to arrhythmias, but most of them exert proarrhythmic effects as well. In addition, there are growing evidence that several non–cardiovascular drugs, such as macrolide antibiotics, antihistamines, gastrointestinal or central nervous system drugs can cause arrhythmias as side effects although with lower incidence.

Arrhythmia is defined as an alteration from physiological heart rhythm or rate. Cardiac arrhythmias can be induced by altered or abnormal impulse generation, or abnormal impulse conduction. In my thesis regarding the cellular mechanism of proarrhythmic action of ibuprofen and cannabidiol, I would concentrate on the abnormal impulse conduction caused by dispersion of repolarization. Dispersion of repolarization is attributed to the different phase 1 and phase 3 repolarization characteristics in epicardial, midmyocardial (M cells) and endocardial cells. These heterogeneities can lead to various arrhythmias such as long QT syndrome related Torsades de Pointes (TdP) ventricular tachyarrhythmia or ventricular fibrillation. Acquired LQTS is most frequently caused by different drugs that prolong repolarization and action potential duration (APD) including anti–depressant (e.g., mirtazapine, citalopram), anti–psychotics (e.g., clozapine, haloperidol) or anti–fungal drugs (e.g., fluconazole, ketoconazole).

1.2. Repolarization reserve

Repolarization reserve is defined as an ability to compensate impaired repolarization. Several inward and outward ion currents contribute to the maintenance of repolarization reserve such as the steady–state component of the fast sodium current ($I_{Na,L}$), L-type inward calcium current ($I_{Ca,L}$), rapid and slow component of delayed rectifier outward potassium current (I_{Kr}, I_{Ks}), inward rectifier potassium current (I_{K1}), transient outward potassium current (I_{to}), sodium–potassium pump current ($I_{Na/K}$) and Na^+/Ca^{2+} exchange current (I_{NCX}). In case of $I_{Na,L}$ or $I_{Ca,L}$ are enhanced, plateau voltage is altered in more positive values causing activation of I_{Kr} which could lead to the shortening of repolarization. I_{Ks} thought to have less influence on repolarization than I_{Kr}

during physiologic conditions, but when APs abnormally increased, I_{Ks} signifies a safety reserve protecting the heart from arrhythmias. I_{K1} prevents depolarization of the membrane potential contributing to repolarization reserve in a special way. Inhibition of I_{K1} allows depolarization and the development of extrasystoles leading to ventricular arrhythmias, plus lengthening APD amplifying repolarization heterogeneity. I_{to} current plays an indirect role in repolarization reserve by altering action potential amplitude (APA) and changing the activation and deactivation manners of other currents. $I_{Na/K}$ is an electrogenic outward current contributing to repolarization reserve as well during the entire cardiac cycle.

Overall, repolarization reserve is a very complex entity that is vulnerable. Inhibition or augmentation of ion currents by different drugs or pathophysiologic conditions could lead to the impairments of it. Repolarization reserve impairments contribute to the development of inhomogeneous repolarization; thus, these impairments increase the chance for the formation of ventricular arrhythmias.

1.3. Proarrhythmic effects of non-steroidal anti-inflammatory drugs

Non–steroidal anti–inflammatory drugs (NSAIDs) are widely prescribed for the treatment of pain, fever and inflammation. Ibuprofen counts as a relatively safe drug among other NSAIDs (e.g., paracetamol or aspirin), the incidence of gastrointestinal side effects (e.g., dyspepsia, nausea, vomiting or constipation) is parallel with COX–2 selective agents.

On the other hand, according to a cohort study — appeared in The American Journal of Cardiology —, treatment with ibuprofen significantly increased the risk of arrhythmic event rate. In addition, in a recent Danish study, the risk of developing cardiac arrest was also increased among patients taking ibuprofen. In a case report, combination therapy with ibuprofen and paracetamol was prescribed for a 13–year–old girl for the treatment of hamstring tendinitis. After experiencing palpitations, ibuprofen administration was ceased causing termination of her symptoms. In another case study, the arrhythmogenic and QT prolonging effects of levofloxacin and ibuprofen combination therapy were emerged after a 43–year–old woman was admitted to the Emergency Care Unit because of syncope.

