THE POSSIBLE PROARRHYTHMIC EFFECTS OF SOME NON–ANTIARRHYTHMIC DRUGS

Bence József Pászti MD

PhD thesis

Szeged

2021

THE POSSIBLE PROARRHYTHMIC EFFECTS OF SOME NON–ANTIARRHYTHMIC DRUGS

Bence József Pászti MD

PhD thesis

Supervisors:
István Koncz MD, PhD
László Virág, PhD

Department of Pharmacology and Pharmacotherapy
Faculty of Medicine, University of Szeged
Graduate School of Multidisciplinary Medical Sciences

Szeged
2021

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.
Impact factor: 1.946 (2019)

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.
Impact factor: 3.998 (2019)

Impact factor of publications related to the thesis: 5.944

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.
Impact factor: 1.946 (2019)

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

Impact factor of other publications: 1.946
Impact factor of all publication: 7.89

Table of contents

Abbreviations

ACh: acetylcholine

AEA: N–arachidonoylethanolamine

AF: atrial fibrillation

AP(s): action potential(s)

APA: action potential amplitude

$APD_{90,75,50,25}$: action potential duration at 90%, 75%, 50% and 25% of repolarization

AV node: atrioventricular node

Bpm: beats per minute

Ca_v: L–type calcium channel

CBC: cannabichromene

CBD: cannabidiol

CBG: cannabigerol

CCh: carbachol

CHO: Chinese Hamster Ovary cells

COX: cyclooxygenase enzyme

CV: cardiovascular

CVDs: cardiovascular diseases

DAD: delayed afterdepolarization

DI: diastolic interval

EAD: early afterdepolarization

ERP: effective refractory period

GI: gastrointestinal

HEK: Human Embryonic Kidney cells

hERG: human ether–a–go–go–related gene channel

$I_{Ca,L}$: inward calcium current

I_f: ‘funny current’ (the hyperpolarizing–activated current)

I_{K1}: inward rectifier potassium current

$I_{K–ACh}$: muscarinic–gated potassium current (acetylcholine–regulated potassium current)

$I_{K–ATP}$: ATP–sensitive potassium current

I_{Kr}: rapid delayed rectifier potassium current

I_{Ks}: slow delayed rectifier potassium current

I_{Kur}: ultra–rapid delayed rectifier potassium current

$I_{Na,L}$: ‘window’ or late sodium current

$I_{Na/K}$: sodium–potassium pump current

I_{Na}: voltage–dependent sodium current

I_{NCX}: sodium/calcium exchange current

$I_{to,f}$: fast component of the transient outward potassium current

$I_{to,s}$: slow component of the transient outward potassium current

K_{ir}: inward rectifier potassium channel

K_v: voltage–dependent potassium channel

LQTS: long QT syndrome

M cell: midmyocardial cells

Na_v: voltage–dependent sodium channel

NSAID(s): non–steroidal anti-inflammatory drug(s)

PGE_2: prostaglandin E_2

$PGF_{2\alpha}$: prostaglandin $F_{2\alpha}$

PGI_2: prostacyclin

RMP: resting membrane potential

SA node: sinoatrial node

TdP: Torsades de Pointes

THC: tetrahydrocannabinol

TRPA: ankyrin–type transient receptor potential

TRPM: melastatin–type transient receptor potential

TRPV: vanilloid–type transient receptor potential

TXA_2: thromboxane A_2

V_{max}: maximum rate of depolarization

1. Introduction

1.1. Epidemiology of cardiovascular diseases

According to the World Health Organization, cardiovascular diseases (CVDs) are the first cause of deaths worldwide. In 2016, approximately 17.9 million people (31% of all deaths in the world) died in, and 45% of all death in Europe was owing to CVDs (Townsend et al., 2016). 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. According to the Cardiac Arrhythmia Suppression Trial (CAST) study (1989), the abolition of ventricular premature complexes after myocardial infarction would increase survival rate, however surprisingly, the use of encainide, flecainide and moricizine (class I/C, sodium channel blocking antiarrhythmic drugs) worsened it. Similarly, another study (SWORD) by Waldo et al. (1996) have investigated the effects of d-sotalol (class III, potassium channel blocking antiarrhythmic drug) in patients with decreased ejection fraction and found that d-sotalol increased mortality due to arrhythmias compared to placebo group. 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. In harmony with these, in my PhD work, I have studied the possible proarrhythmic effects of two commonly used drugs, ibuprofen and cannabidiol, in a cellular level.

1.2. Cardiac ion channels

Several ion channels are expressed in the heart forming the cardiac action potential (AP). Voltage–gated sodium channels are responsible for the development of voltage–dependent sodium current (I_{Na}) during phase 0 of the APs. Among the nine subtypes of sodium channels ($Na_v1.1$–$Na_v1.9$), $Na_v1.5$ (encoded by SCN5A gene) and $Na_v1.8$ are abundantly expressed in the heart (Priest and McDermott, 2015). The initial fast depolarization of the AP is induced by the activation of the $Na_v1.5$ except in the sinoatrial (SA) node and in the atrioventricular (AV) node (Remme et al., 2009; Priest and McDermott, 2015). The steady–state component of I_{Na}, also known as the 'window' sodium current or late sodium current ($I_{Na,L}$) remain activated during plateau phase of the APs (Attwell et al., 1979). Gain–of–function mutations of the SCN5A gene generate higher sodium influx into the cardiac myocytes causing

long QT syndrome, and the loss–of–function mutations of the gene provoke Brugada syndrome with lower expression of $Na_v1.5$ channels (Wilde and Amin, 2018). According to the Vaughan–Williams's classification of antiarrhythmic drugs, sodium channel blockers belong to the Class I group and they inhibit $Na_v1.5$ channels (Vaughan Williams and Somberg, 1998). $Na_v1.8$ channels (encoded by SCN10A) are presented in human cardiac myocytes and in intracardiac neurons as well (Facer et al., 2011), and it was suggested to contribute to the $I_{Na,L}$ current (Yang et al., 2012).

Among the voltage–gated calcium channels, mainly L–type calcium ($Ca_v1.x$) channels are expressed in the heart developing inward calcium current ($I_{Ca,L}$) and shaping phase 2 (plateau phase) of the APs (Priest and McDermott, 2015). $Ca_v1.2$ (encoded by CACNA1C gene) is the only calcium channels expressed in ventricular myocytes, but beside $Ca_v1.2$, $Ca_v1.3$ (encoded by CACNA1D gene) channels are also expressed in atrial myocytes, SA node and AV node (Priest and McDermott, 2015). Furthermore, $Ca_v3.1$ and $Ca_v3.2$ channels forming T–type Ca^{2+} channels are also found in the SA node facilitating slow diastolic depolarization (Ono and Iijima, 2005). Gain–of–function mutations of the CACNA1C gene are likely to cause prolongation of the action potential duration and non–syndromic long QT syndrome (Wemhoner et al., 2015), and the loss–of–function mutations of the CACNA1D are associated bradycardia and impaired SA node function with normal QRS complex and QT interval (Priest and McDermott, 2015). Calcium channel blocker (notedly non–dihydropyridines, such as diltiazem and verapamil) are group in Class IV in the classification of antiarrhythmic drugs decreasing heart rate and contractility (Vaughan Williams and Somberg, 1998).

The repolarization of the APs is characterized by several voltage–gated and inward rectifier potassium channels. Voltage–gated potassium channels form outward currents contributing to various phases of AP repolarization. Phase 1 repolarization of the APs is developed by the fast and slow components of the transient outward potassium current ($I_{to,f}$ and $I_{to,s}$) that are the results of the activation and inactivation of $K_v4.2/K_v4.3$ ($I_{to,f}$) and $K_v1.4$ ($I_{to,s}$) channels (Varro and Baczko, 2011; Priest and McDermott, 2015). In heart failure, I_{to} is reduced causing prolongation of the AP repolarization, but the inhibition of I_{to} may initiate shortening of the action potential duration as well by indirectly influencing the activation of delayed rectifier potassium currents (Grant, 2009). Augmentation of the I_{to} promotes early repolarization and J wave manifestation in dog left ventricular wedge preparations from the inferior wall of the heart (Koncz et al., 2014). Delayed rectifier potassium currents contribute to the phase 3 repolarization of the AP. $K_v1.5$ (encoded by KCNA5 gene), $K_v11.1$/hERG (encoded by KCNH2 gene) and $K_v7.1$/KvLQT1 (encoded by KCNQ1 gene) channels are the

pore forming units of the ultra–rapid delayed rectifier potassium current (I_{Kur}), the rapid delayed rectifier potassium current (I_{Kr}) and the slow delayed rectifier potassium current (I_{Ks}), respectively (Grant, 2009; Priest and McDermott, 2015). I_{Kur} is mainly expressed in atrial myocytes, therefore pharmacological inhibition of the current may have beneficial effects in atrial fibrillation (Priest and McDermott, 2015). I_{Kr} is highly expressed in left atrium and ventricular myocytes (Grant, 2009), and it is considered as one of the most important repolarizing current (Varro and Baczko, 2011). I_{Ks} is expressed in atria and ventricles as well, but the expression of the current is diminished in midmyocardial cells (Grant, 2009). In normal setting, I_{Ks} plays a subsidiary role in AP repolarization, but under certain conditions, when repolarization would be prolonged, the activation of the current maintains AP duration (Varro and Baczko, 2011).

Inward rectifier potassium channels transport potassium ions at hyperpolarized membrane potentials due to the voltage–dependent inhibition of intracellular magnesium ions and cytoplasmic polyamine such as spermine, spermidine or putrescine (Vandenberg, 1987; Lopatin et al., 1994). Inward rectifier potassium current (I_{K1}) are responsible for the termination of phase 3 repolarization and the maintenance of the resting membrane potential of cardiac APs. $K_{ir}2.1$ (encoded by KCNJ2 gene) and $K_{ir}2.3$ are the main elements of I_{K1} current in ventricular and atrial myocytes, respectively (Melnyk et al., 2002). Andersen-Tawil syndrome is caused by the loss–of–function mutations in KCNJ2 gene leading to long QT interval (Donaldson et al., 2004). $K_{ir}3.1$ and $K_{ir}3.4$ are the dominant components of muscarinic–gated potassium current ($I_{K–ACh}$) expressed in atria, sinoatrial and atrioventricular nodes, therefore contributing to the parasympathetic regulation of cardiac pacemaker activity by slowing spontaneous depolarization phase of the AP (Grant, 2009; Mesirca et al., 2013; Priest and McDermott, 2015). ATP–sensitive potassium channels are formed by $K_{ir}6.2$ in atrial and ventricular myocytes, and form ATP–sensitive potassium current ($I_{K–ATP}$) (Priest and McDermott, 2015). The activation of these channels is modulated by the intracellular level of ATP providing a protective factor during ischemic preconditioning (Ashcroft, 1988; Liang, 1996). Acetylcholine inhibits the $I_{K–ATP}$ and thus decreases the ventricle–Purkinje APD dispersion (Magyar et al., 2021).

The spontaneous activity of sinoatrial and atrioventricular cells is generated dominantly by the hyperpolarizing–activated channel, so called I_f current during phase 4 of the action potential (DiFrancesco, 1985). The I_f current is an inward cation current mediated by intracellular cyclic adenosine monophosphate (cAMP) levels. Activation of the these channels by hyperpolarization depolarizes sarcolemmal membrane reaching the threshold potential and activating the L-type calcium current ($I_{Ca,L}$).

1.3. Dispersion of repolarization as a mechanism of cardiac arrhythmias

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 (Antzelevitch et al., 1991). In epicardial and M cells, spike and dome morphology can be observable because of a prominent I_{to} during phase 1 of the AP (Litovsky and Antzelevitch, 1988; Antzelevitch et al., 1991). M cells of canines have a smaller presence of I_{Ks} and a larger presence of $I_{Na,L}$ and Na^+/Ca^{2+} exchange current (I_{NCX}) than epicardial or endocardial cells, thus APs of M cells are prolonged (Liu and Antzelevitch, 1995; Zygmunt et al., 2000 and 2001).

These heterogeneities can lead to various arrhythmias such as long QT syndrome related Torsades de Pointes (TdP) ventricular tachyarrhythmia or ventricular fibrillation. In general, long QT syndromes (LQTS) could be congenital or acquired, but both are associated with long QT interval in the electrocardiogram (ECG) and with an increased risk of TdP ventricular tachycardia or sudden death (Watanabe et al, 2005). 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) (Fazio et al., 2013). Administration of these drugs amplifies spatial dispersion of repolarization and induce early afterdepolarization (EAD) leading to TdP polymorphic ventricular arrhythmia (Belardinelli et al., 2003). Agents that reduce I_{Kr} or I_{Ks} or augment $I_{Ca,L}$ or $I_{Na,L}$ increase the formation of transmural dispersion of repolarization and escalate development of triggered activity causing QT prolongation and reentry (Antzelevitch and Burashnikov, 2011).

1.4. Repolarization reserve

The definition of repolarization reserve is derived from Roden (1998; 2008) who stated: ”The concept of ‘repolarization reserve’, the idea is that the complexity of repolarization includes some redundancy. As a consequence, loss of 1 component (such as I_{Kr}) ordinarily will not lead to failure of repolarization…”.

Several inward and outward ion currents contribute to the maintenance of repolarization reserve. The plateau phase and repolarization of the APs is shaped by 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 (Varro and Baczko, 2011). On the other hand, Virag et al. (2009) have showed that when APs are already prolonged, I_{Kr} (and I_{K1} also) represented a positive feedback mechanism further lengthening repolarization. I_{Ks} thought to have less influence on repolarization than I_{Kr} during physiologic conditions, but when duration of APs abnormally increased, I_{Ks} signifies a safety reserve protecting the heart from arrhythmias (Varró, 2000; Carmeliet, 2006). I_{K1} are open at the end of repolarization and during diastole, thus it prevents depolarization of the membrane potential contributing to repolarization reserve in a special way. Inhibition of I_{K1} (by PA-6) allows depolarization and the development of extrasystoles leading to ventricular arrhythmias, plus lengthening APD amplifying repolarization heterogeneity (Hoeker et al., 2017; Varro and Baczko, 2011). According to Ishihara and coworkers (2009), simultaneous inhibition of I_{Kr} and I_{K1} produced EAD because of the weakening of repolarization reserve. I_{to} current is responsible for the formation of phase 1 repolarization of the APs; thus these channels play an indirect role in repolarization reserve by affecting other currents. I_{to} alters the action potential amplitude (APA) changing the activation and deactivation manners of currents (Varro and Baczko, 2011). Furthermore, the representation of I_{to} current varies among the subepicardial and subendocardial layers of the ventricular wall, supplying the chance for repolarization heterogeneity (Litovsky and Antzelevitch, 1988). $I_{Na/K}$ is an electrogenic outward current transporting Na^+ and K^+ across the cell membrane and contributes to repolarization reserve as well during the entire cardiac cycle (De Weer et al., 1988; Bueno-Orovio et al., 2014). In heart failure, the expression of Na^+/Ca^{2+} exchanger gene is increased (Schillinger et al., 2000), and the increased amount of I_{NCX} could trigger delayed afterdepolarization (DAD) and cardiac arrhythmias (Bers et al., 2002).

According to the simplified model illustrated in Figure 1 (Varro and Baczko, 2011), during physiologic conditions, the duration of APs of cardiomyocytes is long (200–300 ms), thus these cells can not be stimulated again until the end of refractory state, which is characterized by the effective refractory period (ERP). If the repolarization and ERP prolonged heterogeneously —dispersion of repolarization occurred — between the transmural layers or

cardiac regions, cardiac arrythmias (TdP or ventricular fibrillation) were developed due to an extrasystole propagating towards cells with short APD (Figure 1). Repolarization reserve impairments contribute to the development of inhomogeneous repolarization; thus these impairments increase the chance for the formation of ventricular arrhythmias. 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 causing cardiac arrhythmias.

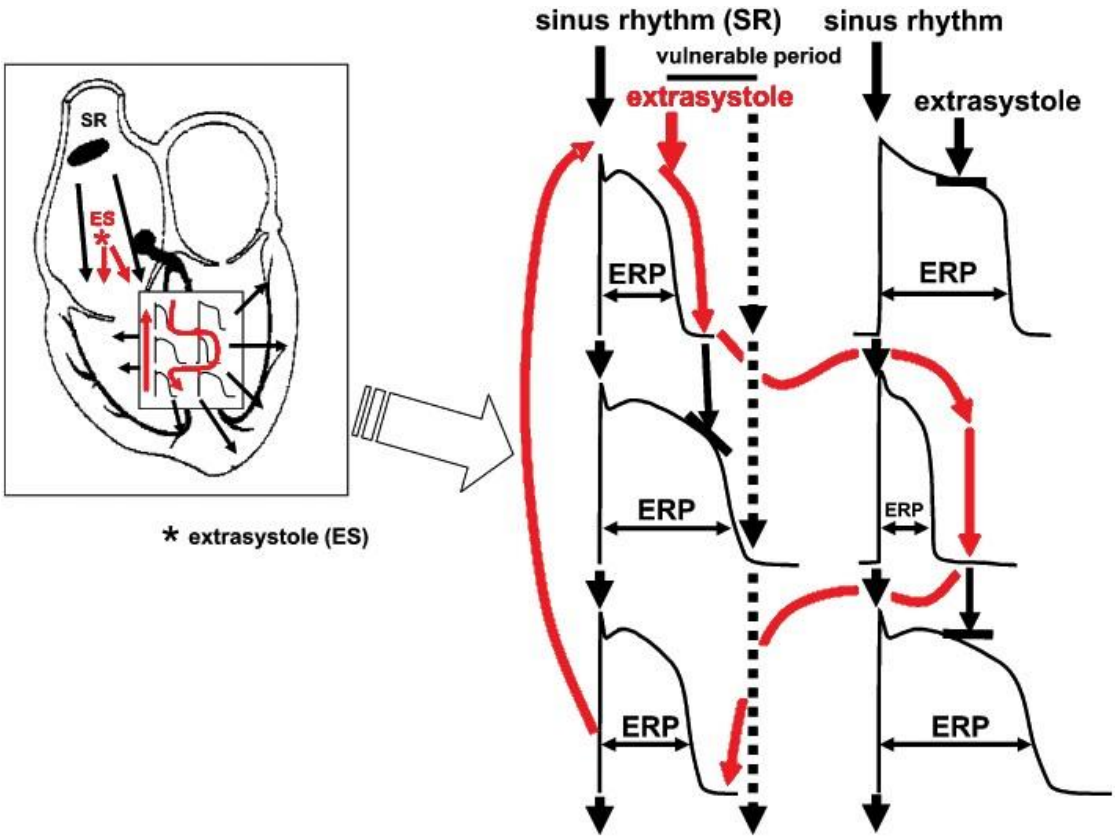


Figure 1 – The mechanism of the development of cardiac arrhythmias due to inhomogeneous repolarization. Action potentials evoked by extrasystoles (ES) during the vulnerable period (red arrows) can travel towards cells with short effective refractory periods (ERP). When action potentials propagate back to the site of origin, reentry occurs leading to ventricular arrhythmias.
Modified from Varro and Baczko (2011), with permission. Abbreviation: SR, sinus rhythm; ES, extrasystole; ERP, effective refractory period.

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

NSAIDs are widely prescribed for the treatment of pain, fever and inflammation. Their mechanism of action is based on the inhibition of cyclooxygenase (COX) enzymes which have two isoforms: COX–1 and COX–2. Traditional NSAIDs suppress both isozymes, but as a higher COX–2 expression can be observed during inflammation, COX–2 selective agents ("coxibs") were developed (Hla and Neilson, 1992). Several studies assessed that COX–2 selective drugs have a lower incidence of gastrointestinal (GI) side effects but a higher incidence of cardiovascular (CV) complications than traditional NSAIDs (Coruzzi et al., 2007; Marsico et al., 2017). For that reason, rofecoxib and valdecoxib were withdrawn from the market (Gunter et al., 2017; Atukorala and Hunter, 2013) and researchers were reconsidering the use of NSAIDs.

In a case-control study, De Caterina et al. (2010) have detected a positive correlation between chronic atrial fibrillation (AF) and long-term use of NSAIDs, and this finding was confirmed by Schmidt et al. (2011) who also reported an elevated risk of AF and flutter in NSAID users compared with non-users. In a population-based cohort of 7 million subjects, COX–2 inhibitors (after the withdrawal of rofecoxib) were investigated and researchers have found significant higher incidence of AF among COX–2 inhibitor users, however, administration of celecoxib and etoricoxib did not reveal significant correlation with myocardial infarction (Back et al., 2012). In contrast to the findings of Back and coworkers, McGettingan and Henry (2011) assessed an increased risk of cardiovascular events with NSAIDs, especially rofecoxib, celecoxib and diclofenac.

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 (Rainsford et al., 2008). The drug could elevate blood pressure among normotensive patients, and it could interfere with antihypertensive drugs such as β-adrenergic receptor blockers or diuretics (Pope et al., 1993; Johnson et al., 1994). According to a cohort study — appeared in The American Journal of Cardiology — treatment with ibuprofen significantly increased the risk of arrhythmic event rate (Pratt et al., 1994). In addition, in a recent Danish study, the risk of developing cardiac arrest was also increased among patients taking ibuprofen (Sondergaard and Gislason, 2017). In a case report, published by Douglas (2010), 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 (Sauza-Sosa, 2016).

Even though ibuprofen is commonly used, only Yang and coworkers (2008) investigated the cardiac electrophysiological effects of ibuprofen in guinea-pig papillary muscle and in rabbit sinoatrial node preparations. They found that ibuprofen dose dependently prolonged the QRS complex and RR interval on the ECG recordings, however, QT interval was significantly decreased in *in vivo* and *in vitro* experiments. In some animals, premature contractions and ventricular fibrillation occurred as well. Using microelectrode technique, they observed that ibuprofen markedly decreased the maximum rate of depolarization (V_{max}), action potential duration (APD) and effective refractory period (ERP), while it did not influence resting membrane potential (RMP) and action potential amplitude (APA) significantly. They presumed that ibuprofen is able to inhibit fast Na^+ channels and slow Ca^{2+} channels during phase 0 of the fast– and slow–response APs.

1.6. Possible proarrhythmic effects of cannabinoids

Cannabis is one of the most used illicit drugs all over the world, and the number of regularly users was gradually increased year by year (Burns et al., 2013). Legalization of cannabis use facilitates consumption according to Goodman et al. (2020). 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) (ElSohly et al., 2017). After the discovery of endocannabinoid system, researchers conducted several studies to reveal the possible therapeutic options of cannabinoids (Fraguas-Sanchez and Torres-Suarez, 2018). Nowadays, there are cannabis–based medicines containing well known amounts of cannabinoids. Beneficial effects of cannabinoids are proved for the treatment of several diseases, such as Parkinson's disease (Stampanoni Bassi et al., 2017), Alzheimer's disease (Talarico et al., 2019), Crohn's disease, irritable bowel syndrome (Goyal et al., 2017), nausea and vomiting due to chemotherapy (Adel, 2017) or chronic pain (Yanes et al., 2019). Besides, 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 (Lall, 2020). 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.

The development of CV side effects is controversial in the literature. According to Pacher et al. (2018) the increased use of medical or recreational cannabinoids is accompanied by the higher prevalence of myocardial infarction, stroke, arrhythmias — including atrial fibrillation and ventricular tachycardia (Rezkalla and Kloner, 2019) — or cardiac arrest. Moreover, cannabis use seems to be an independent predictor of heart failure and cerebrovascular accidents among 18–55 years old patients (Kalla et al., 2018). In the Determinants of Myocardial Infarction Onset Study, the risk of myocardial infarction was 4.8 times higher among marijuana users one hour after consumption (Mittleman et al., 2001). On the other hand, beneficial effects of CBD have reported as well in experimental models of myocardial infarction in which CBD reduced infarct size and the number of ventricular arrhythmias (Kicman and Toczek, 2020). This cardioprotective effect could be observed only in *in vivo* experiments, whilst in *ex vivo* experiments no significant difference could be detected (Durst et al., 2007).

Several case reports were published concerning the association between synthetic cannabinoid use and cardiac arrhythmias. Efe et al. (2017) have reported a 23–year–old man who was admitted to hospital with atrial fibrillation after the first use of synthetic cannabinoid. The case report published by Westin et al. (2016) described a patient with cardiac asystole due to synthetic cannabinoid consumption. Atrioventricular block, left bundle branch block, QT prolongation and ventricular fibrillation could also develop after the use of synthetic cannabinoids (Von Der Haar et al., 2016; Aksel et al., 2015; Yamanoglu et al., 2018).

1.7. 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. Human tissue ethics statement

Non–diseased human hearts were obtained from organ donors whose hearts were unusable for transplantation due to logistical, not patient–related considerations. Before cardiac explanation, organ donors did not receive medication aside from dobutamine, furosemide and plasma expanders. Obtained human cardiac tissues were stored in cardioplegic solution for 4–8 hours at 4°C. The investigations conformed to the principles of the Declaration of Helsinki of the World Medical Association. Experimental protocols were approved by the Scientific and Research Ethical Committee of the Medical Scientific Board at the Hungarian Ministry of Health under ethical approval No. 4991-0/2010-1018EKU (339/PI/010).

2.2. Animal ethics statement

All experiments performed in rabbit, canine and guinea–pig ventricular papillary muscle and Purkinje fiber preparations were carried out in compliance with the 'Guide for the Care and Use of Laboratory Animals' (USA NIH publication NO 85-23, revised 1996) and conformed to the Directive 2010/63/EU of the European Parliament. The protocols were approved by the Review Board of the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development (authority approval number XIII/3331/2017 and XIII/1211/2012) and the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Szeged, Hungary (approval number: I-74-24-2017).

2.3. 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.

Before the surgical intervention, heparin was applied in order to inhibit blood clotting in the animals. Rabbits and guinea–pigs were terminated by rapid cervical dislocation, and dogs were operated under high dose (60 mg/kg intravenously) sodium pentobarbital anesthesia. Animal hearts were removed through a right lateral thoracotomy performed just before the experiments. Ventricular papillary muscles were obtained from the right and left ventricles of

rabbits, dogs and guinea–pigs and free–running (false tendons of) Purkinje fibers were isolated from both right and left ventricles of dog hearts.

The preparations were placed into a bath as soon as possible and perfused with Locke's solution containing 120 mmol/L NaCl, 22 mmol/L $NaHCO_3$, 11 mmol/L D-glucose, 4 mmol/L KCl, 1.8 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$. 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. The papillary muscle preparations were incubated for 1–2 hours to avoid spontaneous alteration of the action potentials. 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. The microelectrodes were coupled to the input of a high–impedance, capacitance–neutralizing amplifier (Experimetria 2011) through a silver (Ag-AgCl) junction. With these microelectrodes, the preparations were impaled, and the intracellular recordings were displayed on a storage oscilloscope (Hitachi V-555). Data were digitized with analogue–to–digital converters (ADA 3300, Real Time Devices Inc.) and processed by a computer system (APES home–made software) designed for on–line determination of action potential parameters.

2.4. Protocols

Test protocol, cycle length dependent protocol and recovery kinetic protocol were applied. To register test protocol, ten action potentials were recorded while the 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. Cycle length dependent protocol was measured when the preparations were stimulated with different constant cycle lengths between 300 and 5000 ms and the twentieth action potential at every cycle length was recorded. To determine the restitution kinetics of action potential duration, extra action potentials were elicited after every twentieth basic (S_1) beat by using single test pulses (S_2) driven at a basic cycle length of 500 ms in Purkinje fibers of dogs. The intervals between basic and extra stimuli (S_1–S_2 coupling interval) were gradually increased from −20 ms to 10000 ms compared to action potential duration at 90% of repolarization (APD_{90}).

2.5. Action potential parameters and concentrations of administered drugs

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}). Action potential amplitude was defined as the voltage difference between resting potential and the peak of the action potential curve. Speed of rapid depolarization phase was characterized by maximum rate of depolarization (V_{max}). Action potential duration (APD) was used to describe the length of the action potential. 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). To exclude the effects of the solvent, DMSO (2‰), was measured on ventricular papillary muscle preparations and Purkinje fibers.

2.6. Statistical analysis

All data expressed as mean value ± standard error of the mean (S.E.M.). Normality of distributions was verified using Shapiro–Wilk test, and homogeneity of variances was verified using Bartlett's test in each treatment group. 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

The effects of ibuprofen on action potential characteristics were investigated in right ventricular papillary muscle preparations of rabbits, dogs and human hearts in concentrations of 50 μM, 100 μM or 200 μM. The isolated preparations were perfused with Locke's solution for 1–2 hours and were stimulated at a basic cycle length of 1000 ms. After the equilibration period, ibuprofen (in different concentrations) was applied, then the parameters of fast response APs were measured after about 30 minutes exposure.

In rabbit ventricular papillary muscle preparations, ibuprofen, investigated at 50 μM concentration, did not significantly change the resting membrane potential (RMP, −88.6 ± 3.3 mV *vs* −87.4 ± 3.5 mV), the action potential amplitude (APA, 104.7 ± 4.2 mV *vs* 105.3 ± 4.0 mV), the maximum rate of depolarization (V_{max}, 155.3 ± 38.2 V/s *vs* 150.9 ± 29.2 V/s) and the action potential durations at 90% and 75% of repolarization (APD_{90} and APD_{75}, 177.0 ± 6.2 ms *vs* 179.7 ± 8.7 ms and 166.3 ± 5.7 ms *vs* 167.7 ± 7.7 ms, respectively). Applying cycle length–dependent protocol, ibuprofen (50 μM) did not alter neither APD_{90} nor V_{max} parameters of the action potentials. Application of 100 μM ibuprofen, the drug slightly but statistically significant manner increased APD_{90} from 163.5 ± 11.1 ms to 168.5 ± 11.0 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 (see later in Table 4/B, Figure 2/A).

Table 1 summarizes the effects of ibuprofen at 50 μM and 200 μM concentrations in canine right ventricular papillary muscle preparations. Ibuprofen at 50 μM did not affect markedly action potential characteristics, including RMP, APA, V_{max} and APD ($n = 8$, Table 1/B), but after the application of ibuprofen at 200 μM concentration (Table 1/C, Figure 2/B), it significantly lengthened the APD_{90} by 4.3 ± 1.0% (from 214.1 ± 5.9 ms to 223.0 ± 4.9 ms; $n = 6$; $p < 0.01$) and the APD_{75} by 4.5 ± 1.3% (from 201.0 ± 6.3 ms to 209.8 ± 5.1 ms; $n = 6$; $p < 0.05$). To exclude the effects of the solvent on action potential parameters, DMSO (2‰) was measured alone as well. It elicited no noticeable changes in the mentioned action potential parameters, regarding RMP, APA, V_{max}, or APD (Table 1/A).

Table 1 – The cardiac electrophysiological effects of 2‰ DMSO (panel A), 50 μM (panel B) and 200 μM (panel C) ibuprofen in canine right ventricular papillary muscle preparations at basic cycle length of 1000 ms.

A

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**	**APD_{90} (%)**
Control (n = 6)	−83.3 ± 2.3	106.7 ± 1.5	136.7 ± 14.2	203.6 ± 7.6	187.2 ± 8.9	157.8 ± 11.6	
DMSO 2‰ (n = 6)	−85.8 ± 1.7	105.3 ± 1.4	123.3 ± 17.0	201.6 ± 8.0	184.9 ± 9.5	153.8 ± 11.5	−1.0 ± 1.2

B

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**	**APD_{90} (%)**
Control (n = 8)	−83.2 ± 1.6	108.1 ± 1.0	175.2 ± 22.3	227.5 ± 9.7	213.3 ± 9.5	187.0 ± 9.3	
Ibuprofen 50 μM (n = 8)	−85.3 ± 2.1	106.7 ± 1.9	172.4 ± 29.9	225.9 ± 8.9	213.9 ± 9.0	187.7 ± 9.7	−0.6 ± 1.0

C

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**	**APD_{90} (%)**
Control (n = 6)	−89.0 ± 1.8	110.6 ± 2.4	174.6 ± 20.3	214.1 ± 5.9	201.0 ± 6.3	173.8 ± 8.0	
Ibuprofen 200 μM (n = 6)	−89.1 ± 3.2	113.4 ± 3.0	192.9 ± 27.1	223.0 ± 4.9$^{\#}$	209.8 ± 5.1*	181.6 ± 6.3	4.3 ± 1.0

Abbreviations: RMP, resting membrane potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{90}, APD_{75} and APD_{50}, action potential duration at 90%, 75% and 50% of repolarization; n, number of experiments. Data are expressed as means ± SEM; *$p < 0.05$ *vs* control, $^{\#}p < 0.01$ *vs* control, Student's t-test for paired data.

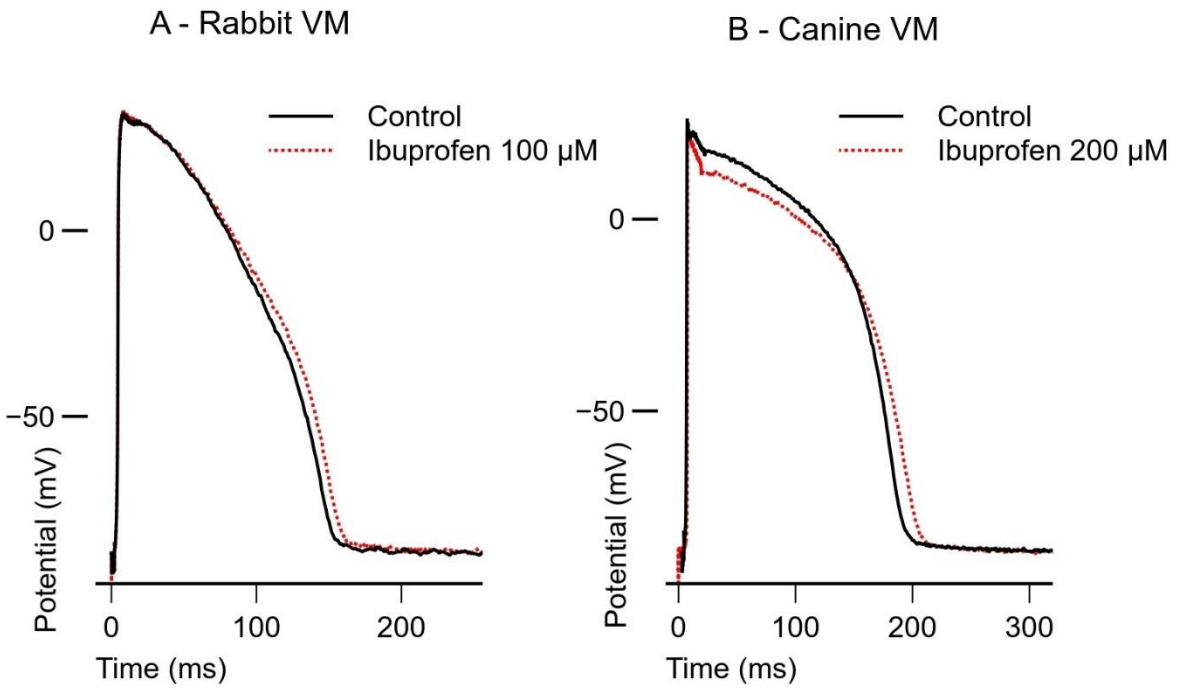


Figure 2 – The effects of ibuprofen on action potential characteristics recorded from rabbit (panel A) and canine (panel B) cardiac preparations. Original action potential records show that ibuprofen slightly but significantly lengthened the action potential duration in rabbit right ventricular papillary muscle preparations at 100 µM concentration (panel A) and in dog right ventricular papillary muscle preparations at 200 µM concentration (panel B) at a basic cycle length of 1000 ms. Abbreviations: VM, ventricular muscle.

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, Table 2 and Figure 3/B) or change other action potential parameters significantly.

Table 2 – The electrophysiological effects of ibuprofen at 50 μM and 150 μM concentrations in human right ventricular papillary muscle preparations at basic cycle length of 1000 ms.

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**
Control (n = 4)	−85.2 ± 4.4	96.9 ± 2.6	113.5 ± 27.2	294.1 ± 14.7	254.8 ± 13.7	203.4 ± 17.0
Ibuprofen 50 μM (n = 4)	−80.7 ± 5.7	91.9 ± 2.2	91.5 ± 26.2	293.1 ± 9.4	247.9 ± 8.5	200.4 ± 8.4
Ibuprofen 150 μM (n = 4)	−82.0 ± 1.9	92.1 ± 2.9	84.2 ± 20.2	292.5 ± 9.3	246.2 ± 10.6	189.9 ± 13.1

Abbreviations: RMP, resting membrane potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{90}, APD_{75} and APD_{50}, action potential duration at 90%, 75% and 50% of repolarization; n, number of experiments. Data are expressed as means ± SEM; ANOVA for repeated measurements followed by Bonferroni's post hoc test.

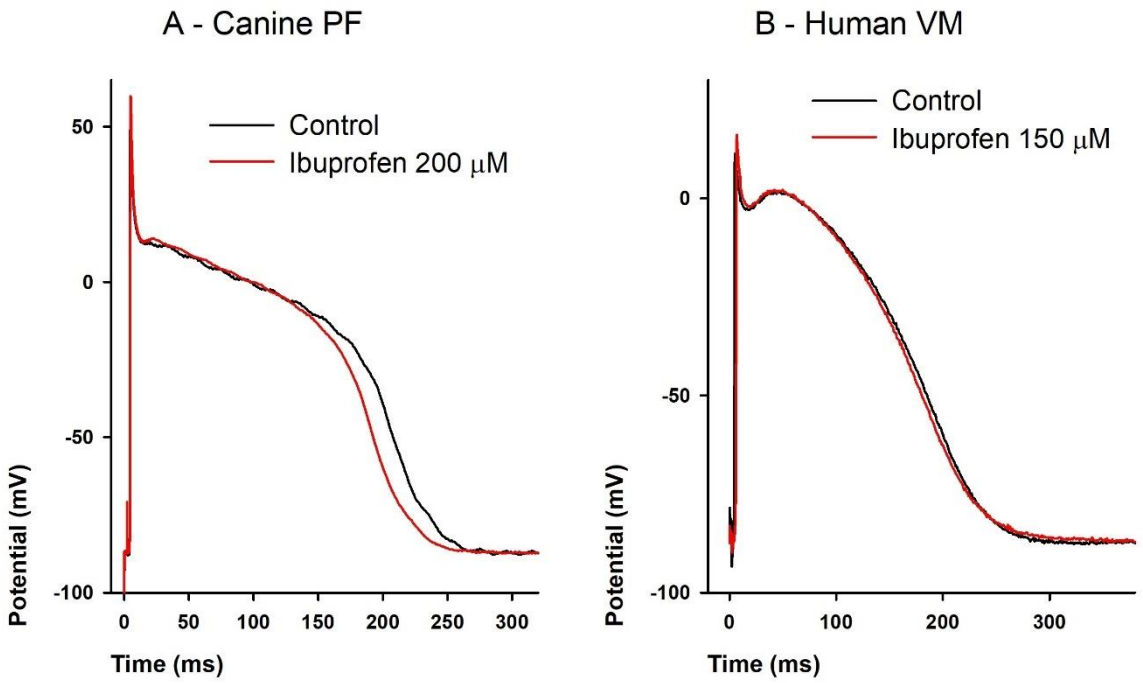


Figure 3 – The effects of ibuprofen on action potential characteristics recorded from canine Purkinje fibers (panel A) and human right ventricular muscle preparations (panel B). Representative traces show that ibuprofen significantly shortened the action potential repolarization at a basic cycle length of 500 ms in Purkinje fibers of dogs (panel A). The drug did not affect action potential characteristics in human papillary muscles at a basic cycle length of 1000 ms (panel B). Abbreviations: VM, ventricular muscle; PF, Purkinje fiber.

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

Purkinje fiber preparations from both ventricles of canines were examined at a basic cycle length of 500 ms. After the equilibration period, the effects of ibuprofen at 50 μM or at 200 μM concentration were measured after 30 minutes elapsed.

All data obtained from test protocol are shown in Table 3. 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 (from 249.8 ± 9.8 ms to 247.0 ± 9.2 ms, $n = 6$, $p < 0.05$, Table 3/B) and by 4.5 ± 0.7% at 200 μM (from 253.4 ± 14.2 ms to 242.0 ± 13.7 ms, $n = 7$, $p < 0.01$, Table 3/C). 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 (Table 3/B and Table 3/C). Representative traces are shown in Figure 3/A in which the shortening of the repolarization could be observed. The solvent, DMSO (2‰) did not alter markedly any of the measured action potential parameters (Table 3/A).

Various stimulation cycle lengths were also applied in canine Purkinje fibers ranging from 300 ms to 1000 ms. As Figure 4 indicates, 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 (Figure 4/A), and statistical significance was reached from 500 ms to 1000 ms ($n = 6$, $p < 0.05$). V_{max} depression was marked at rapid cycle lengths (Figure 4/B), but significance was not detectable ($n = 6$). DMSO (2‰) did not evoke any effects on Purkinje fibers using the cycle length–dependent protocol (not shown in Figure).

Table 3 – The cardiac electrophysiological effects of 2‰ DMSO (panel A) and ibuprofen at 50 μM (panel B) and at 200 μM (panel C) concentration in canine Purkinje fiber preparations from both ventricles at basic cycle length of 500 ms.

A

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**	**APD_{90} (%)**
Control (n = 5)	−85.5 ± 0.6	125.1 ± 3.7	521.5 ± 24.0	228.3 ± 3.4	201.8 ± 5.0	153.7 ± 9.1	
DMSO 2‰ (n = 5)	−83.7 ± 1.8	127.3 ± 6.1	537.6 ± 16.9	227.7 ± 4.5	202.5 ± 6.3	157.9 ± 10.5	−0.3 ± 0.5

B

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**	**APD_{90} (%)**
Control (n = 6)	−84.6 ± 1.9	129.2 ± 9.8	538.3 ± 93.6	249.8 ± 9.8	225.9 ± 9.3	173.0 ± 6.9	
Ibuprofen 50 μM (n = 6)	−83.8 ± 2.1	130.4 ± 9.2	522.5 ± 102.2	247.0 ± 9.2*	224.1 ± 9.0	173.1 ± 5.8	−1.1 ± 0.3

C

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**	**APD_{90} (%)**
Control (n = 7)	−89.7 ± 0.7	133.5 ± 3.3	580.7 ± 36.0	253.4 ± 14.2	226.6 ± 12.3	163.9 ± 10.9	
Ibuprofen 200 μM (n = 7)	−87.3 ± 1.0	135.9 ± 3.4	621.5 ± 93.5	242.0 ± 13.7$^{\#}$	217.8 ± 12.2$^{\#}$	163.7 ± 11.2	−4.5 ± 0.7

Abbreviations: RMP, resting membrane potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{90}, APD_{75} and APD_{50}, action potential duration at 90%, 75% and 50% of repolarization; n, number of experiments. Data are expressed as means ± SEM; *$p < 0.05$ *vs* control, $^{\#}p < 0.01$ *vs* control, Student's t-test for paired data.

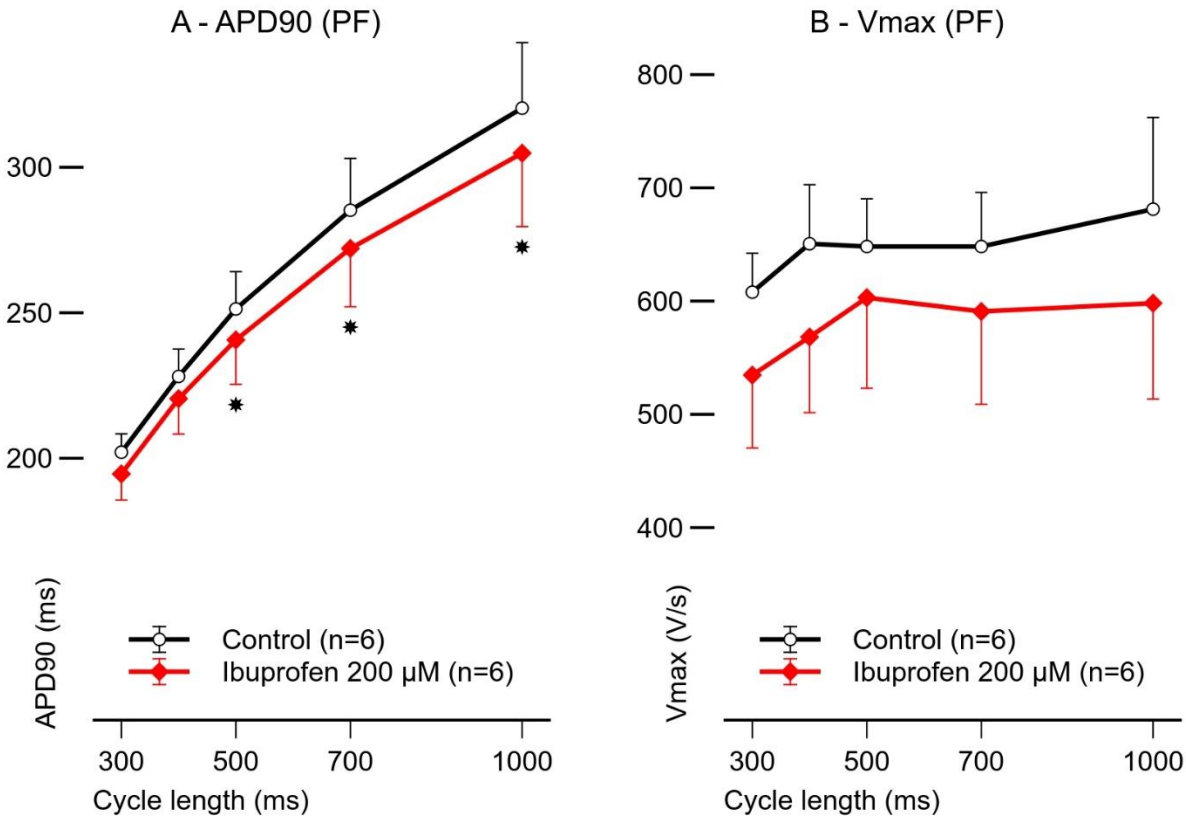


Figure 4 – Cycle length–dependent changes in action potential duration (panel A) and in maximum rate of depolarization (panel B) measured under control conditions and in the presence of 200 µM ibuprofen in dog Purkinje fiber preparations. Values are means ± SEM., asterisks indicate significant changes, *$p < 0.05$ *vs* control. Abbreviations: APD_{90}, action potential duration at 90% of repolarization; V_{max}, maximum rate of depolarization; PF, Purkinje fiber; n, number of experiments.

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. The preparations were stimulated at a basic cycle length of 1000 ms during and after equilibration period. Ibuprofen at 100 μM concentration and levofloxacin at 40 μM concentration were applied in a cumulative manner, and the effects were measure after 30 minutes elapsed.

All the results are summarized in Table 4 and representative action potentials are shown in Figure 5. 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% ($n = 7$, $p < 0.05$), whilst other action potential parameters remained unchanged (Table 4/B). 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} (Table 4/A, Figure 5/A), but the drug intensified the APD_{90} prolongation by 7.5 ± 2.4% (from 168.5 ± 11.0 ms to 182.4 ± 16.0 ms; $n = 7$, $p < 0.05$) evoked by 100 μM ibuprofen (Table 4/B and Figure 5/B). Addition of levofloxacin after ibuprofen significantly increased APD_{25} by 15.0 ± 5.2% (from 75.5 ± 4.3 ms to 87.5 ± 8.0 ms, $n = 7$, $p < 0.05$; not shown in Table 4) and APD_{10} by 24.8 ± 10.5% (from 34.5 ± 2.3 ms to 42.3 ± 3.0 ms, $n = 7$, $p < 0.05$; not shown in Table 4).

Table 4 – The electrophysiological effects of levofloxacin 40 μM (panel A) and the combination of ibuprofen 100 μM with levofloxacin 40 μM (panel B) in rabbit right ventricular papillary muscle preparations at basic cycle length of 1000 ms.