1.4. Possible proarrhythmic effects of cannabinoids

Cannabis is one of the most used illicit drugs all over the world, and the legalization of cannabis use facilitates consumption. The major components of cannabis are cannabinoids, including psychoactive agents — such as tetrahydrocannabinol (THC) — and non–psychoactive compounds like cannabidiol (CBD), cannabichromene (CBC) and cannabigerol (CBG).

Beneficial effects of CBD have reported in experimental models of myocardial infarction in which CBD reduced infarct size and the number of ventricular arrhythmias. Nonetheless, there are cannabis–based medicines containing well known amounts of cannabinoids, the inappropriate use of CBD oils has a rise in popularity in the past years as a wonder substance for the treatment of cancer, autism or epilepsy. The main problem with these over–the–counter food supplements is that these products contain unknown amount of CBD and/or THC exposing patients to high health risk.

1.5. Aims of the study

The effects of ibuprofen and cannabidiol on the cardiac action potential parameters have not yet been reported in larger animals, closer to human in basic electrophysiologic characteristics and size. Thus, the purpose of the present study was to investigate the cardiac electrophysiological effects of ibuprofen and cannabidiol in guinea–pig, rabbit and dog papillary muscle and Purkinje fiber preparations. In order to elucidate their possible proarrhythmic side effects, action potential characteristics — including the resting membrane potential (RMP), the action potential amplitude (APA), the maximum rate of depolarization (V_{max}) and the action potential duration at 90%, 75% and 50% of repolarization (APD_{90}, APD_{75}, APD_{50}) — were measured during *in vitro* experiments.

2. Materials and methods

2.1. Conventional microelectrode technique

Conventional microelectrode technique was used to record action potentials of ventricular papillary muscle and Purkinje fiber preparations of rabbit, canine and guinea–pig. Beagle dogs, New Zealand rabbits and adult guinea–pigs of both sexes, weighing 10–15 kg (canines), 2–3 kg (rabbits) and 600–800 g (guinea–pigs) were used. The preparations were placed into a bath as soon as possible and perfused with Locke's solution. A gas mixture of oxygen (95%) and carbon dioxide (5%) was used to hold the pH between 7.35

and 7.40 at a temperature of 37 °C. During the equilibration period, ventricular papillary muscle preparations were stimulated at a basic cycle length of 1000 ms, and Purkinje fibers were stimulated at a basic cycle length of 500 ms. Electrical pulses (S_1) of 0.5–2 ms in duration were delivered to the preparation through a bipolar platinum electrode, and threshold was adjusted twice as high as the physiological threshold in intensity. During the experiments, glass capillary microelectrodes filled with 3 mol/L KCl solution (tip resistance was 10 to 20 MΩ) were used to record action potentials. With these microelectrodes, the preparations were impaled, and the intracellular recordings were displayed on a storage oscilloscope (Hitachi V-555). Data were processed by a computer system (APES home–made software) designed for on–line determination of action potential parameters.

2.2. Protocols and action potential parameters

Test protocol, cycle length dependent protocol and recovery kinetic protocol were applied. The following parameters were measured: resting membrane potential (RMP), action potential amplitude (APA), maximum rate of depolarization (V_{max}), action potential duration at 90%, 75% and 50% of repolarization (APD_{90}, APD_{75}, APD_{50}). Protocols were measured both in control conditions and after application of the drugs. Control recordings were obtained after equilibration period. The effects of ibuprofen and cannabidiol were determined at the given concentrations, recording after 30 minutes of exposure. For all experiments, ibuprofen and cannabidiol were dissolved in DMSO at stock solution of 25 mmol/L (for ibuprofen) and 10 mmol/L (for cannabidiol).

2.3. Statistical analysis

All data expressed as mean value ± standard error of the mean (S.E.M.). Statistical comparisons were made using Student's t-test for paired data and variance analysis (ANOVA) for repeated measurements, followed by Bonferroni's post–hoc test. To calculate the kinetic time constant of the APD_{90} restitution curves, data curves were fitted by a mono–exponential equation. Significant differences were defined when the p value was under 0.05 ($p < 0.05$) and super significance was determined when the p value was under 0.01 ($p < 0.01$). The number of experiments is indicated as "n" for each experimental group.

3. Results

3.1. Cardiac electrophysiological effects of ibuprofen

3.1.1. Effects of ibuprofen on transmembrane action potential parameters in ventricular papillary muscle preparations

In rabbit ventricular papillary muscle preparations, ibuprofen at 100 μM concentration slightly but statistically significant manner increased APD_{90} from 163.5 ± 11.1 ms to 168.5 ± 11.2 ms ($n = 7$, $p < 0.05$) and APD_{75} from 149.4 ± 10.1 ms to 155.7 ± 10.9 ms ($n = 7$, $p < 0.05$), respectively. In canine right ventricular papillary muscle preparations, ibuprofen at 200 μM concentration, significantly lengthened the APD_{90} by 4.3 ± 1.0% ($n = 6$; $p < 0.01$) and the APD_{75} by 4.5 ± 1.3% ($n = 6$; $p < 0.05$). In human right ventricular papillary muscle preparations, ibuprofen was applied in concentrations of 50 μM and 150 μM, cumulatively. Neither low nor high concentration of ibuprofen prolonged APD ($n = 4$) or change other action potential parameters significantly.

3.1.2. Effects of ibuprofen on transmembrane action potential parameters in dog Purkinje fiber preparations

Ibuprofen (at both 50 μM and 200 μM concentrations) dose–dependently and significantly shortened the APD_{90} by 1.1 ± 0.3% at 50 μM ($n = 6$, $p < 0.05$) and by 4.5 ± 0.7% at 200 μM ($n = 7$, $p < 0.01$). The drug decreased APD_{75} from 225.9 ± 9.3 ms to 224.1 ± 9.0 ms at 50 μM ($n = 6$) and from 226.6 ± 12.3 ms to 217.8 ± 12.2 ms at 200 μM ($n = 7$, $p < 0.01$). All the other parameters (RMP, APA, V_{max}) remained unchanged. Various stimulation cycle lengths were also applied in canine Purkinje fibers ranging from 300 ms to 1000 ms. Ibuprofen at 200 μM concentration decreased V_{max} and shortened the APD_{90} in a frequency–dependent manner. The APD_{90} abbreviation was more pronounced at higher cycle lengths, and V_{max} depression was marked at rapid cycle lengths.

3.1.3. Electrophysiological effects of ibuprofen in combination with levofloxacin or acetylcholine

3.1.3.1. The effects of ibuprofen and levofloxacin combination in rabbit right ventricular papillary muscle preparations

We have examined the effects of ibuprofen and levofloxacin combination in rabbit right ventricular papillary muscle preparations. Ibuprofen (at 100 μM concentration) significantly prolonged APD_{90} by 3.1 ± 1.1% ($n = 7$, $p < 0.05$) and APD_{75} by 4.2 ± 1.5% ($n = 7$, $p < 0.05$). Levofloxacin alone (at 40 μM

concentration) did not elicit any significant electrophysiological effects on action potential parameters including RMP, APA, V_{max}, APD_{90} and APD_{75}, but the drug intensified the APD_{90} prolongation by 7.5 ± 2.4% (n = 7, p < 0.05) evoked by 100 μM ibuprofen. Addition of levofloxacin after ibuprofen significantly increased APD_{25} by 15.0 ± 5.2% (n = 7, p < 0.05) and APD_{10} by 24.8 ± 10.5% (n = 7, p < 0.05).

3.1.3.2. The effects of ibuprofen and acetylcholine combination in canine right Purkinje fibers

We have also investigated the effects of 50 μM ibuprofen after acetylcholine (ACh) pretreatment to mimic increased vagal tone in canine Purkinje fibers at a basic cycle length of 500 ms. ACh pretreatment slightly but not significantly increased APD_{90} by 4.1 ± 2.3% (n = 6) and APD_{75} by 4.2 ± 2.1% (n = 6). Addition of ibuprofen at 50 μM concentration significantly shortened APD_{90} by 3.3 ± 0.6% (n = 6, p < 0.01) and APD_{75} by 3.2 ± 1.0% (n = 6, p < 0.05).