A

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{90} (%)**
Control (n = 7)	−86.9 ± 1.3	109.0 ± 2.6	127.6 ± 7.5	163.8 ± 6.6	153.3 ± 6.8	
Levofloxacin 40 μM (n = 7)	−85.8 ± 2.0	111.3 ± 4.0	128.0 ± 8.0	164.1 ± 7.0	154.3 ± 7.4	0.1 ± 0.8

B

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{90} (%)**
Control (n = 7)	−86.5 ± 2.1	107.2 ± 2.9	137.1 ± 17.3	163.5 ± 11.1	149.4 ± 10.1	
Ibuprofen 100 μM (n = 7)	−84.6 ± 2.0	105.8 ± 2.0	125.3 ± 9.0	168.5 ± 11.0*	155.7 ± 10.9*	3.1 ± 1.1
Levofloxacin 40 μM (n = 7)	−85.5 ± 2.0	110.5 ± 4.0	141.5 ± 15.0	182.4 ± 16.0§	169.1 ± 16.0	7.5 ± 2.4

Abbreviations: RMP, resting membrane potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{90}, APD_{75} and APD_{50}, action potential duration at 90%, 75% and 50% of repolarization; n, number of experiments. Data are expressed as means ± SEM; *$p < 0.05$ *vs* control, §$p < 0.05$ *vs* ibuprofen 100 μM. Student's t-test for paired data (Table 4/A), ANOVA for repeated measurements followed by Bonferroni's post hoc test (Table 4/B).

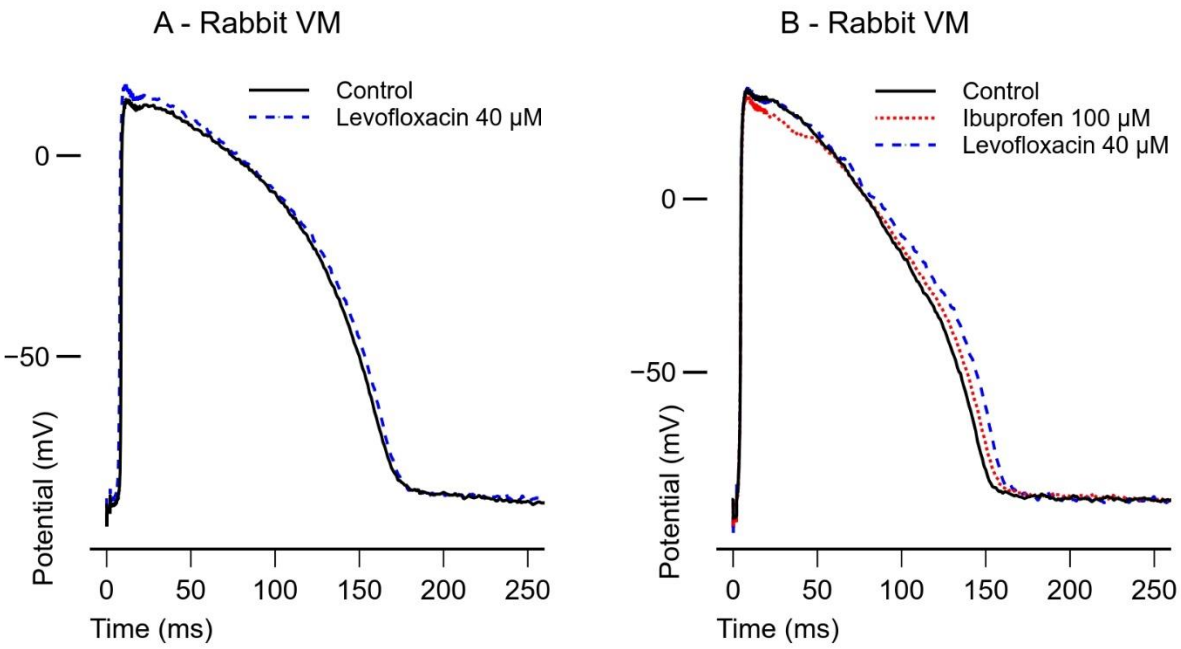


Figure 5 – The electrophysiological effects of 40 µM levofloxacin alone (panel A) and in combination with 100 µM ibuprofen (panel B) on fast–response action potentials in rabbit right ventricular papillary muscle preparations at a basic cycle length of 1000 ms. Original action potential records indicate that 40 µM levofloxacin did not influence the ventricular repolarization in rabbit (panel A), however, in combination with 100 µM ibuprofen levofloxacin significantly lengthened the action potential duration (panel B). Abbreviation: VM, ventricular muscle.

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 (Figure 6). ACh pretreatment slightly but not significantly increased APD_{90} by 4.1 ± 2.3% (from 232.5 ± 7.8 ms to 241.4 ± 4.8 ms, n = 6) and APD_{75} by 4.2 ± 2.1% (from 206.7 ± 5.8 ms to 214.8 ± 3.3 ms, n = 6). Addition of ibuprofen at 50 µM concentration significantly shortened APD_{90} by 3.3 ± 0.6% (to 233.4 ± 5.1 ms, n = 6, $p < 0.01$) and APD_{75} by 3.2 ± 1.0% (to 207.9 ± 3.3 ms, n = 6, $p < 0.05$). The drugs did not change significantly other action potential parameters (RMP, AMP, V_{max}).

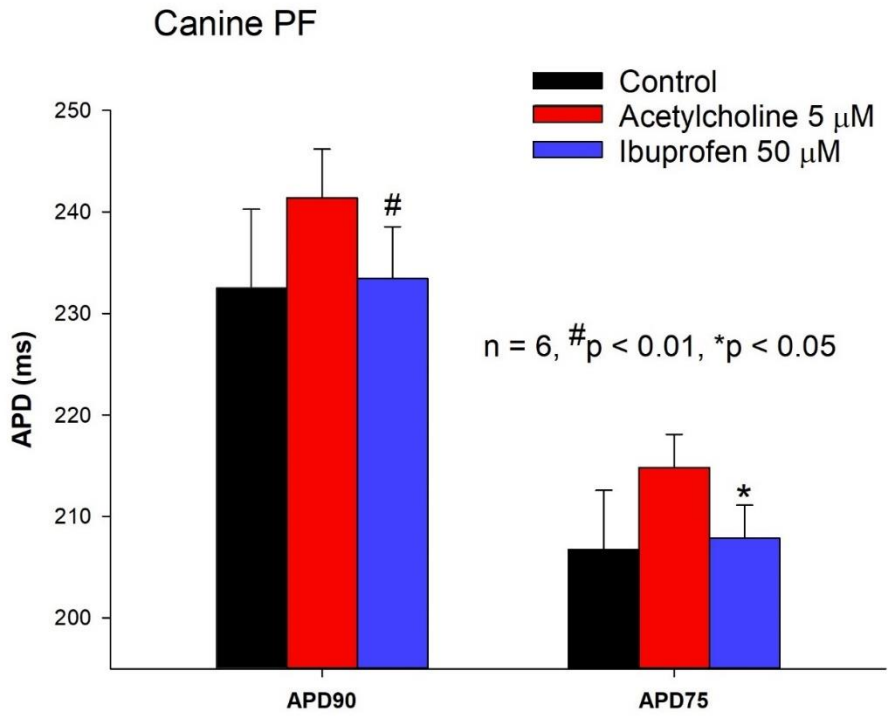


Figure 6 – The effects of acetylcholine (5 μM) and ibuprofen (50 μM) on action potential duration at 90% (left panel) and 75% (right panel) of repolarization in dog Purkinje fibers at basic cycle length of 500 ms. Ibuprofen shortened the action potential duration after pretreatment with acetylcholine nearly back to the control conditions. Values are mean ± SEM. Repeated measures ANOVA followed by Bonferroni's post-hoc test, asterisks indicate significant changes, n = 6, *$p < 0.05$ *vs* acetylcholine 5 μM, $^{\#}p < 0.01$ *vs* acetylcholine 5 μM. Abbreviations: APD_{90}, action potential duration at 90% of repolarization; APD_{75}, action potential duration at 75% of repolarization; n, number of experiments.

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

The cardiac electrophysiologic effects of cannabidiol (CBD) was investigated in ventricular papillary muscle preparations of guinea–pigs, rabbits and dogs, in concentrations of 1 μM, 2.5 μM, 5 μM and 10 μM using the conventional microelectrode technique. All the isolated preparations were stimulated at 1000 ms basic cycle length during experiments. After the equilibration period (1–2 hours), CBD (in different concentrations) was applied, then the parameters (APD, V_{max}, APA, and RMP) of fast response APs were measured after about 30 minutes exposure.

In right ventricular papillary muscle preparations of guinea–pigs, CBD was used in concentrations of 2.5 μM and 5 μM. As Table 5 and Figure 7 shows, CBD at both 2.5 and 5 μM concentrations lengthened slightly but significantly APD_{90} by 3.2 ± 0.4% (from 186.2 ± 6.1 ms to 192.2 ± 6.8 ms, $n = 5$, $p < 0.01$) and by 6.3 ± 2.1% (from 179.9 ± 6.0 ms to 191.5 ± 8.9 ms, $n = 5$, $p < 0.05$), respectively. Furthermore, at 2.5 μM concentration (Table 5/A), 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. At 2.5 μM concentration, CBD slightly but not significantly lengthened APD_{90} dominantly at cycle length from 300 to 2000 ms ($n = 6$; Figure 8/A). At 5 μM concentration, the drug significantly increased APD_{90} at all cycle lengths from 300 to 5000 ms ($n = 5$; Figure 8/B).

Table 5 – The electrophysiological effects of cannabidiol at 2.5 μM (panel A) and at 5 μM concentration (panel B) in guinea–pig right ventricular papillary muscle preparations at basic cycle length of 1000 ms.

A

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**	**APD_{90} (%)**
Control (n = 5)	−86.7 ± 0.4	124.1 ± 2.9	209.5 ± 21.0	186.2 ± 6.1	180.0 ± 6.1	166.6 ± 6.2	
CBD 2.5 μM (n = 5)	−86.9 ± 0.2	126.9 ± 3.9	196.3 ± 24.2	192.2 ± 6.8$^{\#}$	185.7 ± 6.8$^{\#}$	172.5 ± 6.9$^{\#}$	3.2 ± 0.4

B

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**	**APD_{90} (%)**
Control (n = 5)	−86.9 ± 0.3	124.2 ± 2.5	210.9 ± 26.1	179.9 ± 6.0	173.7 ± 6.1	159.5 ± 5.8	
CBD 5 μM (n = 5)	−88.6 ± 0.6	127.3 ± 3.2	180.2 ± 19.2	191.5 ± 8.9*	184.8 ± 9.0	170.2 ± 9.0	6.3 ± 2.1

Abbreviations: RMP, resting membrane potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{90}, APD_{75} and APD_{50}, action potential duration at 90%, 75% and 50% of repolarization; n, number of experiments. Data are expressed as means ± SEM; *$p < 0.05$ *vs* control, $^{\#}p < 0.01$ *vs* control, Student's t-test for paired data.

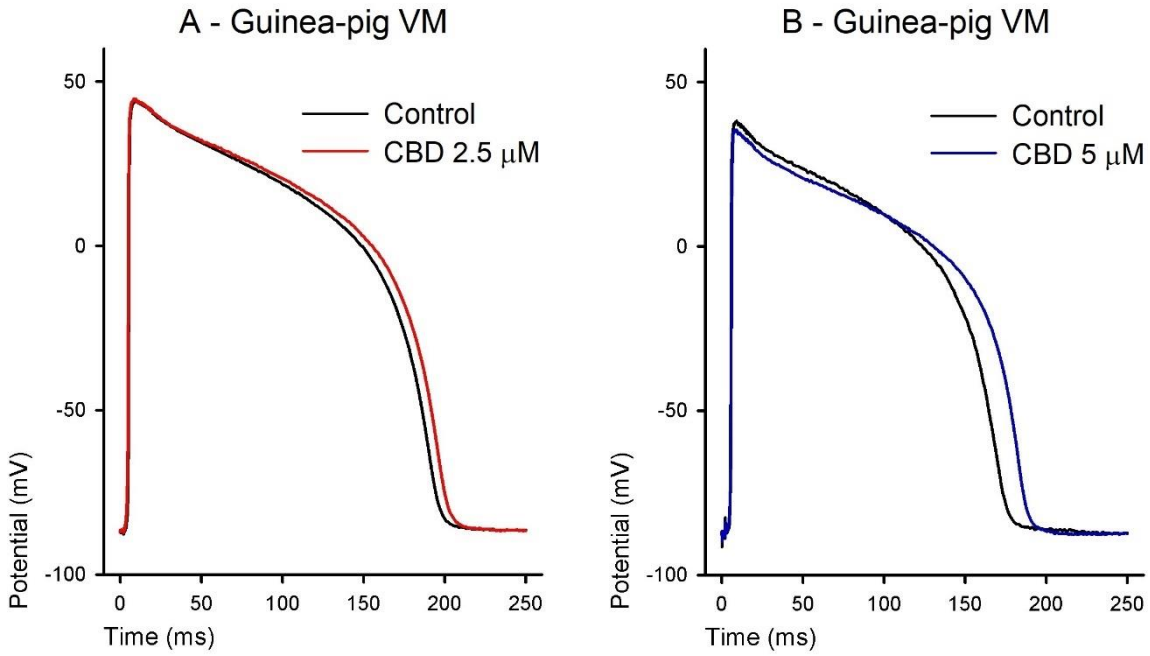


Figure 7 – The effects of cannabidiol (CBD) on action potential characteristics recorded from right ventricular papillary muscles of guinea–pigs. Action potential records indicate that CBD slightly but significantly lengthened the action potential duration at 2.5 μM (panel A) and at 5 μM (panel B) concentrations at a basic cycle length of 1000 ms. Abbreviations: VM, ventricular muscle.

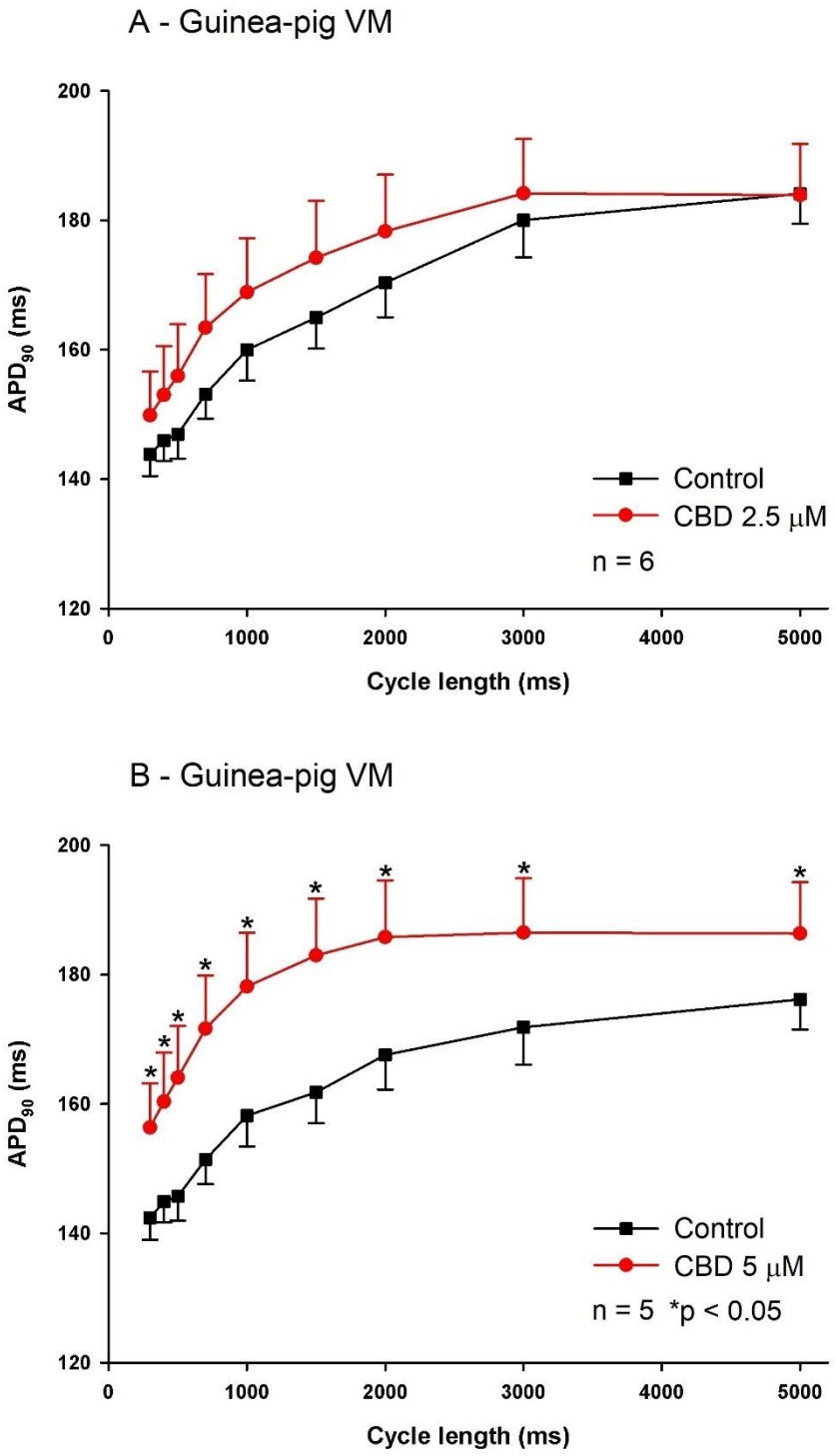


Figure 8 – Cycle length–dependent changes in action potential duration measured under control conditions and in the presence of 2.5 μM (panel A; n = 6) and 5 μM (panel B; n = 5) cannabidiol (CBD) in guinea–pig right ventricular muscle preparations. Values are means ± SEM., asterisks indicate significant changes, *p < 0.05 *vs* control. Student's t-test for paired data. Abbreviations: APD_{90}, action potential duration at 90% of repolarization; VM, ventricular muscle; n, number of experiments.

In rabbit right ventricular papillary muscle preparations, CBD was used in concentrations of 1 μM, 2.5 μM, 5 μM and 10 μM. Figure 9 and Figure 10 show that CBD significantly but not dose–dependently prolonged APD_{90} at 1 μM by 3.0 ± 1.0%; (from 159.5 ± 7.1 ms to 164.5 ± 8.6 ms, n = 5, $p < 0.05$; Figure 9/A and Figure 10/A), at 2.5 μM by 6.8 ± 3.0% (from 164.4 ± 8.3 ms to 175.3 ± 8.8 ms, n = 7, $p < 0.05$; Figure 9/B and Figure 10/B) and at 5 μM concentration by 6.8 ± 1.6% (from 226.2 ± 8.1 ms to 241,2 ± 7.1 ms, n = 5, $p < 0.05$; Figure 9/C and Figure 10/C) at a basic cycle length of 1000 ms. APD_{75} was increased in the same manner, prolongation was 4.0 ± 1.1% (n = 5, $p < 0.05$) at 1 μM, 7.4 ± 3.2% (n = 7, $p < 0.05$) at 2.5 μM and 7.0 ± 2.3% (n = 5, $p < 0.05$) at 5 μM concentration. 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} (e.g., at 1 μM concentration: 87.7 ± 7.3 ms *vs* 90.5 ± 6.5 ms, n = 5). 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} (from 154.8 ± 6.7 ms to 151.7 ± 7.4 ms, n = 5; Figure 9/D and Figure 10/D) or APD_{75} (from 141.3 ± 6.5 ms to 139.7 ± 7.7 ms, n = 5). The APD_{90} lengthening effect of 2.5 μM CBD was depended on the stimulation frequency (Figure 11). 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 (Figure 11).

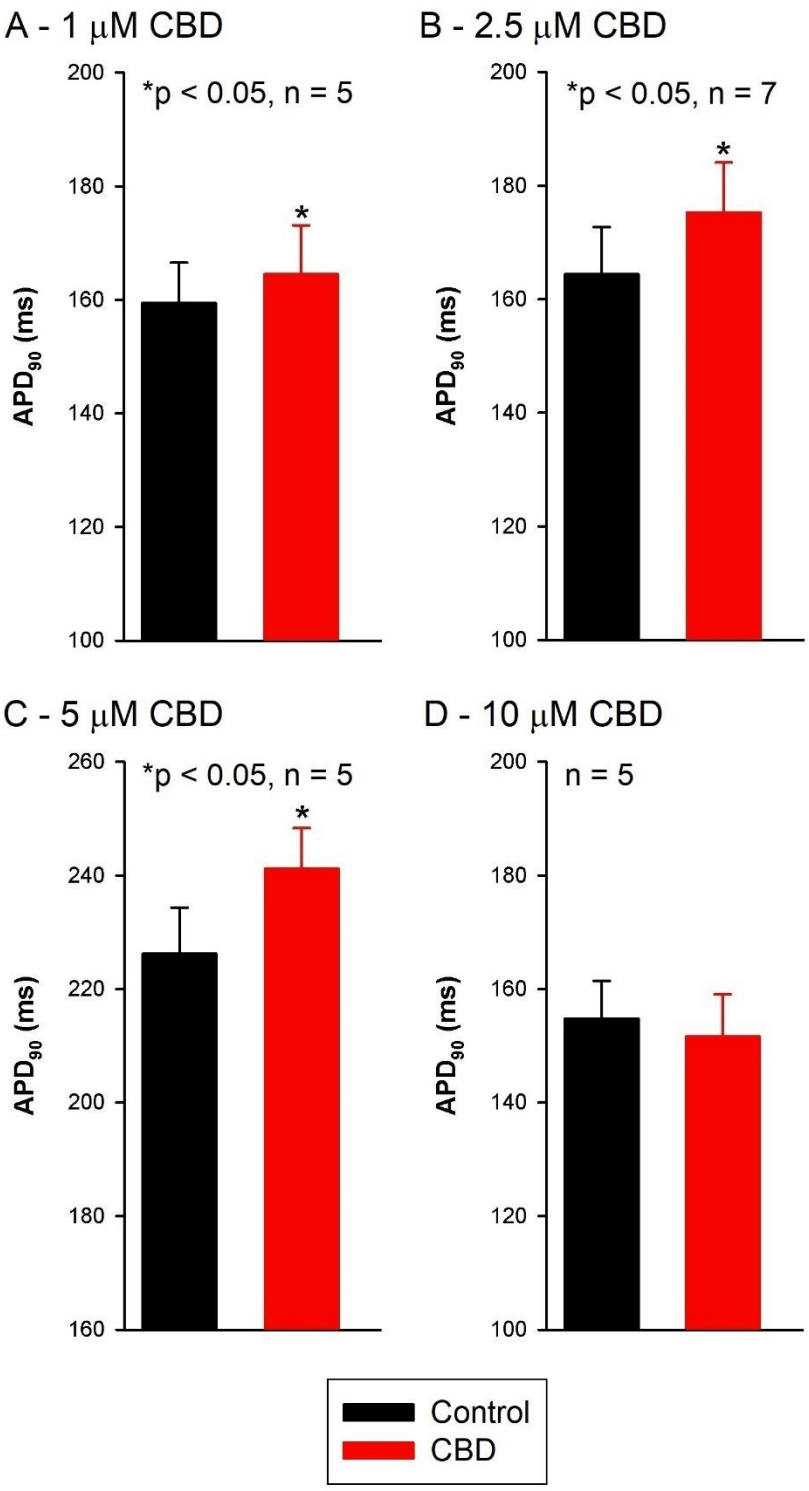


Figure 9 – The effects of CBD on action potential duration at 90% of repolarization (APD_{90}) in rabbit ventricular papillary muscle preparations at basic cycle length of 1000 ms. CBD lengthened the action potential duration at 1 μM (panel A), 2.5 μM (panel B) and 5 μM (panel C) concentrations, but not at 10 μM (panel D). Values are mean ± SEM. Student's t-test for paired data. *p < 0.05 *vs* control. Abbreviations: APD_{90}, action potential duration at 90% of repolarization; n, number of experiments.