3.2. Investigation of the electrophysiological effects of cannabidiol (CBD)

3.2.1. Effects of cannabidiol (CBD) on transmembrane action potential parameters in ventricular papillary muscle preparations

In right ventricular papillary muscle preparations of guinea–pigs, CBD was used in concentrations of 2.5 μM and 5 μM. CBD at both 2.5 and 5 μM concentrations lengthened slightly but significantly APD_{90} by 3.2 ± 0.4% (n = 5, p < 0.01) and by 6.3 ± 2.1% (n = 5, p < 0.05), respectively. Furthermore, at 2.5 μM concentration, CBD significantly increased APD_{75} by 3.1 ± 0.4% (n = 5, p < 0.01), APD_{50} by 3.5 ± 0.5% (n = 5, p < 0.01) and APD_{25} by 3.9 ± 1.1% (n = 5, p < 0.05) beside APD_{90} prolongation. Various cycle length–dependent protocol was also applied in right ventricular papillary muscles of guinea–pigs in which CBD at 2.5 μM concentration slightly but not significantly lengthened APD_{90} dominantly at cycle length from 300 to 2000 ms (n = 6).

In rabbit right ventricular papillary muscle preparations, CBD was used in concentrations of 1, 2.5, 5 and 10 μM. CBD significantly but not dose–dependently prolonged APD_{90}. APD_{90} prolongation were: 3.0 ± 1.0% at 1 μM (n = 5, p < 0.05); 6.8 ± 3.0% at 2.5 μM (n = 7, p < 0.05) and 6.8 ± 1.6% at 5 μM (n = 5, p < 0.05). APD_{75} was increased in the same manner, prolongation were: 4.0 ± 1.1% at 1 μM (n = 5, p < 0.05), 7.4 ± 3.2% at 2.5 μM (n = 7, p < 0.05) and 7.0 ± 2.3% at 5 μM (n = 5, p < 0.05). In some experiments, 1 μM and 2.5 μM CBD caused triangulation of the APs, but not in others reflected

as not significant alteration in APD_{90}–APD_{25}. At high (10 μM) concentration, CBD exerted various effects on AP repolarization — including shortening and lengthening of the APD — causing statistically insignificant alteration of APD_{90} (n = 5) or APD_{75} (n = 5). The APD_{90} lengthening effect of 2.5 μM CBD was depended on the stimulation frequency. Prolongation of APD_{90} could be observed dominantly at rapid pacing rates (at 300–1000 ms basic cycle lengths), and at slow pacing rates it vanished gradually.

CBD was applied also in dog right ventricular papillary muscle preparations and the effects of 2.5 μM or 5 μM CBD were examined. The drug at 2.5 μM concentration slightly increased the APD_{90} by 6.2 ± 3.2% (n = 5) and APD_{75} by 6.5 ± 3.5% (n = 5) but these changes were not statistically significant. On the other hand, at 5 μM concentration, the drug significantly prolonged APD_{90} by 10.2 ± 3.7% (n = 5, $p < 0.05$) and APD_{75} by 11.4 ± 4.3% (n = 5, $p < 0.05$).

3.2.2. Effects of cannabidiol (CBD) on transmembrane action potential parameters in dog Purkinje fiber preparations

CBD dose–dependently and significantly increased APD_{90} from 222.1 ± 7.5 ms to 227.4 ± 7.7 ms (n = 6, $p < 0.01$) at 0.3 μM and to 229.7 ± 8.5 ms (n = 6, $p < 0.05$) at 1 μM concentration. APD_{75} was also significantly changed from 202.3 ± 7.2 ms to 207.3 ± 7.8 ms (n = 6, $p < 0.05$) at 0.3 μM concentration. Application of 3 μM CBD further increased the APD_{90} (to 233.8 ± 9.2 ms, n = 6) and APD_{75} (212.9 ± 8.6 ms, n = 6) parameters of the APs, but these results were not significant. Other action potential parameters (RMP, AMP, V_{max}) remained unchanged.

The restitution kinetics of APD induced by 3 μM CBD was also studied in dog Purkinje fibers at basic stimulation cycle length of 500 ms. Premature beats were produced after every 20th basic beat, and the interval between the basic and extra stimuli (diastolic interval, DI) were gradually increased. The APD–DI curves (restitution curves) show that CBD slightly slowed the restitution kinetics of APD from 317.4 ± 38.0 ms to 431.2 ± 48.6 ms (n = 5).

4. Discussion

4.1. Investigation of the electrophysiological effects of ibuprofen

Ibuprofen is a widely used non–steroidal anti–inflammatory drug (NSAID) all over the world for pain, fever, and inflammation relief. The cardiovascular risk of the drug is relatively low, but due to the high risk of COX–2 selective NSAIDs (e.g., rofecoxib or valdecoxib), traditional NSAIDs,

like ibuprofen, need to be reinterpreted. Even the European Medicines Agency (2015) have started a review of high–dose ibuprofen to evaluate the cardiovascular risks of the drug.

4.1.1. Changes in action potential characteristics induced by ibuprofen and its possible mechanisms

The cellular electrophysiological effects of ibuprofen have been investigated in only one previous study. The drug, in concentrations of 5, 10, 20, 40 and 80 μg/ml (24.2–387.8 μM), dose–dependently shortened APD and effective refractory period (ERP) on APs of guinea–pig ventricular papillary muscle preparations. In addition, V_{max} was also depressed in a dose–dependent and frequency–dependent manner. Furthermore, they have also examined the spontaneous APs of sinus nodes of rabbits and observed that ibuprofen dose–dependently decreased the beating rate, the spontaneous depolarization rate and V_{max}. ECG recorded in *in vivo* and *in vitro* experiments revealed that the drug markedly increased QRS duration and RR intervals, however, QTc was decreased. In some experiments, premature contraction and ventricular fibrillation occurred, but after ibuprofen wash–out, these events were vanished. They concluded that all these findings suggest that ibuprofen is able to block fast Na^+ channels and slow Ca^{2+} channels.

Our findings are partially consistent with the previous investigation: we could confirm the frequency–dependent V_{max} and APD depression evoked by ibuprofen in canine Purkinje fibers. On the other hand, our experiments showed that ibuprofen is able to prolong action potential duration at higher therapeutic concentrations in ventricular papillary muscle preparations of rabbits and dogs, but not in human ventricular preparations (though the drug was not tested above 150 μM concentration). With the use of whole–cell configuration of the patch–clamp technique, it was found that ibuprofen moderately, but significantly decreased the amplitude of the late sodium current ($I_{Na,L}$) and L–type calcium current ($I_{Ca,L}$). In addition of the inhibition of $I_{Na,L}$ and $I_{Ca,L}$, the amplitude of the transient outward potassium current (I_{to}) and the rapidly activating delayed rectifier potassium current (I_{Kr}) were also moderately, but significantly decreased after ibuprofen application.

Despite of the contradictory findings, the net effect of ibuprofen on the repolarization of the APs depends on several factors including the distribution of ionic currents. In guinea–pig ventricular myocytes, no I_{to} is presented and moreover, the slowly activating delayed rectifier potassium current (I_{Ks}) plays a greater role in repolarization than I_{Ks} in rabbit, canine or human preparations.

Consequently, in guinea–pig preparations, inhibition of I_{to} and I_{Kr} by ibuprofen has less impact on AP repolarization, therefore, the inhibitory effects of ibuprofen on $I_{Na,L}$ and $I_{Ca,L}$ are more pronounced resulting in the abbreviation of repolarization. Similar repolarization shortening could be observed in Purkinje fibers due to higher density of $I_{Na,L}$ and lower presence of I_{Kr}. On the other hand, in rabbit and dog ventricular preparations, APD lengthening could be observed because of the higher presence of I_{to}, and I_{Kr} contributing to AP repolarization. Additionally, I_{Kr} and I_{to} play an important role in the development of repolarization reserve, therefore, blockage of one or both ion currents could weaken it.

4.1.2. The pro–arrhythmic risk of ibuprofen and levofloxacin combination

The coadministration of NSAIDs and antibiotics is common in clinical practice. The combination of antibiotics with NSAIDs may have synergistic effects resulting in an improved effectiveness against bacteria. Among fluoroquinolones, we chose levofloxacin due to the fact that the drug counts as a relatively safe antibiotic with low proarrhythmic risk. Electrophysiological studies show that levofloxacin did not induce any APD changes in guinea–pig ventricular myocardia or in rabbit Purkinje fiber preparations.

In the present study, our aim was to examine the potential proarrhythmic risk of ibuprofen and levofloxacin combination. Levofloxacin, when applied alone, did not alter AP parameters including APD, APA, V_{max} and RMP. However, the application of levofloxacin after ibuprofen pretreatment was markedly lengthened APD even though ibuprofen alone caused a moderate APD prolongation. Levofloxacin may inhibit human ether–a–go–go–related gene (hERG) channel which can interfere additively with I_{Kr} blocking property of ibuprofen leading to enhanced APD prolongation. Our experiments, conducted with the combination of ibuprofen and levofloxacin, indicate that even if a drug does not prolong repolarization considerably, the combined effects of two or more drugs could enhance APD prolongation additively by decreasing repolarization reserve. Furthermore, in other situations when repolarization reserve is already weakened, e.g., in heart failure, ischemic heart disease or hypertrophic cardiomyopathy, these drugs may cause further repolarization impairments leading to ventricular arrhythmias or even sudden cardiac death.