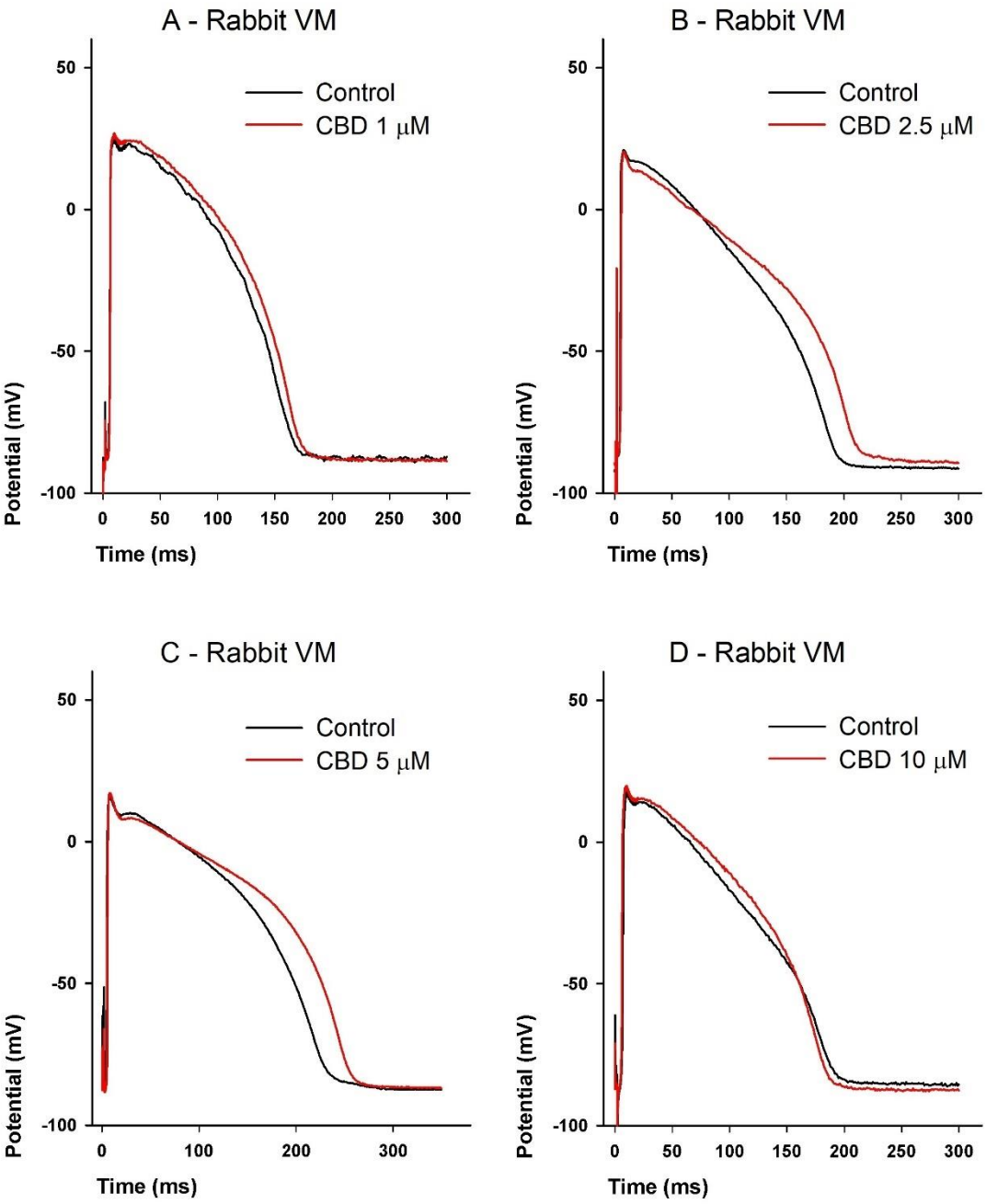


Figure 10 – The effects of cannabidiol (CBD) on action potential characteristics recorded from right ventricular papillary muscles of rabbits. Original action potential records show that CBD significantly increased action potential duration at 1 μM (panel A), at 2.5 μM (panel B) and at 5 μM (panel C) concentrations at a basic cycle length of 1000 ms, but at 10 μM concentration the lengthening of repolarization is vanished (panel D). Abbreviations: VM, ventricular muscle.

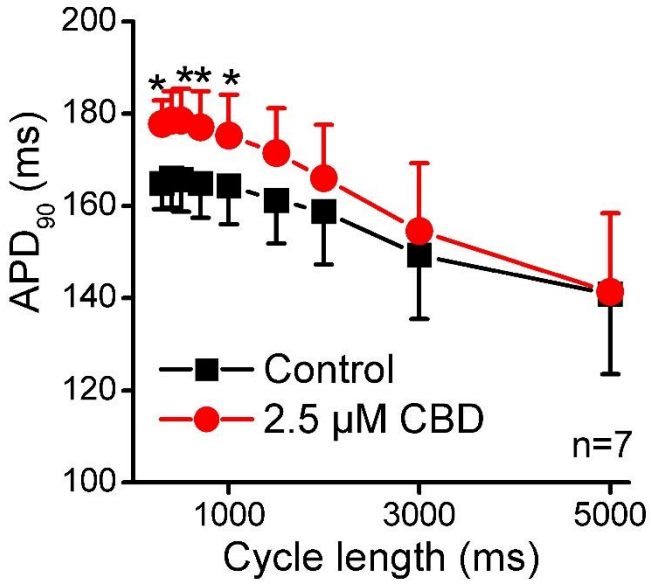


Figure 11 – Cycle length–dependent changes in action potential duration measured under control conditions and in the presence of 2.5 µM cannabidiol (CBD) in rabbit right ventricular muscle preparations. Values are means ± SEM., asterisks indicate significant changes, *p < 0.05 *vs* control. Student's t-test for paired data. Abbreviations: APD_{90}, action potential duration at 90% of repolarization; n, number of experiments.

CBD was applied also in dog right ventricular papillary muscle preparations and results are summarized in Table 6. Representative AP traces are shown in Figure 12. After approximately 30 minutes equilibration period, 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% (from 209.1 ± 6.7 ms to 221.4 ± 3.9 ms, n = 5; Table 6/B) and APD_{75} by 6.5 ± 3.5% (from 197.5 ± 7.0 ms to 209.6 ± 3.7 ms, n = 5; Table 6/B) 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% (from 213.2 ± 10.7 ms to 233.4 ± 5.3 ms, n = 5, $p < 0.05$; Table 6/C) and APD_{75} by 11.4 ± 4.3% (from 200.9 ± 10.8 ms to 222.1 ± 5.2 ms, n = 5, $p < 0.05$; Table 6/C). Neither 2.5 µM nor 5 µM CBD affect other AP parameters including RMP, AMP and V_{max}. The solvent was also examined in dog papillary muscle preparation to verify that CBD was responsible for the emerged effects. DMSO (1‰) did not affect AP parameters and did not lengthened APD_{90} or APD_{75} (Table 6/A).

Table 6 – The cardiac electrophysiological effects of 1‰ DMSO (panel A) and cannabidiol (CBD) at 2.5 μM (panel B) and at 5 μM (panel C) concentrations in canine right ventricular papillary muscle preparations at basic cycle length of 1000 ms.

A

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**	**APD_{90} (%)**
Control (n = 5)	−81.1 ± 2.1	117.1 ± 1.5	161.4 ± 19.2	223.8 ± 8.1	210.9 ± 9.3	186.0 ± 9.6	
DMSO 1‰ (n = 5)	−82.6 ± 1.7	116.7 ± 0.4	151.1 ± 15.7	223.4 ± 7.7	211.4 ± 8.6	185.7 ± 8.7	−0.2 ± 0.5

B

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**	**APD_{90} (%)**
Control (n = 5)	−80.3 ± 1.9	116.2 ± 2.0	196.2 ± 13.8	209.1 ± 6.7	197.5 ± 7.0	172.6 ± 6.6	
CBD 2.5 μM (n = 5)	−82.8 ± 1.9	122.4 ± 1.8	199.2 ± 20.3	221.4 ± 3.9	209.6 ± 3.7	183.3 3.9±	6.2 ± 3.2

C

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**	**APD_{90} (%)**
Control (n = 5)	−84.7 ± 2.0	119.4 ± 3.8	192.9 ± 25.3	213.2 ± 10.7	200.9 ± 10.8	175.8 ± 9.6	
CBD 5 μM (n = 5)	−84.7 ± 2.8	120.5 ± 2.7	207.5 ± 29.9	233.4 ± 5.3*	222.1 ± 5.2*	194.4 ± 5.2	10.2 ± 3.7

Abbreviations: RMP, resting membrane potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{90}, APD_{75} and APD_{50}, action potential duration at 90%, 75% and 50% of repolarization; n, number of experiments. Data are expressed as means ± SEM; *$p < 0.05$ *vs* control, Student's t-test for paired data.

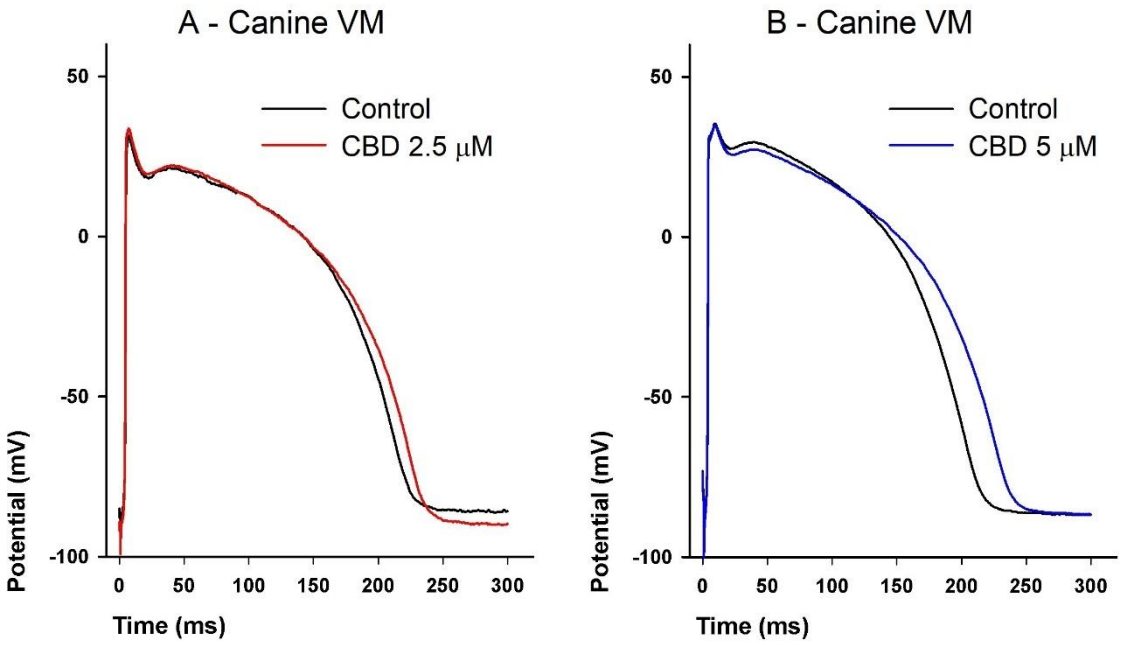


Figure 12 – The effects of cannabidiol (CBD) on action potential characteristics recorded from right ventricular papillary muscles of dogs. Action potential traces show that CBD slightly lengthened the action potential duration at 2.5 μM concentration (panel A) and at 5 μM concentration (panel B) at a basic cycle length of 1000 ms. Abbreviations: VM, ventricular muscle.

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

The effects of CBD on action potentials recorded from canine Purkinje fiber preparations were also studied at a basic cycle length of 500 ms. After the equilibration period, the concentration of CBD was increased from 0.3 μM to 1 μM and to 3μM cumulatively, and all data were obtained after 30 minutes exposure for each concentration.

Table 7 – The electrophysiological effects of cannabidiol (CBD) in concentrations of 0.3 μM, 1 μM and 3 μM in canine Purkinje fiber preparations at basic cycle length of 500 ms.

	RMP (mV)	**APA (mV)**	**V_{max} (V/s)**	**APD_{90} (ms)**	**APD_{75} (ms)**	**APD_{50} (ms)**	**APD_{90} (%)**
Control (n = 6)	−89.1 ± 2.9	130.8 ± 3.7	569.4 ± 60.8	222.1 ± 7.5	202.3 ± 7.2	156.8 ± 5.9	
CBD 0.3 μM (n = 6)	−90.2 ± 0.8	134.1 ± 3.4	567.0 ± 73.9	227.4 ± 7.7$^{\#}$	207.3 ± 7.8*	166.6 ± 8.7	2.4 ± 0.5
CBD 1 μM (n = 6)	−91.2 ± 1.2	135.3 ± 3.1	563.2 ± 80.1	229.7 ± 8.5*	210.1 ± 8.7	171.1 ± 9.2	3.4 ± 1.4
CBD 3 μM (n = 6)	−90.4 ± 0.5	131.2 ± 5.2	573.9 ± 78.7	233.8 ± 9.2	212.9 ± 8.6	169.8 ± 6.3	5.7 ± 5.0

Abbreviations: RMP, resting membrane potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{90} and APD_{75}, action potential duration at 90% and 75% of repolarization; n, number of experiments. Data are expressed as means ± SEM; *$p < 0.05$ *vs* control, $^{\#}p < 0.01$ *vs* control. Student's t-test for paired data.

Results are summerized in Table 7 and representative action potentials are shown in Figure 13. 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 (Figure 13/A) and to 229.7 ± 8.5 ms ($n = 6$, $p < 0.05$) at 1 μM (Figure 13/B) 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 (Figure 13/C), but these results were not significant. Other action potential parameters (RMP, AMP, V_{max}) remained unchanged. Triangulation of the APs could not be observed in these experiments.

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, Figure 14) show that CBD slightly slowed the restitution kinetics of APD from 317.4 ± 38.0 ms to 431.2 ± 48.6 ms ($n = 5$).

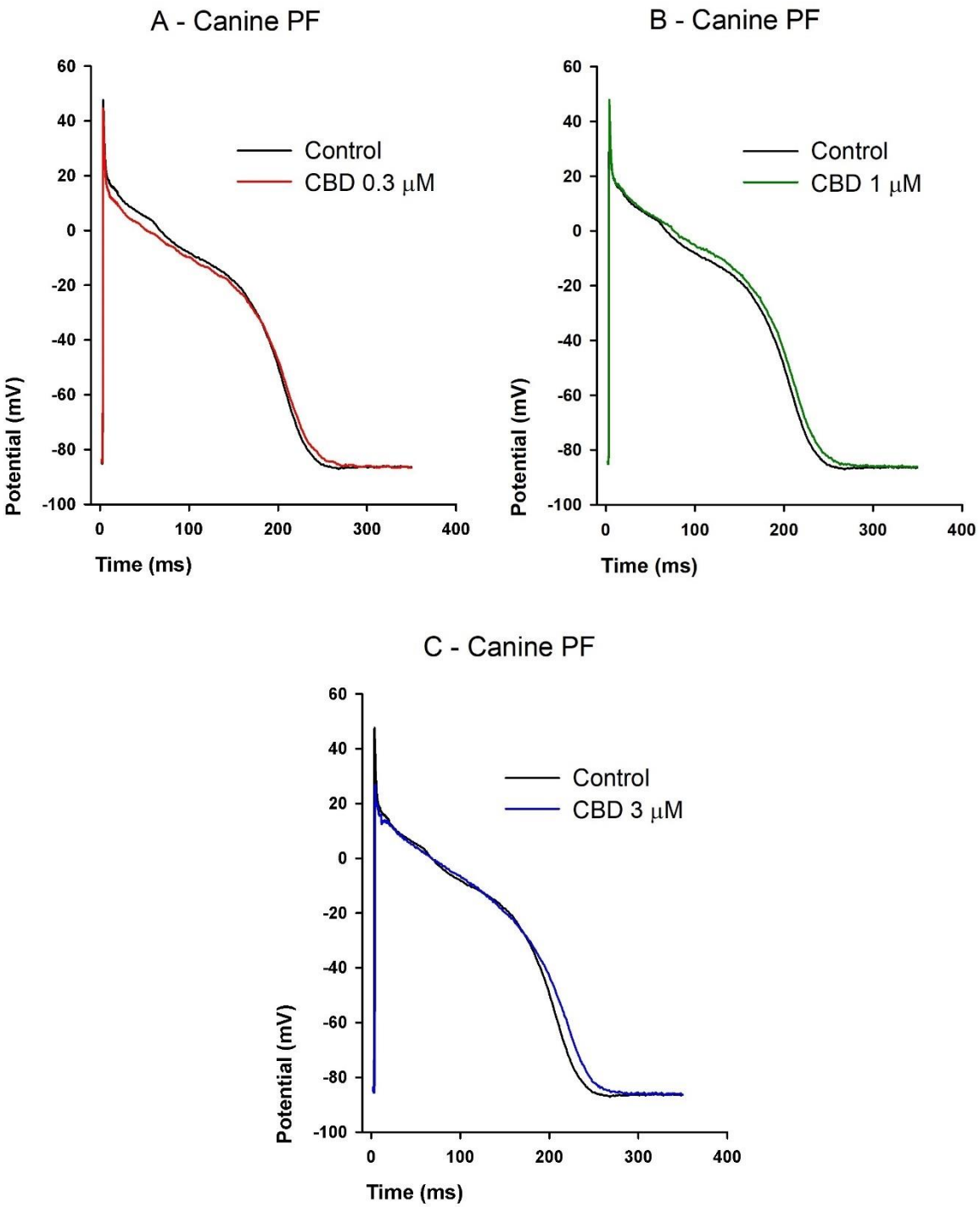


Figure 13 – Electrophysiological effects of cannabidiol (CBD) on action potential characteristics recorded from canine Purkinje fibers at a basic cycle length of 500 ms. Original action potentials demonstrate that CBD slightly prolonged action potential duration at 0.3 and 1 μM concentrations (panel A and panel B). Moreover, at 3 μM concentration (panel C) lengthening was more pronounced. Abbreviations: PF, Purkinje fiber.

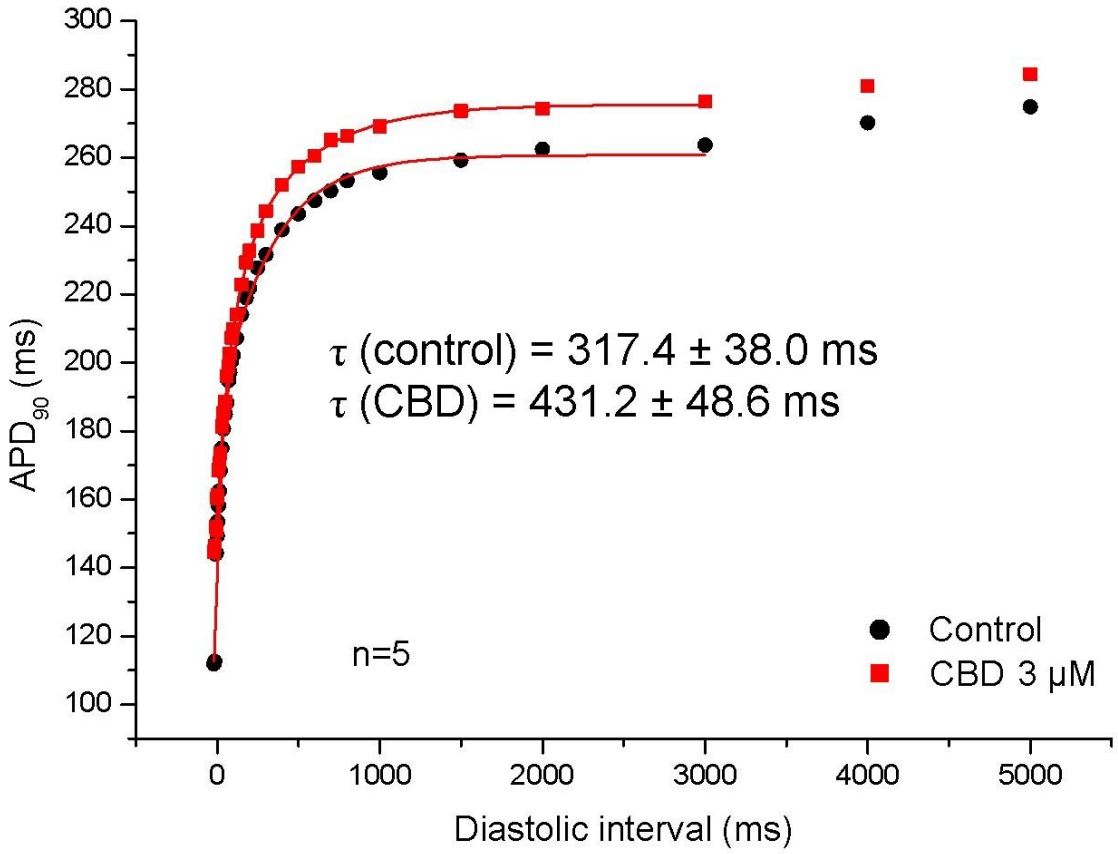


Figure 14 – The effects of cannabidiol (CBD) on restitution of action potential duration (APD) in canine Purkinje fiber preparations. Data points up to 3000 ms diastolic interval were fitted by single exponential function. Kinetical time constants (τ) are shown in control conditions and after drug application. Abbreviations: n, number of experiments.

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 (Rainsford, 1999). Ibuprofen was first used in The United Kingdom in 1969, then from the 1970s it is sold as a prescription only medication. Initially, it was prescribed in low doses (400–1200 mg/day) for the relief of musculoskeletal pain or the inflammation of joints, but over the years the recommended dose was gradually increased to 2400 mg/day (Rainsford, 1999; Rainsford, 2009). Nowadays, low–dose, over–the–counter formulations are available in over 80 countries (Rainsford, 2013). The mechanism of action of ibuprofen incorporates the alteration of different inflammatory pathways in both acute and chronic inflammations (Rainsford, 1992). Main effects exerted by the drug — like some other NSAIDs — are mediated by the inhibition of cyclooxygenase–1 (COX–1) and cyclooxygenase–2 (COX–2) (Rainsford, 1992). Boneberg et al. (1996) found that S(+) enantiomer has a higher efficacy for COX–1 and COX–2 compared with R(−) enantiomer.

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. Yang et al. (2008) found that 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 fast– and slow–response APs of guinea–pig ventricular papillary muscle preparations. In addition, V_{max} was also depressed in a dose–dependent and frequency–dependent manner, however, the RMP and APA were unchanged. 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} (Yang et al., 2008). ECG recorded in *in vivo* and *in vitro* experiments revealed that the drug markedly increased QRS duration and RR intervals, however, QTc was decreased (Yang et al., 2008). 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.

In our study, we have investigated the cardiac electrophysiological effects of ibuprofen in different preparations, i.e., in papillary muscles and Purkinje fibers of rabbits and dogs using the conventional microelectrode technique. The applied concentrations of ibuprofen fall into the therapeutic range of 10–50 μg/ml (48.5–242.4 μM) observed in patients (Holubek et al., 2007), however, in patients serum plasma concentrations of the drug could exceed therapeutic range in certain situations, including liver or kidney dysfunction, drug interactions or high age (Kim et al., 1995). Our findings are partially consistent with the previous investigation reported by Yang et al. (2008). We could confirm the frequency–dependent V_{max} and APD depression evoked by ibuprofen in canine Purkinje fibers (Figure 4). 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 (Figure 2) and dogs (Table 1 and Figure 2), but not in human ventricular preparations (though the drug was not tested above 150 μM concentration) or in dog Purkinje fibers (Figure 3, Table 3).

In addition, 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 ($I_{Ca,L}$) (Paszti et al, 2020). All these effects on ion currents could contribute to the shortening of AP repolarization. In contrast to these observations, Yarishkin et al. (2009) proved that diclofenac, but neither ibuprofen nor naproxen, inhibited $I_{Na,L}$ (reversibly) and $I_{Ca,L}$ (irreversibly) in a dose–dependent manner in rat ventricular myocytes. 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 leading to the lengthening of repolarization (Paszti et al, 2020).

Despite of the contradictory findings, the net effect of ibuprofen on the repolarization of the APs depends on several factors including experimental species (rat, guinea–pig, rabbit or dog), experimental conditions (room temperature or 37°C), preparations (ventricular myocytes or Purkinje fibers) and the distribution of ionic currents. In guinea–pig ventricular myocytes, no I_{to} is presented (Zicha et al., 2003) 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 (Bartos et al., 2015). 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} (Bartos et al., 2015). 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 (Virag et al., 2011; Jost et al., 2013).