4.1.3. Other possible mechanism of cardiac actions of ibuprofen

The cardiac rhythm is partially regulated by the balance between eicosanoids in the heart. It is also worth to mention that beside direct effects on cardiac ion channels, ibuprofen could exert its effects on the development of arrhythmias by the inhibition of COX enzymes because by this inhibition, the levels of prostanoids are decreased.

Previous studies have showed that left atrial injection of thromboxane A_2 (TXA_2) could induce ventricular arrhythmias via direct action on cardiac myocytes. On the other hand, the occurrence of ventricular fibrillation could be reduced by prostacyclin (PGI_2) in a canine model of sudden cardiac death. Moreover, PGI_2 seems to have antiarrhythmic properties on aconitine–induced arrhythmias in rats. Furthermore, PGI_2 can significantly reduce the amplitude of early afterdepolarization and the prevalence of ventricular tachycardia in anesthetized dogs, although PGI_2 may increase the atrial and ventricular tachycardias in patients. Prophylactic administration of PGE_2 decreased in severity of catecholamine–induced arrhythmias by 37% (compared with 91% propranolol and 34% ajmaline). PGE_2 dose–dependently decreased the incidence of premature ventricular beats in men and reduced drug–induced TdP ventricular tachyarrhythmia. Evidence about the effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is controversial in the literature. The arrhythmogenic effects of $PGF_{2\alpha}$ depend on the parasympathetic innervation of the heart in anesthetized cats. $PGF_{2\alpha}$ predominantly decreased the prevalence of ouabain–induced arrhythmias in non–vagotomised cats and aggravated them in the vagotomised group. Furthermore, atropine pretreatment considerably decreased the antiarrhythmic effect, and significantly increased the pro–arrhythmic effect of $PGF_{2\alpha}$. In experimental conditions, acetylcholine (ACh) and carbachol (CCh) produces positive inotropic effects in isolated rat hearts and biphasic inotropic response — transient decrease in contractility followed by an increase — in isolated mice left atria. The increase in contractile force is mediated by type 3 muscarinic acetylcholine receptors via activation of COX–2 enzyme. Moreover, acetylcholine, $PGF_{2\alpha}$ and PGD_2 lengthened action potential duration in a same manner.

In order to investigate the interaction between muscarinic agonists and COX enzyme inhibitors, we examined the electrophysiological effects of ibuprofen after acetylcholine pretreatment in dog Purkinje fiber preparations. We have found that acetylcholine moderately but statistically insignificantly increased APD_{90} and APD_{75}. Addition of ibuprofen after acetylcholine significantly decreased APD nearly back to the control conditions while other

action potential characteristics remained unchanged. The shortening of the repolarization was more pronounced compared with the effects of ibuprofen when it was applied alone. These observations suggest that parasympathetic predominance alter the electrophysiological effects of ibuprofen, thus these effects are mediated not only by direct actions on cardiac ion channels but other mechanisms as well.

4.2. The investigation of the cardiac electrophysiological effects of cannabidiol

Cannabidiol (CBD) products have become a popular possibility as an over–the–counter medication in various medical conditions despite the fact that they contain inaccurate quantity of the cannabinoid. In 2018, Epidiolex — containing CBD as an active agent — was approved by the US Food and Drug Administration for the treatment of Lennox-Gastaut syndrome, Dravet syndrome or tuberous sclerosis complex. Beneficial effects of CBD were observed in other disorders as well such as Alzheimer's disease, Parkinson's disease and multiple sclerosis, or substance use disorders, chronic psychosis, anxiety and chronic pain. Moreover, many benign effects were observed in experimental models of cardiovascular diseases such as myocardial infarction, cardiomyopathy or myocarditis as well. The chronic use of cannabidiol (up to 1500 mg/day) is well tolerated in humans but the cannabinoid can interfere with hepatic drug metabolism. Notwithstanding that the clinical use of CBD has risen in the past years, the cardiac side effects of CBD have not yet been reported expansively. Thus, our goal was to deepen our knowledge concerning the possible cardiac electrophysiological effects of CBD using *in vitro* experimental models.