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 (Chan et al., 2017; Altaf et al., 2019). Fluoroquinolones are a class of antibiotics with different indications, including respiratory tract infections, skin and soft tissue infections, abdominal infections or urinary tract infections (Van Bambeke et al., 2005). The cardiovascular side effects of fluoroquinolones are well known: Chiba et al. (2000) reported that sparfloxacin, but not levofloxacin prolonged ERP and ventricular repolarization and induced TdP ventricular arrhythmia leading to ventricular fibrillation in dogs. In another study, quinolone antibiotics significantly increased dispersion of repolarization and induced triangulation of APs and TdP tachyarrhythmia (Milberg et al., 2007). Among fluoroquinolones, we chose levofloxacin due to the fact that the drug counts as a relatively safe antibiotic with low proarrhythmic risk (Chiba et al., 2000; Milberg et al., 2007). To support this hypothesis, Lapi et al. (2012) — in a population–based study — found that the use of levofloxacin did not increase the risk of serious arrhythmias. Electrophysiological studies show that levofloxacin did not induce any APD changes in guinea–pig ventricular myocardia or in rabbit Purkinje fiber preparations (Hagiwara et al., 2001; Adamantidis et al., 1998).

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 characteristics including APD, APA, V_{max} and RMP (Table 4/A, Figure 5/A). These findings are in accordance with the results of Hagiwara et al. (2001), and Adamantidis et al. (1998). However, the application of levofloxacin after ibuprofen pretreatment was markedly lengthened APD even though ibuprofen alone caused a moderate APD prolongation (Table 4/B, Figure 5/B). Levofloxacin may inhibit human ether–a–go–go–related gene (hERG) channel (Kang et al., 2001) 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 (Varró and Baczkó, 2011), 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 (Mest et al., 1987). 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 (Rainsford, 2009).

Previous studies have showed that left atrial injection of thromboxane A_2 (TXA_2) could induce ventricular arrhythmias via direct action on cardiac myocytes (Wacker et al., 2006 and 2009). On the other hand, the occurrence of ventricular fibrillation could be reduced by prostacyclin (PGI_2) in a canine model of sudden cardiac death (Fiedler et Mardin, 1986). Moreover, PGI_2 seems to have antiarrhythmic properties on aconitine–induced arrhythmias in rats (Mest and Forster, 1978). Therefore, in aconitine–induced arrhythmias, alteration of the balance between TXA_2 and PGI_2 in favour of PGI_2 could be beneficial in arrhythmia treatment (Riedel et al., 1988). Furthermore, PGI_2 can significantly reduce the amplitude of early afterdepolarization and the prevalence of ventricular tachycardia in anesthetized dogs (Miyazaki et al., 1990), although PGI_2 may increase the occurrence of non–sustained ventricular tachycardias in patients (Brembilla–Perrot et al., 1985).

The antiarrhythmic properties of prostaglandin E_2 (PGE_2) was examined in several investigations. Mest et al. (1977) compared antiarrhythmic effects of PGE_2 with that of propranolol and ajmaline on catecholamine–induced arrhythmias in guinea–pigs. Prophylactic administration of PGE_2 decreased in severity of arrhythmias by 37% (compared with 91% propranolol and 34% ajmaline) (Mest et al., 1977). PGE_2 dose–dependently decreased the incidence of premature ventricular beats in men (Mest et Rausch, 1983) and reduced drug–induced TdP ventricular tachyarrhythmia that action was not mediated by ATP–dependent K^+ channels (Farkas and Coker, 2003).

Evidence about the effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is controversial in the literature. According to Förster et al. (1973), $PGF_{2\alpha}$ improved or even normalized $CaCl_2$–induced

arrhythmias in rats, $BaCl_2$–induced arrhythmias in rabbits and ouabain–induced arrhythmias in cats. In addition, Mann et al. (1973) have found that $PGF_{2\alpha}$ could completely abolished extrasystoles in 5 of 6 patients. The results of Mann et al. (1973) are consistent with the clinical evaluation performed by Sziegoleit et al. (1983) who stated that infusion of $PGF_{2\alpha}$ decreased the incidence of extrasystoles in men, although in one patient ventricular tachycardia occurred. On the other hand, $PGF_{2\alpha}$ may increase the beating rate in isolated atria of mice (Takayama et al., 2005) or in cultured neonatal rats (Li et al., 1997), but in anesthetized cats it could induce episodes of sinus bradycardia as well. In addition, it can even increase the incidence of ouabain–induced arrhythmias in isolated guinea–pig hearts (Moffat et al., 1987). In the experiments of Rao et al. (1987), the arrhythmogenic effects of $PGF_{2\alpha}$ depend on the parasympathetic innervation of the heart in anesthetized cats. According to them, $PGF_{2\alpha}$ 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 $PGF_{2\alpha}$ (Rao et al., 1987).

In experimental conditions, acetylcholine (ACh) and carbachol (CCh) produces positive inotropic effects in isolated rat hearts (Ates et Kaygisiz, 1998) and biphasic inotropic response — transient decrease in contractility followed by an increase — in isolated mice left atria (Tanaka et al., 2001; Hara et al., 2009). The increase in contractile force is mediated by type 3 muscarinic acetylcholine receptors via activation of COX–2 enzyme (Harada et al., 2012). According to Tanaka et al (2001), prostaglandins ($PGF_{2\alpha}$, PGD_2, PGE_2) with the exception of PCI_2 had positive inotropic effect in mice left atria. Moreover, acetylcholine, $PGF_{2\alpha}$ and PGD_2 lengthened action potential duration in a same manner (Tanaka et al., 2003).

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 (Figure 6). 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) — a non–psychoactive cannabinoid — was isolated in the 1940s from marihuana and Cannabis sativa (Mechoulam and Shvo, 1963). In recent years 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 (VanDolah et al., 2019). 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 (VanDolah et al., 2019). Beneficial effects of CBD were observed in other disorders as well such as Alzheimer's disease, Parkinson's disease and multiple sclerosis (Mannucci et al, 2017), or substance use disorders, chronic psychosis and anxiety (Bonaccorso et al., 2019). CBD might have a potential role in the management of chronic pain through the modulation of endocannabinoid, inflammatory and nociceptive systems (Hammell et al., 2016; Boyaji et al., 2020). Moreover, many benign effects were observed in experimental models of cardiovascular diseases such as myocardial infarction, cardiomyopathy or myocarditis as well (Kicman and Toczek, 2020). In general, the chronic use of cannabidiol (up to 1500 mg/day) is well tolerated in humans but the cannabinoid can interfere with hepatic drug metabolism (Bergamaschi et al., 2011). Beside hepatotoxicity, CBD was found to able to cause diarrhea, fatigue, vomiting and somnolence in humans (Huestis et al., 2019). 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. Concentrations of CBD used in our experiments match the plasma levels of CBD measured by Deiana et al. (2012), however, the actual concentration of CBD could be lower than the target concentration because of the high lipophilicity and adherence of CBD to plastic surfaces (Le Marois et al., 2020).

In ventricular papillary muscles of guinea–pigs (Table 5 and Figure 7) and dogs (Table 6 and Figure 12), CBD — applied in two different concentrations (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 (Figure 9 and 10). 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, Figure 9/D and 10/D). CBD exerted either shortening or lengthening of the AP repolarization referring to that CBD might have multiple impact on cardiac ion channels. These effects are similar to that of quinidine — a Class I antiarrhythmic drug — which can prolong APD_{90} at 1 μM concentration but at 10 μM concentration the lengthening is counterbalanced by the V_{max} depression (Roden and Hoffman, 1985). Furthermore, the APD lengthening seemed to be frequency–dependent in papillary muscle preparations of guinea–pigs (Figure 8) and rabbits (Figure 11), 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 (Table 7 and Figure 13) and we have found that CBD dose–dependently increased APD in a concentration range from 0.3 to 3 μM. In contrast to our findings, Le Marois et al. (2020) investigated the effects of CBD in Purkinje fibers of rabbits in different concentrations and at different pacing rates. 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 (Le Marois et al., 2020). 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 (Le Marois et al., 2020).

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 (Figure 14). CBD increased APD and slowed the restitution curve kinetics from $\tau = 317.4 \pm 38.0$ ms to $\tau = 431.2 \pm 48.6$ ms ($n = 5$). Similar effects were earlier described by sotalol and E–4031, — inhibitors of the I_{Kr} current — in human undiseased ventricular muscle preparations (Arpadffy–Lovas et al., 2020). 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. According to the restitution hypothesis, 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) (Garfinkel et al., 2000). The principal determinant of the slope of the restitution curve might be the lengths of APD of the previous basic beats (Shattock et al., 2017), which depends mainly on repolarization currents. On the other hand, other transmembrane currents — such as I_{to}, I_{Na} and $I_{Ca,L}$ — may contribute to slope of it (Elharrar et al., 1984; Arpadffy–Lovas et al., 2020). 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 and synthetic cannabinoids can interfere with transmembrane ion channels. Anandamide — also known as N–arachidonoylethanolamine (AEA) — could inhibit the voltage–dependent sodium channels, L–type calcium channels, cardiac sodium/calcium exchanger (NCX)–mediated currents (Al Kury et al., 2014 and 2014) and the transient outward potassium current (I_{to}), and augment ATP–sensitive potassium current (I_{K-ATP}) in rat ventricular myocytes (Li et al., 2012). Moreover, AEA potently blocked I_{to} in isolated myocytes of human atria (Amoros et al., 2010) and inhibited human cardiac $K_v1.5$ channels — which generate the ultrarapid delayed rectifier current (I_{Kur}) — in a cannabinoid receptor–independent manner (Barana et al., 2010). In addition, JWH–030 — a synthetic cannabinoid — was found to inhibit hERG channels (with an IC_{50} value of 88.36 μM) and lengthen QT interval in anaesthetized rats (Yun et al., 2016). On the other hand, it is also worth to note that the repolarization of rat ventricles mainly depends on the fast component of transient outward potassium current ($I_{to,f}$) and the ultrarapid delayed rectifier potassium current (I_{Kur}), thus inhibition of hERG/I_{Kr} channels seems not so important (Varro et al., 1993; Yeola and Snyders, 1997). Therefore, JWH–030 may exert its effects by the depression of $K_v4.2$ (I_{to}) and/or $K_v1.5$ (I_{Kur})channels.

Not only endocannabinoids, but 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 (Qin et at., 2008;

De Petrocellis et al., 2011 and 2012; Iannotti et al., 2014). According to previous researches, CBD can inhibit human voltage–dependent sodium currents (I_{Na}/$Na_v1.1$–1.7) and human T–type calcium channels (I_{CaT}/$Ca_v3.1$–3.3) (Ross et al., 2008; Ghovanloo et al., 2018).

Le Marois et al (2020) have investigated the effects of CBD on the individual currents of cardiac APs and they found that CBD inhibited I_{Na}, $I_{Na,L}$ ($Na_v1.5$), $I_{Ca,L}$ ($Ca_v1.2$), I_{to} ($K_v4.3$), I_{Kr} ($K_v11.1$) and I_{Ks} ($K_v7.1$) but did not affect I_{K1} ($K_{ir}2.1$) 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 (Le Marois et al., 2020).

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 (Orvos et al., 2020). Furthermore, the observation that 10 μM CBD did not prolong the APD further lead us to measure the effects of CBD on $I_{Na,L}$ and $I_{Ca,L}$. CBD at 10 μM concentration significantly inhibited $I_{Na,L}$ and $I_{Ca,L}$, thus these effects might contribute to the observed AP alterations (Orvos et al., 2020). 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 that was higher than that of tetrahydrocannabinol (THC) (Orvos et al., 2020). These observations are fully in–line with the previous studies reported by Al Kury et al. (2014) and Ghovanloo et al. (2018) but in contrast to the findings of Le Marois et al. (2020), hERG inhibition was more pronounced with a lower IC_{50} value. The different observed IC_{50} value of hERG inhibition could be the cause of the various effects of CBD on AP characteristics in dog Purkinje fiber preparations compared to CBD effects in rabbit Purkinje fiber preparations reported by Le Marois et al. (2020).

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

Redfern et al. (2003) 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. According to Millar et al. (2018), 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 (Millar et al., 2018). In our experiment the IC_{50} value was 2.07 μM for the inhibition of hERG channels and 6.5 μM for the inhibition 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 (Brown and Winterstein, 2019). 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 (Varró and Baczkó, 2011).

5. Conclusion

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

1. Ibuprofen, a very widely used non–steroidal anti-inflammatory drug, 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. In human cardiac preparations, the 200–250 μM (high and maximum) therapeutic concentrations were not tested. The drug did not prolong the APD_{90} at 150 μM in human cardiac preparations (though n numbers should be increased in this protocol). 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, a well–known fluoroquinolone antibiotic, 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. On the other hand, ibuprofen could exert its effects by the inhibition of COX enzymes resulting in altered levels of prostanoids which is in connection with the parasympathetic innervation in the heart. To test this hypothesis, 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, a non–psychoactive cannabinoid, 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 flattening 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.

Acknowledgements

I would like to express my special thanks of gratitude to my supervisors, senior assistant professor (adjunct) István Koncz MD, PhD and associate professor László Virág PhD, for their invaluable support and professional guidance during my PhD work, and for introducing me into a research area involving critical thinking.

I am very grateful to Professor András Varró MD, PhD, DSc and associate professor István Baczkó MD, PhD the former and present Heads of the Department of Pharmacology and Pharmacotherapy, Faculty of Medicines, University of Szeged, for providing me the opportunity to work as a PhD student at the Department and for the continuous support, advices and suggestions. I always admire their optimistic attitude to solve scientific problems.

I am thankful to all my colleagues at the Department, especially for Tibor Magyar MD, Zsuzsa Molnárné and Tamás Árpádffy-Lovas MD, for their technical advice.

Last but not least, I wish to thank all the support and care of my family and friends.

References

Adamantidis MM, Dumotier BM, Caron JF, Bordet R. Sparfloxacin but not levofloxacin or ofloxacin prolongs cardiac repolarization in rabbit Purkinje fibers. *Fundam Clin Pharmacol*. 1998;12(1):70-6.

Adel N. Overview of chemotherapy-induced nausea and vomiting and evidence-based therapies. *Am J Manag Care*. 2017;23(14 Suppl):S259-S65.

Agency EM. Updated advice on use of high-dose ibuprofen 2015 [Available from: https://www.ema.europa.eu/en/news/updated-advice-use-high-dose-ibuprofen].

Aksel G, Guneysel O, Tasyurek T, Kozan E, Cevik SE. Intravenous Lipid Emulsion Therapy for Acute Synthetic Cannabinoid Intoxication: Clinical Experience in Four Cases. *Case Rep Emerg Med*. 2015;2015:180921.

Al Kury LT, Voitychuk OI, Yang KH, Thayyullathil FT, Doroshenko P, Ramez AM, Shuba YM, Galadari S, Howarth FC, Oz M. Effects of the endogenous cannabinoid anandamide on voltage-dependent sodium and calcium channels in rat ventricular myocytes. *Br J Pharmacol*. 2014;171(14):3485-98.

Al Kury LT, Yang KH, Thayyullathil FT, Rajesh M, Ali RM, Shuba YM, Howarth FC, Galadari S, Oz M. Effects of endogenous cannabinoid anandamide on cardiac Na(+)/Ca(2)(+) exchanger. *Cell Calcium*. 2014;55(5):231-7.

Altaf M, Ijaz M, Ghaffar A, Rehman A, Avais M. Antibiotic susceptibility profile and synergistic effect of non-steroidal anti-inflammatory drugs on antibacterial activity of resistant antibiotics (Oxytetracycline and Gentamicin) against methicillin resistant Staphylococcus aureus (MRSA). *Microb Pathog*. 2019;137:103755.

Amoros I, Barana A, Caballero R, Gomez R, Osuna L, Lillo MP, Tamargo J, Delpon E. Endocannabinoids and cannabinoid analogues block human cardiac Kv4.3 channels in a receptor-independent manner. *J Mol Cell Cardiol*. 2010;48(1):201-10.

Antzelevitch C, Burashnikov A. Overview of Basic Mechanisms of Cardiac Arrhythmia. *Card Electrophysiol Clin*. 2011;3(1):23-45.

Antzelevitch C, Sicouri S, Litovsky SH, Lukas A, Krishnan SC, Di Diego JM, Gintant GA, Liu DW. Heterogeneity within the ventricular wall. Electrophysiology and pharmacology of epicardial, endocardial, and M cells. *Circ Res*. 1991;69(6):1427-49.

Arpadffy-Lovas T, Baczko I, Balati B, Bitay M, Jost N, Lengyel C, Nagy N, Takacs J, Varro A, Virag L. Electrical Restitution and Its Modifications by Antiarrhythmic Drugs in Undiseased Human Ventricular Muscle. *Front Pharmacol*. 2020;11:479.

Ashcroft FM. Adenosine 5'-triphosphate-sensitive potassium channels. *Annu Rev Neurosci*. 1988;11:97-118.

Ates S, Kaygisiz Z. Positive inotropic, negative chronotropic, and coronary vasoconstrictor effects of acetylcholine in isolated rat hearts: role of muscarinic receptors, prostaglandins, protein kinase C, influx of extracellular ca2+, intracellular Ca2+ release, and endothelium. *Jpn J Physiol*. 1998;48(6):483-91.

Attwell D, Cohen I, Eisner D, Ohba M, Ojeda C. The steady state TTX-sensitive ("window") sodium current in cardiac Purkinje fibres. *Pflugers Arch*. 1979;379(2):137-42.

Atukorala I, Hunter DJ. Valdecoxib : the rise and fall of a COX-2 inhibitor. *Expert Opin Pharmacother*. 2013;14(8):1077-86.

Back M, Yin L, Ingelsson E. Cyclooxygenase-2 inhibitors and cardiovascular risk in a nation-wide cohort study after the withdrawal of rofecoxib. *Eur Heart J*. 2012;33(15):1928-33.

Barana A, Amoros I, Caballero R, Gomez R, Osuna L, Lillo MP, Blazquez C, Guzman M, Delpon E, Tamargo J. Endocannabinoids and cannabinoid analogues block cardiac hKv1.5 channels in a cannabinoid receptor-independent manner. *Cardiovasc Res*. 2010;85(1):56-67.

Bartos DC, Grandi E, Ripplinger CM. Ion Channels in the Heart. *Compr Physiol*. 2015;5(3):1423-64.

Belardinelli L, Antzelevitch C, Vos MA. Assessing predictors of drug-induced torsade de pointes. *Trends Pharmacol Sci*. 2003;24(12):619-25.

Bergamaschi MM, Queiroz RH, Zuardi AW, Crippa JA. Safety and side effects of cannabidiol, a Cannabis sativa constituent. *Curr Drug Saf*. 2011;6(4):237-49.

Bers DM, Pogwizd SM, Schlotthauer K. Upregulated Na/Ca exchange is involved in both contractile dysfunction and arrhythmogenesis in heart failure. *Basic Res Cardiol*. 2002;97 Suppl 1:I36-42.

Bonaccorso S, Ricciardi A, Zangani C, Chiappini S, Schifano F. Cannabidiol (CBD) use in psychiatric disorders: A systematic review. *Neurotoxicology*. 2019;74:282-98.

Boneberg EM, Zou MH, Ullrich V. Inhibition of cyclooxygenase-1 and -2 by R(-)- and S(+)-ibuprofen. *J Clin Pharmacol*. 1996;36(12 Suppl):16S-9S.

Boyaji S, Merkow J, Elman RNM, Kaye AD, Yong RJ, Urman RD. The Role of Cannabidiol (CBD) in Chronic Pain Management: An Assessment of Current Evidence. *Curr Pain Headache Rep*. 2020;24(2):4.

Brembilla-Perrot B, Terrier de la Chaise A, Clozel J, Cherrier F, Faivre G. Proarrhythmic and antiarrhythmic effects of intravenous prostacyclin in man. *Eur Heart J*. 1985;6(7):609-14.

Brown JD, Winterstein AG. Potential Adverse Drug Events and Drug-Drug Interactions with Medical and Consumer Cannabidiol (CBD) Use. *J Clin Med*. 2019;8(7).

Bueno-Orovio A, Sanchez C, Pueyo E, Rodriguez B. Na/K pump regulation of cardiac repolarization: insights from a systems biology approach. *Pflugers Arch*. 2014;466(2):183-93.

Burns RM, Caulkins JP, Everingham SS, Kilmer B. Statistics on cannabis users skew perceptions of cannabis use. *Front Psychiatry*. 2013;4:138.

Cardiac Arrhythmia Suppression Trial I. Preliminary report: effect of encainide and flecainide on mortality in a randomized trial of arrhythmia suppression after myocardial infarction. *N Engl J Med*. 1989;321(6):406-12.

Carmeliet E. Repolarization reserve in cardiac cells. *Journal of Medical and Biological Engineering*. 2006;26(3):97-105.

Chan EWL, Yee ZY, Raja I, Yap JKY. Synergistic effect of non-steroidal anti-inflammatory drugs (NSAIDs) on antibacterial activity of cefuroxime and chloramphenicol against methicillin-resistant Staphylococcus aureus. *J Glob Antimicrob Resist*. 2017;10:70-4.

Chiba K, Sugiyama A, Satoh Y, Shiina H, Hashimoto K. Proarrhythmic effects of fluoroquinolone antibacterial agents: in vivo effects as physiologic substrate for torsades. *Toxicol Appl Pharmacol*. 2000;169(1):8-16.

Coruzzi G, Venturi N, Spaggiari S. Gastrointestinal safety of novel nonsteroidal antiinflammatory drugs: selective COX-2 inhibitors and beyond. *Acta Biomed*. 2007;78(2):96-110.

De Caterina R, Ruigomez A, Rodriguez LA. Long-term use of anti-inflammatory drugs and risk of atrial fibrillation. *Arch Intern Med*. 2010;170(16):1450-5.

De Petrocellis L, Ligresti A, Moriello AS, Allara M, Bisogno T, Petrosino S, Stott CG, Di Marzo V. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. *Br J Pharmacol*. 2011;163(7):1479-94.

De Petrocellis L, Orlando P, Moriello AS, Aviello G, Stott C, Izzo AA, Di Marzo V. Cannabinoid actions at TRPV channels: effects on TRPV3 and TRPV4 and their potential relevance to gastrointestinal inflammation. *Acta Physiol (Oxf)*. 2012;204(2):255-66.

De Weer P, Gadsby DC, Rakowski RF. Voltage dependence of the Na-K pump. *Annu Rev Physiol*. 1988;50:225-41.

Deiana S, Watanabe A, Yamasaki Y, Amada N, Arthur M, Fleming S, Woodcock H, Dorward P, Pigliacampo B, Close S, Platt B, Riedel G. Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Delta(9)-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. *Psychopharmacology (Berl)*. 2012;219(3):859-73.

DiFrancesco D. The cardiac hyperpolarizing-activated current, if. Origins and developments. *Prog Biophys Mol Biol*. 1985;46(3):163-83.

Donaldson MR, Yoon G, Fu YH, Ptacek LJ. Andersen-Tawil syndrome: a model of clinical variability, pleiotropy, and genetic heterogeneity. *Ann Med*. 2004;36 Suppl 1:92-7.

Douglas RJ. Palpitations following regular ibuprofen dosing in a 13-year-old girl: a case report. *J Med Case Rep*. 2010;4:76.

Durst R, Danenberg H, Gallily R, Mechoulam R, Meir K, Grad E, Beeri R, Pugatsch T, Tarsish E, Lotan C. Cannabidiol, a nonpsychoactive Cannabis constituent, protects against myocardial ischemic reperfusion injury. *Am J Physiol Heart Circ Physiol*. 2007;293(6):H3602-7.

Efe TH, Felekoglu MA, Cimen T, Dogan M. Atrial fibrillation following synthetic cannabinoid abuse. *Turk Kardiyol Dern Ars*. 2017;45(4):362-4.

Elharrar V, Atarashi H, Surawicz B. Cycle length-dependent action potential duration in canine cardiac Purkinje fibers. *Am J Physiol*. 1984;247(6 Pt 2):H936-45.

ElSohly MA, Radwan MM, Gul W, Chandra S, Galal A. Phytochemistry of Cannabis sativa L. *Prog Chem Org Nat Prod*. 2017;103:1-36.

Facer P, Punjabi PP, Abrari A, Kaba RA, Severs NJ, Chambers J, Kooner JS, Anand P. Localisation of SCN10A gene product Na(v)1.8 and novel pain-related ion channels in human heart. *Int Heart J*. 2011;52(3):146-52.

Farkas A, Coker SJ. Prevention of clofilium-induced torsade de pointes by prostaglandin E2 does not involve ATP-dependent K+ channels. *Eur J Pharmacol*. 2003;472(3):189-96.

Fazio G, Vernuccio F, Grutta G, Re GL. Drugs to be avoided in patients with long QT syndrome: Focus on the anaesthesiological management. *World J Cardiol*. 2013;5(4):87-93.

Fiedler VB, Mardin M. Prostacyclin prevents ventricular fibrillation in a canine model of sudden cardiac death. *Basic Res Cardiol*. 1986;81(1):40-53.

Forster W, Mest HJ, Mentz P. The influence of PGF2 alpha on experimental arrhythmias. *Prostaglandins*. 1973;3(6):895-904.

Fraguas-Sanchez AI, Torres-Suarez AI. Medical Use of Cannabinoids. *Drugs*. 2018;78(16):1665-703.

Garfinkel A, Kim YH, Voroshilovsky O, Qu Z, Kil JR, Lee MH, Karagueuzian HS, Weiss JN, Chen PS. Preventing ventricular fibrillation by flattening cardiac restitution. *Proc Natl Acad Sci U S A*. 2000;97(11):6061-6.

Ghovanloo MR, Shuart NG, Mezeyova J, Dean RA, Ruben PC, Goodchild SJ. Inhibitory effects of cannabidiol on voltage-dependent sodium currents. *J Biol Chem*. 2018;293(43):16546-58.

Goodman S, Wadsworth E, Leos-Toro C, Hammond D, International Cannabis Policy Study t. Prevalence and forms of cannabis use in legal vs. illegal recreational cannabis markets. *Int J Drug Policy*. 2020;76:102658.

Goyal H, Singla U, Gupta U, May E. Role of cannabis in digestive disorders. *Eur J Gastroenterol Hepatol*. 2017;29(2):135-43.

Grant AO. Cardiac ion channels. *Circ Arrhythm Electrophysiol*. 2009;2(2):185-94.

Gunter BR, Butler KA, Wallace RL, Smith SM, Harirforoosh S. Non-steroidal anti-inflammatory drug-induced cardiovascular adverse events: a meta-analysis. *J Clin Pharm Ther*. 2017;42(1):27-38.

Hagiwara T, Satoh S, Kasai Y, Takasuna K. A comparative study of the fluoroquinolone antibacterial agents on the action potential duration in guinea pig ventricular myocardia. *Jpn J Pharmacol*. 2001;87(3):231-4.

Hammell DC, Zhang LP, Ma F, Abshire SM, McIlwrath SL, Stinchcomb AL, Westlund KN. Transdermal cannabidiol reduces inflammation and pain-related behaviours in a rat model of arthritis. *Eur J Pain*. 2016;20(6):936-48.

Hara Y, Ike A, Tanida R, Okada M, Yamawaki H. Involvement of cyclooxygenase-2 in carbachol-induced positive inotropic response in mouse isolated left atrium. *J Pharmacol Exp Ther*. 2009;331(3):808-15.

Harada N, Ochi K, Yaosaka N, Teraoka H, Hiraga T, Iwanaga T, Unno T, Komori S, Yamada M, Kitazawa T. Immunohistochemical and functional studies for M(3) muscarinic receptors and cyclo-oxygenase-2 expressed in the mouse atrium. *Auton Autacoid Pharmacol*. 2012;32(3 Pt 4):41-52.

Hla T, Neilson K. Human cyclooxygenase-2 cDNA. *Proc Natl Acad Sci U S A*. 1992;89(16):7384-8.

Hoeker GS, Skarsfeldt MA, Jespersen T, Poelzing S. Electrophysiologic effects of the IK1 inhibitor PA-6 are modulated by extracellular potassium in isolated guinea pig hearts. *Physiol Rep*. 2017;5(1).

Holubek W, Stolbach A, Nurok S, Lopez O, Wetter A, Nelson L. A report of two deaths from massive ibuprofen ingestion. *J Med Toxicol*. 2007;3(2):52-5.

Huestis MA, Solimini R, Pichini S, Pacifici R, Carlier J, Busardo FP. Cannabidiol Adverse Effects and Toxicity. *Curr Neuropharmacol*. 2019;17(10):974-89.

Iannotti FA, Hill CL, Leo A, Alhusaini A, Soubrane C, Mazzarella E, Russo E, Whalley BJ, Di Marzo V, Stephens GJ. Nonpsychotropic plant cannabinoids, cannabidivarin (CBDV) and cannabidiol (CBD), activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels in vitro: potential for the treatment of neuronal hyperexcitability. *ACS Chem Neurosci*. 2014;5(11):1131-41.

Ishihara K, Sarai N, Asakura K, Noma A, Matsuoka S. Role of Mg(2+) block of the inward rectifier K(+) current in cardiac repolarization reserve: A quantitative simulation. *J Mol Cell Cardiol*. 2009;47(1):76-84.

Johnson AG, Nguyen TV, Day RO. Do nonsteroidal anti-inflammatory drugs affect blood pressure? A meta-analysis. *Ann Intern Med*. 1994;121(4):289-300.

Jost N, Virag L, Comtois P, Ordog B, Szuts V, Seprenyi G, Bitay M, Kohajda Z, Koncz I, Nagy N, Szel T, Magyar J, Kovacs M, Puskas LG, Lengyel C, Wettwer E, Ravens U, Nanasi PP, Papp JG, Varro A, Nattel S. Ionic mechanisms limiting cardiac repolarization reserve in humans compared to dogs. *J Physiol*. 2013;591(17):4189-206.

Kalla A, Krishnamoorthy PM, Gopalakrishnan A, Figueredo VM. Cannabis use predicts risks of heart failure and cerebrovascular accidents: results from the National Inpatient Sample. *J Cardiovasc Med (Hagerstown)*. 2018;19(9):480-4.

Kang J, Wang L, Chen XL, Triggle DJ, Rampe D. Interactions of a series of fluoroquinolone antibacterial drugs with the human cardiac K+ channel HERG. *Mol Pharmacol*. 2001;59(1):122-6.

Kicman A, Toczek M. The Effects of Cannabidiol, a Non-Intoxicating Compound of Cannabis, on the Cardiovascular System in Health and Disease. *Int J Mol Sci*. 2020;21(18).

Kim J, Gazarian M, Verjee Z, Johnson D. Acute renal insufficiency in ibuprofen overdose. *Pediatr Emerg Care*. 1995;11(2):107-8.

Koncz I, Gurabi Z, Patocskai B, Panama BK, Szel T, Hu D, Barajas-Martinez H, Antzelevitch C. Mechanisms underlying the development of the electrocardiographic and arrhythmic manifestations of early repolarization syndrome. *J Mol Cell Cardiol*. 2014;68:20-8.

Lall S. CBD oil. *Br Dent J*. 2020;228(6):395.

Lapi F, Wilchesky M, Kezouh A, Benisty JI, Ernst P, Suissa S. Fluoroquinolones and the risk of serious arrhythmia: a population-based study. *Clin Infect Dis*. 2012;55(11):1457-65.

Le Marois M, Ballet V, Sanson C, Maizieres MA, Carriot T, Chantoiseau C, Partiseti M, Bohme GA. Cannabidiol inhibits multiple cardiac ion channels and shortens ventricular action potential duration in vitro. *Eur J Pharmacol*. 2020;886:173542.

Li Q, Ma HJ, Song SL, Shi M, Ma HJ, Li DP, Zhang Y. Effects of anandamide on potassium channels in rat ventricular myocytes: a suppression of I(to) and augmentation of K(ATP) channels. *Am J Physiol Cell Physiol*. 2012;302(6):C924-30.

Li Y, Kang JX, Leaf A. Differential effects of various eicosanoids on the production or prevention of arrhythmias in cultured neonatal rat cardiac myocytes. *Prostaglandins*. 1997;54(2):511-30.

Liang BT. Direct preconditioning of cardiac ventricular myocytes via adenosine A1 receptor and KATP channel. *Am J Physiol*. 1996;271(5 Pt 2):H1769-77.

Litovsky SH, Antzelevitch C. Transient outward current prominent in canine ventricular epicardium but not endocardium. *Circ Res*. 1988;62(1):116-26.

Liu DW, Antzelevitch C. Characteristics of the delayed rectifier current (IKr and IKs) in canine ventricular epicardial, midmyocardial, and endocardial myocytes. A weaker IKs contributes to the longer action potential of the M cell. *Circ Res*. 1995;76(3):351-65.

Lopatin AN, Makhina EN, Nichols CG. Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. *Nature*. 1994;372(6504):366-9.

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.

Mann D, Meyer HG, Forster W. Preliminary clinical experience with the antiarrhythmic effect of PGF2 alpha. *Prostaglandins*. 1973;3(6):905-12.

Mannucci C, Navarra M, Calapai F, Spagnolo EV, Busardo FP, Cas RD, Ippolito FM, Calapai G. Neurological Aspects of Medical Use of Cannabidiol. *CNS Neurol Disord Drug Targets*. 2017;16(5):541-53.

Marsico F, Paolillo S, Filardi PP. NSAIDs and cardiovascular risk. *J Cardiovasc Med (Hagerstown)*. 2017;18 Suppl 1: Special Issue on The State of the Art for the Practicing Cardiologist: The 2016 Conoscere E Curare Il Cuore (CCC) Proceedings from the CLI Foundation:e40-e3.

McGettigan P, Henry D. Cardiovascular risk with non-steroidal anti-inflammatory drugs: systematic review of population-based controlled observational studies. *PLoS Med*. 2011;8(9):e1001098.

Mechoulam R, Shvo Y. Hashish. I. The structure of cannabidiol. *Tetrahedron*. 1963;19(12):2073-8.

Melnyk P, Zhang L, Shrier A, Nattel S. Differential distribution of Kir2.1 and Kir2.3 subunits in canine atrium and ventricle. *Am J Physiol Heart Circ Physiol*. 2002;283(3):H1123-33.

Mesirca P, Marger L, Toyoda F, Rizzetto R, Audoubert M, Dubel S, Torrente AG, Difrancesco ML, Muller JC, Leoni AL, Couette B, Nargeot J, Clapham DE, Wickman K, Mangoni ME. The G-protein-gated K+ channel, IKACh, is required for regulation of pacemaker activity and recovery of resting heart rate after sympathetic stimulation. *J Gen Physiol*. 2013;142(2):113-26.

Mest HJ, Forster W. The antiarrhythmic action of prostacyclin (PGI2) on aconitine induced arrhythmias in rats. *Acta Biol Med Ger*. 1978;37(5-6):827-8.

Mest HJ, Rausch J. The antiarrhythmic effect of PGE2 on premature ventricular beats (PVBs) in man. *Prostaglandins Leukot Med*. 1983;10(3):279-87.

Mest HJ, Schneider S, Riedel A. Relevance of eicosanoids for biochemical regulation of cardiac rhythm disturbances. *Biomed Biochim Acta*. 1987;46(8-9):S534-8.

Mest HJ, Winkler J, Forster W. The antiarrhythmic effect of prostaglandin E2 on catecholamine-induced arrhythmias. *Acta Biol Med Ger*. 1977;36(7-8):1193-6.

Milberg P, Hilker E, Ramtin S, Cakir Y, Stypmann J, Engelen MA, Monnig G, Osada N, Breithardt G, Haverkamp W, Eckardt L. Proarrhythmia as a class effect of quinolones: increased dispersion of repolarization and triangulation of action potential predict torsades de pointes. *J Cardiovasc Electrophysiol*. 2007;18(6):647-54.

Millar SA, Stone NL, Yates AS, O'Sullivan SE. A Systematic Review on the Pharmacokinetics of Cannabidiol in Humans. *Front Pharmacol*. 2018;9:1365.

Mittleman MA, Lewis RA, Maclure M, Sherwood JB, Muller JE. Triggering myocardial infarction by marijuana. *Circulation*. 2001;103(23):2805-9.

Miyazaki T, Pride HP, Zipes DP. Prostaglandin modulation of early afterdepolarizations and ventricular tachyarrhythmias induced by cesium chloride combined with efferent cardiac sympathetic stimulation in dogs. *J Am Coll Cardiol*. 1990;16(5):1287-95.

Moffat MP, Karmazyn M, Ferrier GR. Role of prostaglandins in the arrhythmogenic effects of ouabain on isolated guinea pig hearts. *Eur J Pharmacol*. 1987;141(3):383-93.

Ono K, Iijima T. Pathophysiological significance of T-type Ca2+ channels: properties and functional roles of T-type Ca2+ channels in cardiac pacemaking. *J Pharmacol Sci*. 2005;99(3):197-204.

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.

Pacher P, Steffens S, Hasko G, Schindler TH, Kunos G. Cardiovascular effects of marijuana and synthetic cannabinoids: the good, the bad, and the ugly. *Nat Rev Cardiol*. 2018;15(3):151-66.

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.

Pope JE, Anderson JJ, Felson DT. A meta-analysis of the effects of nonsteroidal anti-inflammatory drugs on blood pressure. *Arch Intern Med*. 1993;153(4):477-84.

Pratt CM, Hertz RP, Ellis BE, Crowell SP, Louv W, Moye L. Risk of developing life-threatening ventricular arrhythmia associated with tefenadine in comparison with over-the-counter antihistamines, ibuprofen and clemastine. *Am J Cardiol*. 1994;73(5):346-52.

Priest BT, McDermott JS. Cardiac ion channels. *Channels (Austin)*. 2015;9(6):352-9.

Qin N, Neeper MP, Liu Y, Hutchinson TL, Lubin ML, Flores CM. TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. *J Neurosci*. 2008;28(24):6231-8.

Rainsford KD. Pharmacology and toxicology of ibuprofen. In: Rainsford KD, editor. Ibuprofen A critical bibliographic review. London: Taylor and Francis; 1992. p. 145-275.

Rainsford KD. History and development of ibuprofen. In: KD R, editor. Ibuprofen A critical bibliographic review. London: Taylor and Francis; 1999. p. 3-24.

Rainsford KD. Ibuprofen: pharmacology, efficacy and safety. *Inflammopharmacology*. 2009;17(6):275-342.

Rainsford KD. Ibuprofen: from invention to an OTC therapeutic mainstay. *Int J Clin Pract Suppl*. 2013(178):9-20.

Rainsford KD KI, Kean WF. Gastro-intestinal complications of anti-rheumatic drugs. In: Ramos-Casals M KM, Britó-Zeron P, Atzeni F, Rodés Teixidor J, editor. Handbook of systemic autoimmune diseases The digestive involvement in systemic autoimmune diseases. Amsterdam: Elsevier; 2008. p. 243-75.

Rao TS, Seth SD, Nayar U, Manchanda SC. The vagal involvement in the antiarrhythmic and arrhythmogenic effects of prostaglandin F2 alpha on ouabain-induced cardiac arrhythmias in cats. *Life Sci*. 1987;41(21):2363-72.

Redfern WS, Carlsson L, Davis AS, Lynch WG, MacKenzie I, Palethorpe S, Siegl PK, Strang I, Sullivan AT, Wallis R, Camm AJ, Hammond TG. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. *Cardiovasc Res*. 2003;58(1):32-45.

Remme CA, Verkerk AO, Hoogaars WM, Aanhaanen WT, Scicluna BP, Annink C, van den Hoff MJ, Wilde AA, van Veen TA, Veldkamp MW, de Bakker JM, Christoffels VM, Bezzina CR. The cardiac sodium channel displays differential distribution in the conduction system and transmural heterogeneity in the murine ventricular myocardium. *Basic Res Cardiol*. 2009;104(5):511-22.

Rezkalla S, Kloner RA. Cardiovascular effects of marijuana. *Trends Cardiovasc Med*. 2019;29(7):403-7.

Riedel A, Schneider S, Mest HJ. The effect of substances influencing the arachidonic acid cascade on experimental cardiac arrhythmias. *Biomed Biochim Acta*. 1988;47(10-11):S27-30.

Roden DM. Taking the "idio" out of "idiosyncratic": predicting torsades de pointes. *Pacing Clin Electrophysiol*. 1998;21(5):1029-34.

Roden DM. Repolarization reserve: a moving target. *Circulation*. 2008;118(10):981-2.

Roden DM, Hoffman BF. Action potential prolongation and induction of abnormal automaticity by low quinidine concentrations in canine Purkinje fibers. Relationship to potassium and cycle length. *Circ Res*. 1985;56(6):857-67.

Ross HR, Napier I, Connor M. Inhibition of recombinant human T-type calcium channels by Delta9-tetrahydrocannabinol and cannabidiol. *J Biol Chem*. 2008;283(23):16124-34.

Sauza-Sosa. Torsades De Pointes Induced by Levofloxacin in an Unknown Long QT Syndrome. *Cardiovasc Pharm*. 2016;5(4).

Schillinger W, Janssen PM, Emami S, Henderson SA, Ross RS, Teucher N, Zeitz O, Philipson KD, Prestle J, Hasenfuss G. Impaired contractile performance of cultured rabbit ventricular myocytes after adenoviral gene transfer of Na(+)-Ca(2+) exchanger. *Circ Res*. 2000;87(7):581-7.

Schmidt M, Christiansen CF, Mehnert F, Rothman KJ, Sorensen HT. Non-steroidal anti-inflammatory drug use and risk of atrial fibrillation or flutter: population based case-control study. *BMJ*. 2011;343:d3450.

Shattock MJ, Park KC, Yang HY, Lee AWC, Niederer S, MacLeod KT, Winter J. Restitution slope is principally determined by steady-state action potential duration. *Cardiovasc Res*. 2017;113(7):817-28.

Sondergaard KB, Gislason G. NSAIDs and cardiac arrest: Non-steroidal anti-inflammatory drug use is associated with increased risk of Out-of-hospital Cardiac Arrest: A nationwide Case-Time-Control study. *Eur Heart J*. 2017;38(23):1788-9.

Stampanoni Bassi M, Sancesario A, Morace R, Centonze D, Iezzi E. Cannabinoids in Parkinson's Disease. *Cannabis Cannabinoid Res*. 2017;2(1):21-9.

Sziegoleit W, Hofmann H, Lange R, Mann D, Pundrich R, Machill K, Hafemeister G, Forster W. Evaluation of antiarrhythmic activity of prostaglandin F2 alpha in man. *Prostaglandins Leukot Med*. 1983;11(4):457-69.

Takayama K, Yuhki K, Ono K, Fujino T, Hara A, Yamada T, Kuriyama S, Karibe H, Okada Y, Takahata O, Taniguchi T, Iijima T, Iwasaki H, Narumiya S, Ushikubi F. Thromboxane A2 and prostaglandin F2alpha mediate inflammatory tachycardia. *Nat Med*. 2005;11(5):562-6.

Talarico G, Trebbastoni A, Bruno G, de Lena C. Modulation of the Cannabinoid System: A New Perspective for the Treatment of the Alzheimer's Disease. *Curr Neuropharmacol*. 2019;17(2):176-83.

Tanaka H, Nishimaru K, Kobayashi M, Matsuda T, Tanaka Y, Shigenobu K. Acetylcholine-induced positive inotropy mediated by prostaglandin released from endocardial endothelium in mouse left atrium. *Naunyn Schmiedebergs Arch Pharmacol*. 2001;363(5):577-82.

Tanaka H, Nishimaru K, Makuta R, Hirayama W, Kawamura T, Matsuda T, Tanaka Y, Kawanishi T, Shigenobu K. Possible involvement of prostaglandins F(2alpha) and D(2) in acetylcholine-induced positive inotropy in isolated mouse left atria. *Pharmacology*. 2003;67(3):157-62.

Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. *Eur Heart J*. 2016;37(42):3232-45.

Van Bambeke F, Michot JM, Van Eldere J, Tulkens PM. Quinolones in 2005: an update. *Clin Microbiol Infect*. 2005;11(4):256-80.

Vandenberg CA. Inward rectification of a potassium channel in cardiac ventricular cells depends on internal magnesium ions. *Proc Natl Acad Sci U S A*. 1987;84(8):2560-4.

VanDolah HJ, Bauer BA, Mauck KF. Clinicians' Guide to Cannabidiol and Hemp Oils. *Mayo Clin Proc*. 2019;94(9):1840-51.

Varro A, Baczko I. Cardiac ventricular repolarization reserve: a principle for understanding drug-related proarrhythmic risk. *Br J Pharmacol*. 2011;164(1):14-36.

Varro A, Balati B, Iost N, Takacs J, Virag L, Lathrop DA, Csaba L, Talosi L, Papp JG. The role of the delayed rectifier component IKs in dog ventricular muscle and Purkinje fibre repolarization. *J Physiol*. 2000;523 Pt 1:67-81.

Varro A, Lathrop DA, Hester SB, Nanasi PP, Papp JG. Ionic currents and action potentials in rabbit, rat, and guinea pig ventricular myocytes. *Basic Res Cardiol*. 1993;88(2):93-102.

Vaughan Williams EM, Somberg, J. C. Conrol of cardiac rhythm: Lippincott-Raven; 1998.

Virag L, Acsai K, Hala O, Zaza A, Bitay M, Bogats G, Papp JG, Varro A. Self-augmentation of the lengthening of repolarization is related to the shape of the cardiac action potential: implications for reverse rate dependency. *Br J Pharmacol*. 2009;156(7):1076-84.

Virag L, Jost N, Papp R, Koncz I, Kristof A, Kohajda Z, Harmati G, Carbonell-Pascual B, Ferrero JM, Jr., Papp JG, Nanasi PP, Varro A. Analysis of the contribution of I(to) to repolarization in canine ventricular myocardium. *Br J Pharmacol*. 2011;164(1):93-105.

Von Der Haar J, Talebi, S., Ghobadi, F., Singh, S., Chirurgi, R., Rajeswari, P., Kalantari, H., Hassen, G.W. Synthetic Cannabinoids and Their Effects on the Cardiovascular System. *The Journal of Emergency Medicine*. 2016;50(2):258-62.

Wacker MJ, Best SR, Kosloski LM, Stachura CJ, Smoot RL, Porter CB, Orr JA. Thromboxane A2-induced arrhythmias in the anesthetized rabbit. *Am J Physiol Heart Circ Physiol*. 2006;290(4):H1353-61.

Wacker MJ, Kosloski LM, Gilbert WJ, Touchberry CD, Moore DS, Kelly JK, Brotto M, Orr JA. Inhibition of thromboxane A2-induced arrhythmias and intracellular calcium changes in cardiac myocytes by blockade of the inositol trisphosphate pathway. *J Pharmacol Exp Ther*. 2009;331(3):917-24.

Waldo AL, Camm AJ, deRuyter H, Friedman PL, MacNeil DJ, Pauls JF, Pitt B, Pratt CM, Schwartz PJ, Veltri EP. Effect of d-sotalol on mortality in patients with left ventricular dysfunction after recent and remote myocardial infarction. The SWORD Investigators. Survival With Oral d-Sotalol. *Lancet*. 1996;348(9019):7-12.

Watanabe A, Nakamura K, Morita H, Kusano KF, Ohe T. [Long QT syndrome]. *Nihon Rinsho*. 2005;63(7):1171-7.

Wemhoner K, Friedrich C, Stallmeyer B, Coffey AJ, Grace A, Zumhagen S, Seebohm G, Ortiz-Bonnin B, Rinne S, Sachse FB, Schulze-Bahr E, Decher N. Gain-of-function mutations in the calcium channel CACNA1C (Cav1.2) cause non-syndromic long-QT but not Timothy syndrome. *J Mol Cell Cardiol*. 2015;80:186-95.

Westin AA, Frost J, Brede WR, Gundersen PO, Einvik S, Aarset H, Slordal L. Sudden Cardiac Death Following Use of the Synthetic Cannabinoid MDMB-CHMICA. *J Anal Toxicol*. 2016;40(1):86-7.

Wilde AAM, Amin AS. Clinical Spectrum of SCN5A Mutations: Long QT Syndrome, Brugada Syndrome, and Cardiomyopathy. *JACC Clin Electrophysiol*. 2018;4(5):569-79.