4.2.1. The effects of cannabidiol on action potential characteristics

Our experiments describe the APD lengthening effects of CBD in guinea–pig, rabbit and dog papillary muscle and Purkinje fiber preparations. In ventricular papillary muscles of guinea–pigs and dogs, CBD (2.5 and 5 μM) dose–dependently lengthened action potential duration while other action potential parameters (APA, RMP, V_{max}) were not affected by the cannabinoid. In rabbit ventricular papillary muscles, CBD was examined in concentrations of 1, 2.5, 5 and 10 μM. Similar to the results found in guinea–pigs or in dogs, APD prolongation was gradual in the concentration range of 1–5 μM, but CBD did not increase APD further at the highest tested concentrations (10 μM). CBD exerted either shortening or lengthening of the AP repolarization referring to that CBD might have multiple impact on cardiac ion channels.

Furthermore, the APD lengthening seemed to be frequency–dependent in papillary muscle preparations of guinea–pigs and rabbits, i.e., more pronounced prolongation was observed at rapid cycle lengths than at slow pacing rates. The cardiac electrophysiological effects of CBD were also investigated in Purkinje fibers of canines and we have found that CBD dose–dependently increased APD in a concentration range from 0.3 to 3 μM.

In another study, the effects of CBD were investigated in Purkinje fibers of rabbits in different concentrations and at different pacing rates. In contrast to our findings, they found that CBD at low concentration (0.3 μM) did not alter action potential parameters (APD, V_{max}, RMP, APA) at pacing rate of 15, 60 or 180 beats per minute (bpm) but it shortened action potential duration (APD_{50} and APD_{90}) in a dose–dependent manner at high concentrations (3 and 10 μM) at all pacing rates. These results remained stable after CBD was washed out. Moreover, CBD significantly decreased APA and it seemed to decrease V_{max} at 10 μM concentration at pacing rates of 60 bpm and 180 bpm while RMP remained the same except at the highest, 10 μM concentration at the most rapid, 180 bpm pacing rate.

Furthermore, the restitution kinetics of APD induced by 3 μM CBD was also examined in Purkinje fiber preparations of dogs driven at 500 ms basic cycle length. CBD increased APD and slowed the restitution curve kinetics. Basically, the restitution kinetics of the action potential duration is the process of AP adaptation to extrasystoles occurring with different diastolic intervals. As diastolic interval increases, the APD of the following extrasystole becomes longer. Flattening of the restitution curves prevents fibrillation through prohibition of AP wave break (antiarrhythmic property), and steep restitution curves induce unstable wave propagation resulting in AP wave break and ventricular fibrillation (pro–arrhythmic property). The principal determinant of the slope of the restitution curve might be the lengths of APD of the previous basic beats, which depends mainly on repolarization currents. Applying these observations, CBD, by flattening the slope of APD restitution kinetics, may decrease pro–arrhythmic consequences and prevent ventricular fibrillation in cases of propagating extrasystoles.

4.2.2. Effects of cannabidiol on cardiac ion channels

Previous studies have shown that endocannabinoids, synthetic cannabinoids and CBD can interfere with transmembrane ion channels. The cannabinoid can stimulate non–selective cation ion channels such as human vanilloid–type transient receptor potential (TRPV1, TRPV2 and TRPV3) and

the ankyrin–type TRPA1 channels and inhibit melastatin–type TRPM8 channel contributing to the antiepileptic, analgesic, anti–inflammatory and anti–cancer effects of CBD. In a previous study, CBD inhibited I_{Na}, $I_{Na,L}$, $I_{Ca,L}$, I_{to}, I_{Kr} and I_{Ks} but did not affect I_{K1} on Chinese Hamster Ovary (CHO) and Human embryonic Kidney (HEK) cells. CBD exerted the weakest inhibitory effect on $K_v11.1$ (hERG) channels with IC_{50} value of 15 μM, and the strongest on $K_v7.1$ channels with IC_{50} value of 2.7 μM on CHO cells. In our study we have investigated the inhibitory effects of CBD on cardiac transmembrane ion channels.