Yamanoglu A, Celebi Yamanoglu NG, Evran T, Sogut O. How much can synthetic cannabinoid damage the heart? A case of cardiogenic shock following resistant ventricular fibrillation after synthetic cannabinoid use. *J Clin Ultrasound*. 2018;46(9):605-9.

Yanes JA, McKinnell ZE, Reid MA, Busler JN, Michel JS, Pangelinan MM, Sutherland MT, Younger JW, Gonzalez R, Robinson JL. Effects of cannabinoid administration for pain: A meta-analysis and meta-regression. *Exp Clin Psychopharmacol*. 2019;27(4):370-82.

Yang T, Atack TC, Stroud DM, Zhang W, Hall L, Roden DM. Blocking Scn10a channels in heart reduces late sodium current and is antiarrhythmic. *Circ Res*. 2012;111(3):322-32.

Yang ZF, Wang HW, Zheng YQ, Zhang Y, Liu YM, Li CZ. Possible arrhythmiogenic mechanism produced by ibuprofen. *Acta Pharmacol Sin*. 2008;29(4):421-9.

Yarishkin OV, Hwang EM, Kim D, Yoo JC, Kang SS, Kim DR, Shin JH, Chung HJ, Jeong HS, Kang D, Han J, Park JY, Hong SG. Diclofenac, a Non-steroidal Anti-inflammatory Drug, Inhibits L-type Ca Channels in Neonatal Rat Ventricular Cardiomyocytes. *Korean J Physiol Pharmacol*. 2009;13(6):437-42.

Yeola SW, Snyders DJ. Electrophysiological and pharmacological correspondence between Kv4.2 current and rat cardiac transient outward current. *Cardiovasc Res*. 1997;33(3):540-7.

Yun J, Yoon KS, Lee TH, Lee H, Gu SM, Song YJ, Cha HJ, Han KM, Seo H, Shin J, Park HK, Kim HS, Kim YH. Synthetic cannabinoid, JWH-030, induces QT prolongation through hERG channel inhibition. *Toxicol Res (Camb)*. 2016;5(6):1663-71.

Zicha S, Moss I, Allen B, Varro A, Papp J, Dumaine R, Antzelevich C, Nattel S. Molecular basis of species-specific expression of repolarizing K+ currents in the heart. *Am J Physiol Heart Circ Physiol*. 2003;285(4):H1641-9.

Zygmunt AC, Eddlestone GT, Thomas GP, Nesterenko VV, Antzelevitch C. Larger late sodium conductance in M cells contributes to electrical heterogeneity in canine ventricle. *Am J Physiol Heart Circ Physiol*. 2001;281(2):H689-97.

Zygmunt AC, Goodrow RJ, Antzelevitch C. I(NaCa) contributes to electrical heterogeneity within the canine ventricle. *Am J Physiol Heart Circ Physiol*. 2000;278(5):H1671-8.

I.

NRC Research Press

ARTICLE

Cardiac electrophysiological effects of ibuprofen in dog and rabbit ventricular preparations: possible implication to enhanced proarrhythmic risk[1]

Bence Pászti, János Prorok, Tibor Magyar, Tamás Árpádffy-Lovas, Balázs Györe, Leila Topál, Péter Gazdag, Jozefina Szlovák, Muhammad Naveed, Norbert Jost, Norbert Nagy, András Varró, László Virág, and István Koncz

Abstract: Ibuprofen is a widely used nonsteroidal anti-inflammatory drug, which has recently been associated with increased cardiovascular risk, but its electrophysiological effects have not yet been properly studied in isolated cardiac preparations. We studied the effects of ibuprofen on action potential characteristics and several transmembrane ionic currents using the conventional microelectrode technique and the whole-cell configuration of the patch-clamp technique on cardiac preparations and enzymatically isolated ventricular myocytes. In dog (200 μM; $n = 6$) and rabbit (100 μM; $n = 7$) papillary muscles, ibuprofen moderately but significantly prolonged repolarization at 1 Hz stimulation frequency. In dog Purkinje fibers, repolarization was abbreviated and maximal rate of depolarization was depressed in a frequency-dependent manner. Levofloxacin (40 μM) alone did not alter repolarization, but augmented the ibuprofen-evoked repolarization lengthening in rabbit preparations ($n = 7$). In dog myocytes, ibuprofen (250 μM) did not significantly influence I_{K1}, but decreased the amplitude of I_{to} and I_{Kr} potassium currents by 28.2% (60 mV) and 15.2% (20 mV), respectively. Ibuprofen also depressed I_{NaL} and I_{Ca} currents by 19.9% and 16.4%, respectively. We conclude that ibuprofen seems to be free from effects on action potential parameters at lower concentrations. However, at higher concentrations it may alter repolarization reserve, contributing to the observed proarrhythmic risk in patients.

Key words: ibuprofen, levofloxacin, repolarization reserve.

Résumé : L'ibuprofène est un anti-inflammatoire non stéroïdien largement utilisé, qui a récemment été associé avec un accroissement du risque cardiovasculaire, mais ses effets électrophysiologiques n'ont pas encore été étudiés adéquatement dans des préparations de cœur isolé. À l'aide de la technique de microélectrode classique et de la technique de « patch-clamp » avec configuration sur cellules entières, nous avons étudié les effets de l'ibuprofène sur les caractéristiques du potentiel d'action ainsi que sur plusieurs courants ioniques transmembranaires dans des préparations de cœur et de myocytes ventriculaires isolés enzymatiquement. Dans le muscle papillaire de chien (200 μM; $n = 6$) et de lapin (100 μM; $n = 7$) stimulé à une fréquence de 1 Hz, l'ibuprofène entraînait une prolongation de la repolarisation notable, bien que modérée. Dans les fibres de Purkinje canines, la durée et la vitesse maximale de la repolarisation diminuaient de manière fréquence-dépendante. Chez le lapin ($n = 7$), la lévofloxacine (40 μM) administrée seule n'entraînait pas de modification de la repolarisation, mais bien une augmentation du prolongement de la repolarisation obtenu avec l'ibuprofène. Dans les myocytes canins, l'ibuprofène (250 μM) n'avait pas d'influence marquée sur I_{K1}, mais entraînait une diminution de l'amplitude des courants potassiques I_{to} et I_{Kr} de 28,2 (60 mV) et de 15,2 % (20 mV), respectivement. L'ibuprofène entraînait aussi une dépression des courants I_{NaL} et I_{Ca} de 19,9 et de 16,4 %, respectivement. Nous en arrivons à la conclusion que l'ibuprofène ne semble pas avoir d'effet sur les paramètres du potentiel d'action à de faibles concentrations. Cependant, à des concentrations plus élevées, il pourrait porter atteinte à la réserve de repolarisation, participant ainsi au risque proarythmique observé chez les patients. [Traduit par la Rédaction]

Mots-clés : ibuprofène, lévofloxacine, réserve de repolarisation.

Received 7 July 2020. Accepted 31 August 2020.

B. Pászti,* T. Magyar, T. Árpádffy-Lovas, L. Topál, P. Gazdag, J. Szlovák, M. Naveed, and I. Koncz.† Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, Szeged, Hungary.

J. Prorok* and N. Nagy. Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, Szeged, Hungary; MTA-SZTE Research Group of Cardiovascular Pharmacology, Szeged, Hungary.

B. Györe. Department of Oral Surgery, Faculty of Dentistry, University of Szeged, Szeged, Hungary.

N. Jost, A. Varró, and L. Virág.† Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, Szeged, Hungary; Department of Pharmacology and Pharmacotherapy, Interdisciplinary Excellence Centre, University of Szeged, Szeged, Hungary; MTA-SZTE Research Group of Cardiovascular Pharmacology, Szeged, Hungary.

Corresponding author: András Varró (email: varro.andras@med.u-szeged.hu).

*These authors share first authorship.

†These authors share senior authorship.

[1]This paper is part of a special issue of selected papers from the Joint North American/European IACS 2019.

Introduction

Ibuprofen is one of the most widely used nonsteroidal anti-inflammatory drugs (NSAIDs) (Rainsford 2009). However, a recent Danish nationwide case–time–control study (Sondergaard et al. 2017) found that short-term therapy with ibuprofen was associated with an increased risk of cardiac arrest. It is important to mention that this study also concluded that there was an increased risk of out-of-hospital cardiac arrest in diclofenac users. In an observational, historical cohort evaluation (Pratt et al. 1994), it was found that the ibuprofen cohort had a significantly higher arrhythmic event rate. A case report outlined a probable relationship between standard ibuprofen dosing and palpitations (Douglas 2010). Surprisingly, very little is known about the cardiac electrophysiological effect of ibuprofen, and, to our knowledge, the cellular cardiac electrophysiological effects of ibuprofen have been investigated only in one study on guinea pig papillary muscle and sinoatrial node (Yang et al. 2008). In these preparations, ibuprofen dose-dependently shortened action potential duration (APD) and decreased the maximal rate of depolarization (V_{max}) at therapeutically relevant and at high concentrations. The effects of ibuprofen on the action potential parameters and the underlying transmembrane currents have not yet been reported in other cardiac preparations, including those obtained from larger animals (e.g., rabbit or dog), closer to human in heart size, in spontaneous frequency, and in basic electrophysiological properties. Repolarization prolonging properties have also been reported among fluoroquinolone antibiotic agents (Chiba et al. 2000; Garnett and Johannesen 2016; Komatsu et al. 2019), and combination of such antibiotics and NSAIDs is a common practice in the treatment of infections. Therefore, the purpose of our work was to further characterize the cellular electrophysiological effects of ibuprofen and levofloxacin using preparations obtained from the hearts of large animals, namely dogs and rabbits. We found that 50 μM ibuprofen did not influence the action potential parameters including APD in dog and rabbit ventricular muscle preparations but at higher concentrations (100–200 μM), especially when repolarization reserve (Varró et al. 2000; Roden 2006; Varró and Baczkó 2011) had been previously attenuated, some repolarization lengthening occurred. Therefore, although at low therapeutic concentrations the drug could be considered safe regarding its cardiac electrophysiological effects, it is important to further improve our understanding concerning the possible unfavorable association between ibuprofen and increased cardiovascular risk reported in clinical studies.

Methods

Conventional microelectrode technique

All experiments were conducted in compliance with the *Guide for the Care and Use of Laboratory Animals* (USA NIH publication No. 85-23, revised 1996) and conformed to Directive 2010/63/EU of the European Parliament. The protocols were approved by the Review Board of the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development, Hungary (XIII/1211/2012). Ventricular (papillary) muscles were obtained from the right ventricle of rabbits and dogs. Free-running (false tendons of) Purkinje fibers were isolated from both ventricles of dog hearts removed through a right lateral thoracotomy. Male New Zealand rabbits (body mass 2–3 kg) were terminated by rapid cervical dislocation, and Beagle dogs (body mass 10–15 kg) of both sexes were anesthetized and sacrificed using high-dose sodium pentobarbital (60 mg/kg i.v.). The preparations were placed in a tissue bath and allowed to equilibrate for at least 2 h while superfused (flow rate 4–5 mL/min) with Locke's solution containing (in mM): NaCl 120, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 1, $NaHCO_3$ 22, and glucose 11. The pH of this solution was 7.35 to 7.40 when gassed with 95% O_2 and 5% CO_2 at 37 °C. During the equilibration period, the ventricular muscle tissues were stimulated at a basic cycle length of 1000 ms, Purkinje fibers were stimulated at a basic cycle length of 500 ms. Electrical pulses of 0.5–2 ms in duration and twice diastolic threshold in intensity (S_1) were delivered to the preparations through bipolar platinum electrodes. Transmembrane potentials were recorded with the use of glass capillary microelectrodes filled with 3 M KCl (tip resistance 5–15 MΩ). The microelectrodes were coupled through an Ag–AgCl junction to the input of a high-impedance, capacitance-neutralizing amplifier (Experimetria, Type 309, Budapest, Hungary). Intracellular recordings were displayed on a storage oscilloscope (Hitachi V-555) and led to a computer system (APES) designed for online determination of the following parameters: resting membrane potential, action potential amplitude, APD at 50% (APD_{50}) and 90% (APD_{90}) repolarization, and the maximum rate of rise of the action potential upstroke (V_{max}). The following types of stimulation were applied in the course of the experiments: stimulation with a constant cycle length of 1000 ms (ventricular muscles); stimulation with a constant cycle length of 500 ms (Purkinje fibers). In case of Purkinje fibers, stimulation with different constant cycle lengths ranging from 300 to 1000 ms were also applied. Control recordings were obtained after the equilibration period. The effects of ibuprofen and dimethyl sulfoxide (DMSO) not exceeding 18.8% were determined at the given concentrations, after the addition of each compound until 30 min elapsed, in a cumulative manner. Compounds were purchased from Sigma/Merck for all experiments.

Whole-cell configuration of the patch-clamp technique

Untreated adult beagle dogs of either sex (body mass 8–15 kg) were used for the study. All experiments were conducted in compliance with the *Guide for the Care and Use of Laboratory Animals* (USA NIH publication No. 85-23, revised 1996) and conformed to the Directive 2010/63/EU of the European Parliament. The protocols were approved by the review board of Committee on Animal Research of the Albert Szent-Györgyi Medical University (54/1999 OEj).

The isolation and preparation of dog ventricular myocytes were described earlier in detail (Varró et al. 2000). One drop of cell suspension was placed in a transparent recording chamber mounted on the stage of an inverted microscope. The myocytes were allowed to settle and adhere to the bottom for at least 5–10 min before superfusion was initiated with Tyrode solution containing (in mM): NaCl 144, NaH_2PO_4 0.4, KCl 4.0, $CaCl_2$ 1.8, $MgSO_4$ 0.53, glucose 5.5, and HEPES 5.0 (pH 7.4, NaOH). Temperature was set to 37 °C. Only rod-shaped cells with clear cross-striations were used. Patch-clamp micropipettes were fabricated from borosilicate glass capillaries using a micropipette puller (Flaming/Brown, type P-97; Sutter Instrument, Novato, California, USA). These electrodes had resistances between 1.5 and 2.5 MΩ. Membrane currents were recorded with Axopatch 200B patch-clamp amplifiers (Molecular Devices Inc., Sunnyvale, California, USA) using the whole-cell configuration of the patch-clamp technique. After establishing a high resistance (1–10 GΩ) seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by suction or application of short electrical pulses. Membrane currents were digitized after low-pass filtering at 1 kHz using analog-to-digital converters (Digidata 1440 A; Molecular Devices Inc., Sunnyvale, California, USA) under software control (pClamp 10; Molecular Devices Inc., Sunnyvale, California, USA). The various ion currents were measured as described earlier in detail (Kohajda et al. 2016). The same software was used for offline analysis.

Statistical analysis

Results are expressed as mean ± SEM. Normality of distributions was verified using the Shapiro–Wilk test, and homogeneity of variances was verified using Bartlett's test in each treatment group. Statistical comparisons were made using Student's *t* test for Tables 1, 2A, and 2B. Variance analysis (ANOVA) for repeated measurements was performed, followed by Bonferroni's post hoc

Table 1. The electrophysiological effects of dimethyl sulfoxide (DMSO, 2.2‰) and ibuprofen (50 μM and 200 μM) in dog right ventricular papillary muscle preparations (VM; A, B, and C), at basic cycle length of 1000 ms; and ibuprofen (200 μM) in dog Purkinje fibers (PF; D) at basic cycle length of 500 ms.

(A)	Sample	RP (mV)	APA (mV)	V_{max} (V/s)	APD_{50} (ms)	APD_{90} (ms)	APD_{90} (%)
Control	Dog VM (n = 6)	−83.3 ± 2.3	106.7 ± 1.5	136.7 ± 14.2	157.8 ± 11.6	203.6 ± 7.6	—
DMSO (2.2‰)	Dog VM (n = 6)	−85.8 ± 1.7	105.3 ± 1.4	123.3 ± 17.0	153.8 ± 11.5	201.6 ± 8.0	−1.0 ± 1.2
(B)	**Sample**	**RP (mV)**	**APA (mV)**	**V_{max} (V/s)**	**APD_{50} (ms)**	**APD_{90} (ms)**	**APD_{90} (%)**
Control	Dog VM (n = 8)	−83.2 ± 1.6	108.1 ± 1.0	175.2 ± 22.3	187.0 ± 9.3	227.5 ± 9.7	—
Ibuprofen (50 μM)	Dog VM (n = 8)	−85.3 ± 2.1	106.7 ± 1.9	172.4 ± 29.9	187.7 ± 9.7	225.9 ± 8.9	−0.6 ± 1.0
(C)	**Sample**	**RP (mV)**	**APA (mV)**	**V_{max} (V/s)**	**APD_{50} (ms)**	**APD_{90} (ms)**	**APD_{90} (%)**
Control	Dog VM (n = 6)	−89.0 ± 1.8	110.6 ± 2.4	174.6 ± 20.3	173.8 ± 8	214.1 ± 5.9	—
Ibuprofen (200 μM)	Dog VM (n = 6)	−89.1 ± 3.2	113.4 ± 3.0	192.9 ± 27.1	181.6 ± 6.3	223.0 ± 4.9*	4.3 ± 1.0
(D)	**Sample**	**RP (mV)**	**APA (mV)**	**V_{max} (V/s)**	**APD_{50} (ms)**	**APD_{90} (ms)**	**APD_{90} (%)**
Control	Dog PF (n = 7)	−89.7 ± 0.7	133.5 ± 3.3	580.7 ± 36.0	163.9 ± 10.9	253.4 ± 14.2	—
Ibuprofen (200 μM)	Dog PF (n = 7)	−87.3 ± 1.0	135.9 ± 3.4	621.5 ± 93.5	163.7 ± 11.2	242.0 ± 13.7*	−4.5 ± 0.7

Note: Results are expressed as means ± SEM. RP, resting potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{50} and APD_{90}, action potential durations at 50% and 90% of repolarization.

*$p < 0.05$, Student's t test for paired data.

Fig. 1. The effects of ibuprofen and dimethyl sulfoxide (DMSO) on action potentials recorded from different cardiac preparations. Original action potential records show that ibuprofen (at 100 μM) slightly but significantly lengthened the action potential duration in rabbit right ventricular papillary muscle (panel C) and in dog right ventricular papillary muscle (at 200 μM, panel A) at a basic cycle length of 1000 ms. However, in dog Purkinje fiber (panel B) the drug significantly shortened the action potential repolarization at a basic cycle length of 500 ms. DMSO at 2.2‰ did not alter action potential duration in any of the preparations at the same cycle lengths (panels D–F). [Color online.]

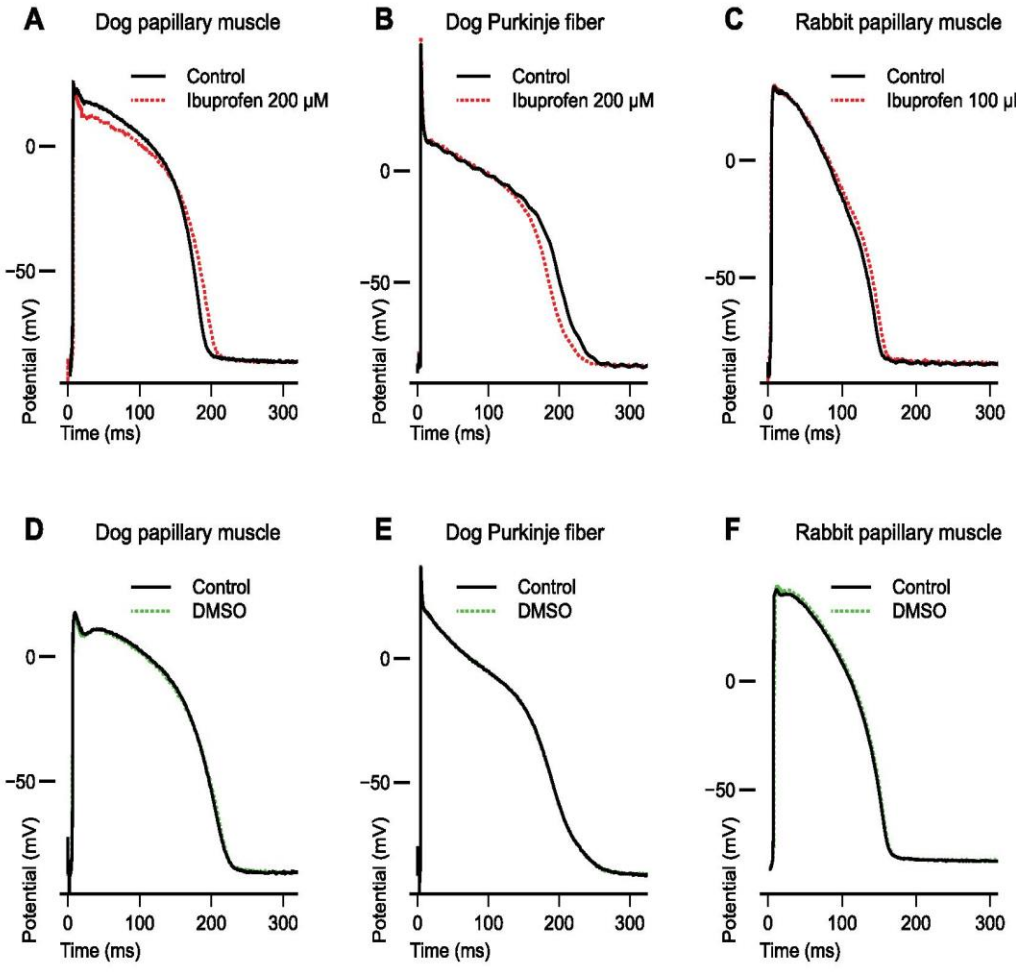


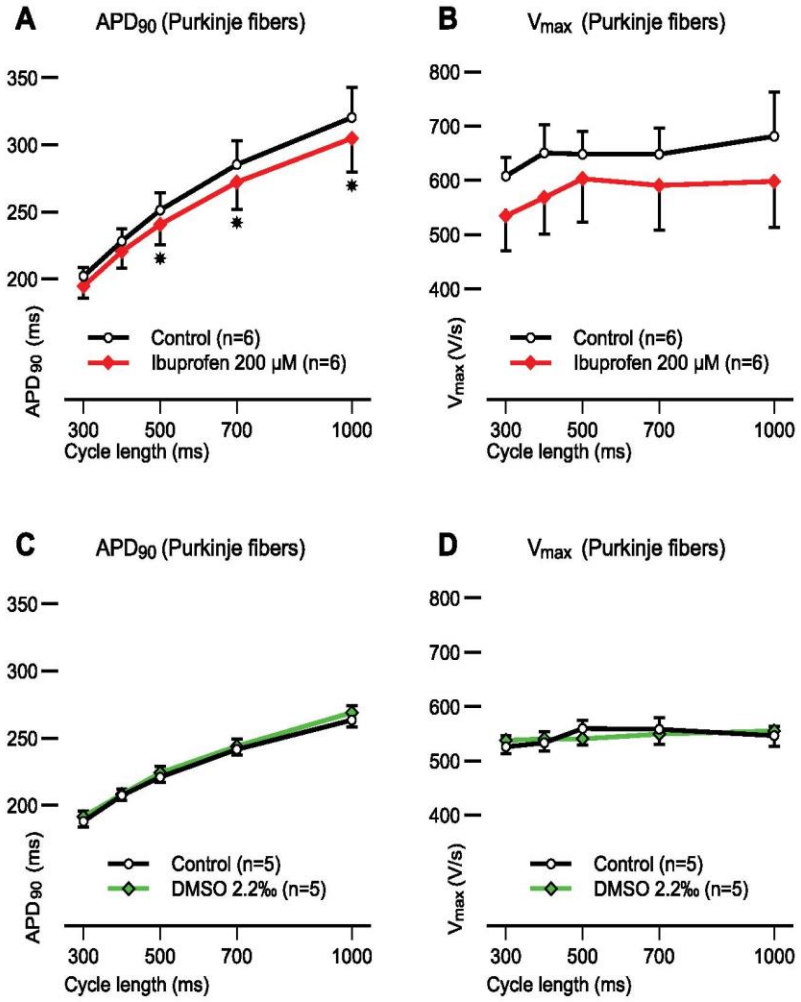
Table 2. The electrophysiological effects of dimethyl sulfoxide (DMSO, 2.2‰), levofloxacin (40 μM), and ibuprofen (100 μM) in rabbit right ventricular papillary muscle (VM) preparations at a basic cycle length of 1000 ms.