Whole–cell patch clamp experiments showed inhibition of I_{Kr} (with IC_{50} value of 6.5 μM) evoked by CBD in rabbit native ventricular myocytes contributing to the lengthening of the AP repolarization. Furthermore, CBD at 10 μM concentration significantly inhibited $I_{Na,L}$ and $I_{Ca,L}$, thus these effects might contribute to the observed AP alterations. In HEK 293 cell line, CBD exerted inhibitory effect on hERG potassium channels with an estimated IC_{50} value of 2.07 ± 0.12 μM. Accordingly, these results suggest that at lower concentrations (1, 2.5 and 5 μM), I_{Kr} and I_{Ks} inhibition together might be responsible for the prolongation of APD which was compensated by the depression of $I_{Ca,L}$ and $I_{Na,L}$ at 10 μM concentration in rabbit ventricular myocytes.

4.2.3. Clinical implications

Researchers have investigated the anticipated risk of TdP ventricular tachyarrhythmia based on the comparison of hERG activity, action potential duration and QT prolongation with QT effects and reports of TdP in humans for 100 drugs. They assessed that at least a 30–fold margin between hERG IC_{50} and C_{max} is adequate to avoid arrhythmogenic consequences. Based on human pharmacokinetic data, the mean maximal measured plasma concentration (C_{max}) of CBD was 0.58 μM and 0.7 μM (at 3 hours) after oral administration of 400 mg and 800 mg of CBD, respectively. The highest plasma concentration was 0.35 μM after cigarette smoking containing 19.2 mg CBD. In our experiment the IC_{50} value was 2.07 μM for the inhibition of hERG channels and 6.5 μM for the inhihibition of I_{Kr} in rabbit ventricular myocytes, i.e., these estimated IC_{50} values of CBD were higher than the reported C_{max} values in patients. The ratios of IC_{50} and C_{max} values are in the range of 2.96–18.57 meaning that CBD may have pro–arrhythmic risks in clinical settings. On the other hand, the electrophysiological effects of CBD on other cardiac transmembrane ion channels can mitigate or aggravate the lengthening of the

APD resulting in altered pro–arrhythmic risk of the cannabinoid. Moreover, C_{max} values of CBD could be elevated in certain diseases or due to drug–drug interactions —such as ketoconazole, amiodarone, verapamil or cimetidine — further increasing the risk of arrhythmogenesis. Furthermore, the co–administration of CBD with drugs lengthening AP repolarization results in an enhanced weakening of the repolarization reserve leading to ventricular arrhythmias or even sudden cardiac death.

5. Conclusion

The most important findings of this PhD thesis of the followings:

1. Ibuprofen, increased action potential duration in ventricular papillary muscle preparations of rabbits and dogs at intermediate therapeutic and higher therapeutic concentrations (100 or 200 μM). Thereby it could decrease repolarization reserve and as such it may represent so far unrecognized enhanced proarrhythmic risk. The drug did not prolong the APD_{90} at 150 μM in human cardiac preparations. In Purkinje fibers of canines, the drug shortened action potential repolarization at low and high therapeutic concentrations and suppressed maximum rate of depolarization at rapid cycle lengths. Moreover, levofloxacin further prolonged action potential duration after the application of ibuprofen even though levofloxacin did not alter action potential characteristics when it was applied alone. These electrophysiological effects of ibuprofen might be the results of direct interaction between the drug and cardiac ion channels. Acetylcholine and ibuprofen combination was applied in canine Purkinje fibers, and we have found that ibuprofen shortened action potential duration after acetylcholine pretreatment in a higher degree compared with the effects of the drug when it was applied without acetylcholine.
2. Cannabidiol prolonged action potential duration in ventricular papillary muscle preparation of guinea–pigs, rabbits and dogs in a concentration range from 1 to 5 μM in a frequency–dependent manner at rapid cycle length, but at higher concentration (10 μM), it did not lengthen action potential repolarization further in rabbit ventricular myocytes. In Purkinje fiber preparations of dogs, CBD increased action potential repolarization in concentrations of 0.3, 1 and 3 μM, and flattened the restitution curve of action potential duration. These alterations could decrease repolarization reserve of the cardiac action potentials contributing to the pro–arrhythmic

risks of CBD resulting in Torsades de Pointes ventricular tachyarrhythmia or even sudden cardiac death.

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