(A)	Sample	RP (mV)	APA (mV)	V_{max} (V/s)	APD_{50} (ms)	APD_{90} (ms)	APD_{90} (%)
Control	Rabbit VM ($n = 6$)	−81.7 ± 1.9	119.1 ± 2.3	222.0 ± 21.9	123.8 ± 7.9	158.7 ± 7.7	—
DMSO (2.2‰)	Rabbit VM ($n = 6$)	−81.6 ± 2.6	115.8 ± 4.7	181.9 ± 20.5	123.0 ± 7.2	159.3 ± 7.1	0.7 ± 1.9
(B)	**Sample**	**RP (mV)**	**APA (mV)**	**V_{max} (mV)**	**APD_{50} (ms)**	**APD_{90} (ms)**	**APD_{90} (%)**
Control	Rabbit VM ($n = 7$)	−86.9 ± 1.3	109.0 ± 2.6	127.6 ± 7.5	149.6 ± 9.6	163.8 ± 6.6	—
Levofloxacin (40 μM)	Rabbit VM ($n = 7$)	−85.8 ± 2.0	111.3 ± 4.0	128.0 ± 8.0	156.9 ± 26.0	164.1 ± 7.0	0.1 ± 0.8
(C)	**Sample**	**RP (mV)**	**APA (mV)**	**V_{max} (V/s)**	**APD_{50} (ms)**	**APD_{90} (ms)**	**APD_{90} (%)**
Control	Rabbit VM ($n = 9$)	−86.5 ± 1.6	108.0 ± 3.7	147.7 ± 21.2	121.8 ± 7.1	164.7 ± 8.7	—
Ibuprofen (100 μM)	Rabbit VM ($n = 9$)	−85.6 ± 2.7	107.3 ± 2.3	145.2 ± 19.3	123.3 ± 7.5	169.3 ± 8.7*	2.9 ± 0.9
Levofloxacin (40 μM)	Rabbit VM ($n = 9$)	−87.4 ± 2.2	111.0 ± 3.7	148.5 ± 19.4	138.2 ± 13.1	183.2 ± 12.5*	7.6 ± 1.9

Note: Results are expressed as means ± SEM. APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{50} and APD_{90}, action potential durations at 50% and 90% of repolarization.

*$p < 0.05$, Student's t test for paired data (Tables 2A and 2B), ANOVA for repeated measurements followed by Bonferroni's post hoc test (Table 2C).

Fig. 2. Cycle-length-dependent changes in action potential duration at 90% of repolarization (APD_{90}, panels A and C) and in maximal rate of depolarization (V_{max}, panels B and D) measured under control conditions and in the presence of 200 μM ibuprofen and dimethyl sulfoxide (DMSO) at 2.2‰ in dog Purkinje fiber preparations. Values are means ± SEM. Asterisks indicate significant changes ($p < 0.05$). [Color online.]



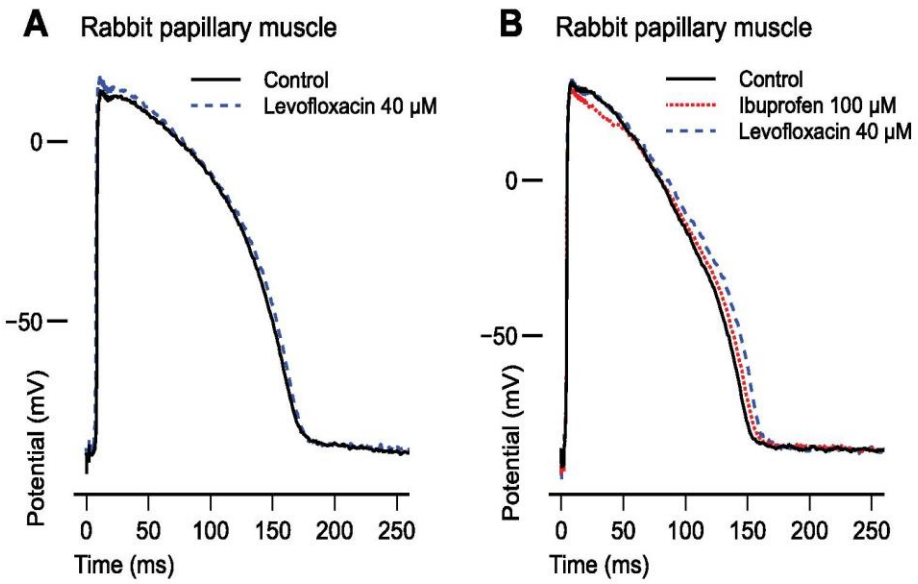
test for Table 2C. Differences were considered significant when $p < 0.05$.

Results

Effects of ibuprofen on transmembrane action potentials

We have investigated the effects of ibuprofen on cardiac action potentials in the concentration range of 50–200 μM (10.3–41.2 μg/mL) in rabbit and dog right ventricular papillary muscle using the conventional microelectrode technique. As Tables 1B and 1C and Figs. 1A and 2C show, ibuprofen in dog right ventricular papillary muscle at 50 and 200 μM and at 1 Hz stimulation frequency did not change the resting membrane potential, the action potential amplitude, or V_{max}, but at 200 μM it moderately lengthened the APD_{50} and APD_{90}. The solvent DMSO at the applied concentration did not

Fig. 3. The effects of levofloxacin alone (panel A) and in combination with 100 μM ibuprofen (panel B) on action potentials recorded from rabbit right ventricular papillary muscle preparation. Original action potential records indicate that 40 μM levofloxacin did not influence the ventricular repolarization in rabbit (panel A); however, in combination with 100 μM ibuprofen, levofloxacin significantly lengthened the action potential duration (panel B). [Color online.]



affect any of the measured action potential parameters (Table 1A and Fig. 1E).

In dog Purkinje fibers, action potentials were studied at a 500 ms constant cycle length (Table 1D) and also at various stimulation cycle lengths, ranging from 300–1000 ms (Fig. 2A). At constant cycle length stimulation, ibuprofen at 200 μM concentration elicited significant abbreviation of APD_{90}, while all other characteristics, including the resting potential, action potential amplitude, and V_{max}, remained unchanged. As Fig. 2B indicates, in Purkinje fibers, V_{max} was decreased and APD was shortened in a frequency-dependent manner. The decrease in APD_{90} was more pronounced at slower cycle lengths, being significant from 500 to 1000 ms. V_{max} depression was observed only at high stimulation rate corresponding to 300 ms cycle length. DMSO elicited no changes in the action potential characteristics of the Purkinje fibers at any cycle length (Table 1A; Figs. 1E, 2C, and 2D).

Levofloxacin, a widely known antibiotic, at 40 μM did not change action potential parameters, including APD_{90} in rabbit papillary muscles at 1 Hz stimulation rate (Table 2B and Fig. 3A). However, when levofloxacin was applied in combination with 100 μM ibuprofen, the extent of APD lengthening evoked by levofloxacin was greater than that observed without the application of ibuprofen (Table 2C and Fig. 3B).

To elucidate the mechanism of the changes induced by ibuprofen in the action potential, the effects of ibuprofen on the transmembrane ionic currents were investigated by the whole-cell configuration of the patch-clamp technique in dog ventricular myocytes at 250 μM (51.5 μg/mL). The solvent DMSO at the applied concentration did not influence the amplitude or kinetics of the measured transmembrane ionic currents (Figs. 4–5). In dog ventricular myocytes, 250 μM ibuprofen did not significantly alter the inward rectifier (I_{K1}) potassium (Fig. 4A) and moderately but significantly decreased the transient outward (I_{to}, Figs. 4B and 4D) and rapid delayed rectifier (I_{Kr}, Figs. 4C and 4E) potassium currents.

Because cardiac repolarization is determined not only by outward potassium currents but also by late inward sodium (I_{NaL}) and L-type inward calcium (I_{Ca}) currents, the effect of ibuprofen was also studied on I_{NaL} and I_{Ca} in dog ventricular myocytes. As Fig. 5 indicates, 250 μM ibuprofen moderately, but in a statistically significant manner, decreased the amplitude of both I_{NaL} and I_{Ca}.

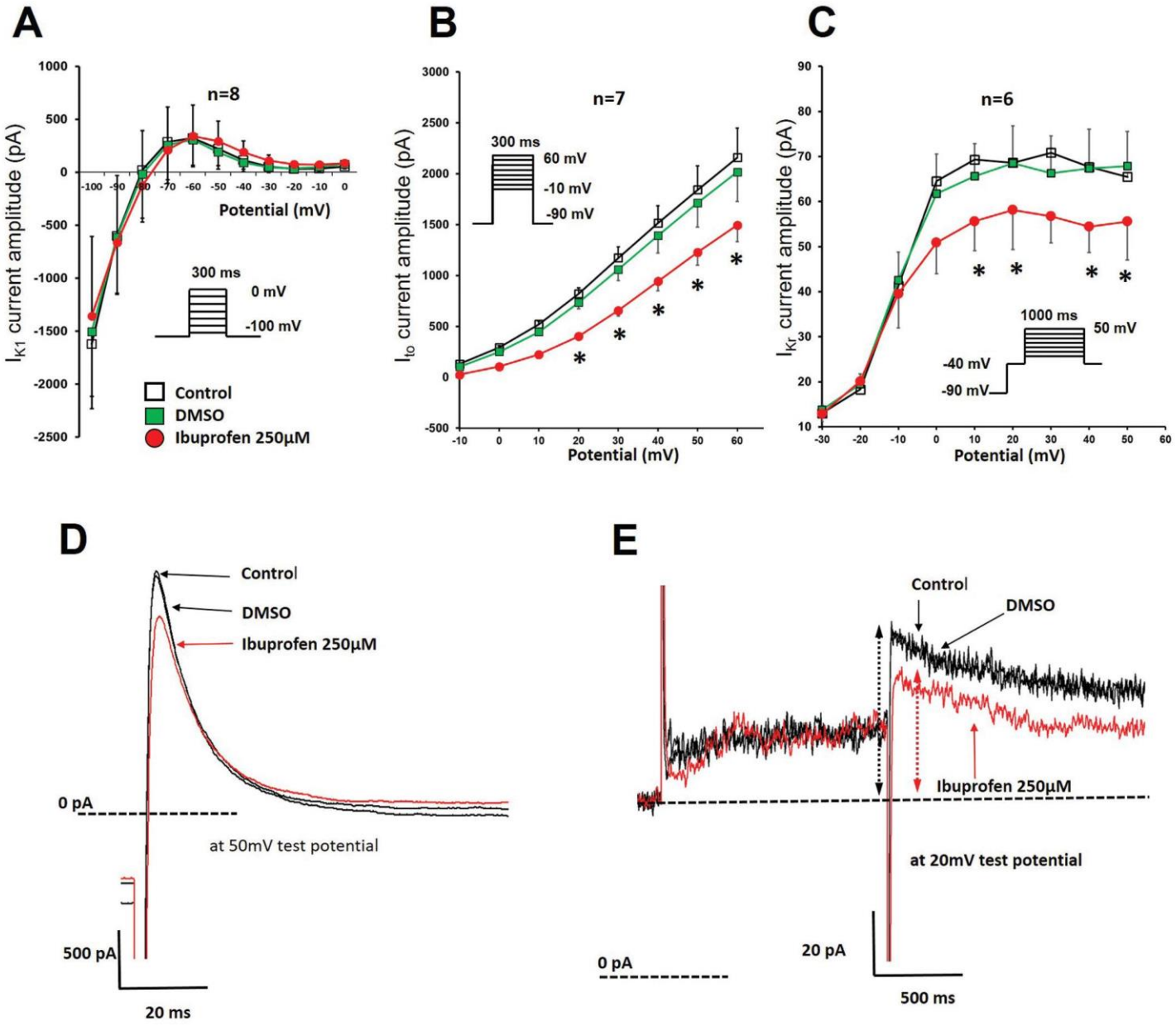
Discussion

The most important message of the present study is to show that ibuprofen in normal situations and therapeutically relevant concentrations exerts none or only moderate repolarization lengthening in ventricular muscle preparations, but in a situation where repolarization reserve has been attenuated, the degree of repolarization lengthening was further increased. This raises the possibility that under such conditions it may enhance proarrhythmic risk and consequent sudden cardiac death.

The paucity of reports regarding the cardiac electrophysiological effects of ibuprofen is surprising in spite of its worldwide use and the two decades of concerns regarding increased risk associated with NSAID drugs in general (Bombardier et al. 2000; Huang et al. 2006*a*, 2006*b*). In addition, in a recent meta-analysis, it has been reported that two NSAID drugs, diclofenac and ibuprofen, increase out-of-hospital cardiac arrest and consequent sudden deaths (Sondergaard et al. 2017). Although the mechanism of these observations is not clear and can be linked to causes other than direct ion channel modulation, the possibility of direct effect of ibuprofen on transmembrane ion channels should be also considered. This argument is further strengthened by a previous experimental study (Kristóf et al. 2012), which indicated that diclofenac decreased repolarization reserve by inhibiting I_{Ks} and I_{Kr} in dog heart. In this paper, it has also been shown that diclofenac also facilitated Torsades de pointes ventricular tachycardia (TdP)-like arrhythmia in in vivo rabbit experiments (Kristóf et al. 2012).

The applied concentrations in the present study were similar to those of the work of Yang et al. (2008), and fall into the range of low and high therapeutic plasma levels (10–50 μg/mL) observed in patients (Holubek et al. 2007). It is also worth mentioning that, in certain situations, including high age or altered metabolism caused by disease or drug interactions, plasma levels may rise beyond normal. In addition, much higher (260 and 352 μg/mL)

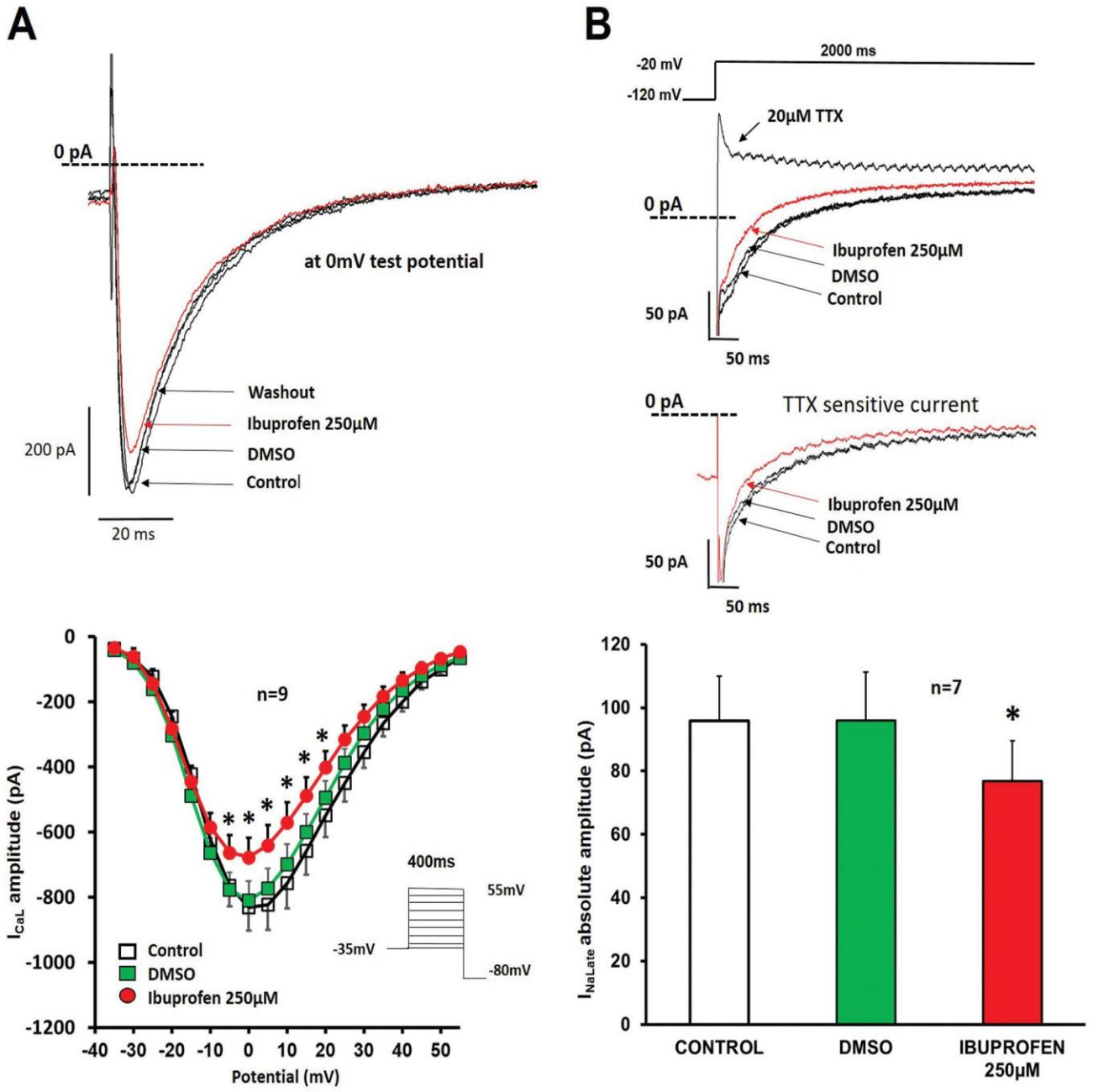
Fig. 4. Panels A–C show the effects of the solvent dimethyl sulfoxide (DMSO) at 2.2‰ and ibuprofen at 250 μM on the potassium currents I_{K1}, I_{to}, and I_{Kr}, respectively, in ventricular myocytes; the insets show the applied voltage protocols. Values are means ± SEM. Asterisks indicate $p < 0.05$, ANOVA for repeated measurements followed by Bonferroni's post hoc test. Panels D and E show original current traces of the I_{to} and I_{Kr}, currents, respectively, recorded in control conditions and in the presence of DMSO and after the application of 250 μM ibuprofen. In panel E, the dotted arrows indicate the amplitude of I_{Kr} tail currents at –40 mV. I_{K1}, inward rectifier potassium current; I_{Kr}, rapidly activating delayed rectifier potassium current; I_{to}, transient outward potassium current. [Color online.]



plasma levels have also been reported after drug intoxication (Holubek et al. 2007).

In guinea pig ventricle, it has been previously shown (Yang et al. 2008) that ibuprofen in the concentration range of 10–80 μg/mL shortened APD and depressed V_{max} in a frequency-dependent manner. In addition, ibuprofen also depressed slow response action potentials and sinus nodal frequency both indicative of I_{Ca} inhibition (Yang et al. 2008) with concomitant increase of PP and QRS intervals in the ECG, but with a shorter QTc (Yang et al. 2008). Our present results are in partial agreement with the ones reported by Yang et al. (2008). In the present study, we could confirm the frequency-dependent V_{max} and I_{Ca} inhibition reported by Yang et al. (2008), and we also found inhibition of I_{NaL}. All these effects would lead to shortening in repolarization. However, contrary to the findings reported by Yang et al. (2008), in our experiments moderate but statistically significant repolarization lengthening was observed in ventricular muscle, but not in Purkinje fibers. Also, Yarishkin et al. (2009) reported that diclofenac but not ibuprofen decreased I_{NaL} and I_{Ca} in rat ventricular myocytes. These dissimilarities are most likely due to the difference in the species (neonatal rat vs. rabbit), in the experimental conditions (room temperature vs. 37 °C), and in the preparations (1-day cultured trabecules vs. isolated papillary muscles) used. Unlike rabbit and dog, guinea pig ventricle lacks I_{to} (Zicha et al. 2003), and expresses very strong I_{Ks} (Bartos et al. 2015). Consequently, in guinea pig ventricle, I_{to} and I_{Kr} inhibition have less impact on repolarization when compared with that in rabbit or dog. Therefore, in guinea pig ventricle, the ibuprofen-evoked

Fig. 5. Panels A and B show the effects of the solvent dimethyl sulfoxide (DMSO) at 2.2‰ and ibuprofen at 250 μM on the L-type calcium current (I_{Ca}) and the late sodium current (I_{NaL}), respectively, in ventricular myocytes; the insets show the applied voltage protocols. Values are means ± SEM. Asterisks indicate $p < 0.05$, ANOVA for repeated measurements followed by Bonferroni's post hoc test (A), Student's t test (B). Upper panels show original current traces of the I_{NaL} and I_{Ca} currents, respectively, recorded in control conditions and in the presence of DMSO and after the application of 250 μM ibuprofen. I_{NaL} was defined as tetrodotoxin-sensitive current by subtracting current traces recorded in the presence of 20 μM tetrodotoxin from traces of control, DMSO, and ibuprofen recordings. [Color online.]



I_{Ca} and I_{NaL} inhibition would change the balance of inward and outward currents, favoring relative augmentation of outward currents, with an overall result of shortened repolarization. Similar effect should be expected in dog Purkinje fibers, in which relatively strong I_{NaL} exists. The opposite effect is expected in rabbit and dog ventricle, where the density of I_{Ks} is weaker than in the guinea pig; therefore, I_{Kr} should have a stronger contribution to repolarization (Jost et al. 2013). In addition, ibuprofen inhibits I_{to}, which also plays an important role in the repolarization reserve (Virág et al. 2011). It should be mentioned that NSAIDs, including ibuprofen, are often used in patients with fever. Therefore, it would be worthwhile to study the effect of ibuprofen and other NSAIDs under hyperthermic conditions as well.

It is well known that fluoroquinolone antibiotics have some repolarization prolonging and proarrhythmic potency (Chiba et al. 2000; Garnett and Johannesen 2016; Komatsu et al. 2019). To test potential interaction between ibuprofen and these antibiotics, we chose to study levofloxacin, which has been reported to possess relatively low proarrhythmic risk (Chiba et al. 2000; Milberg et al. 2007), due to its unpronounced repolarization lengthening (Hagiwara et al. 2001) and human ether-a-go-go-related gene channel inhibiting (Kang et al. 2001) properties compared with others, especially sparfloxacin (Chiba et al. 2000;

Hagiwara et al. 2001). In our experiments, in good agreement with the results of Hagiwara et al., levofloxacin did not evoke significant changes when applied alone. However, when levofloxacin was applied in combination with ibuprofen, noteworthy APD prolongation was observed. Ibuprofen alone elicited a moderate prolongation of APD, and this was increased even further by levofloxacin. It should be emphasized that the observed APD prolongation by ibuprofen, with or without levofloxacin, is not marked, and it is far from being excessive. Nevertheless, this effect should draw attention to the possibility that the combined effect of two drugs with low or even minimal effect on repolarization, and seemingly marginal potassium channel blocking properties may still be additive by collectively decreasing the repolarization reserve. Therefore, in certain situations where repolarization reserve is already attenuated (Varró and Baczkó 2011), e.g., in specific genetic disorders, heart failure, hypertophic cardiomyopathy, low serum potassium concentrations, or ischemic heart disease, this may lead to marked repolarization defects. This may ultimately contribute to enhanced proarrhythmic risk and consequent sudden cardiac death.

In conclusion, it seems that ibuprofen in normal situations, at least regarding its cardiac electrophysiological properties, is a relatively safe drug. However, in certain conditions characterized by attenuated repolarization reserve, ibuprofen may enhance proarrhythmic risk, and may even contribute to the incidence of sudden cardiac death observed in clinical studies. This possibility should be considered and taken into account in clinical practice, because ibuprofen is a very commonly used over-the-counter drug, taken every day by several million people without medical control.

Conflict of interest

The authors declare that there is no conflict of interest associated with this work.

Acknowledgements

This work was funded by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (for I.K., No. BO/00581/17) and the ÚNKP-18-4 and 19-4 (Bolyai+) New National Excellence Program of the Ministry for Innovation and Technology (for I.K.) and the National Research, Development and Innovation Office – NKFIH PD-116011 (for I.K.), K-119992 (for A.V.), FK-129117 (for N.N.), and the Hungarian Government-Ministry of Human Resources (Grant EFOP-3.6.2-16-2017-00006, LIVE LONGER, and EFOP 3.6.3-VEKOP-16-2017-00009 for T.Á.-L.), GINOP-2.3.2.-15-2016-00048, the Ministry of Human Capacities Hungary (20391-3/2018/FEKUSTRAT), and János Bolyai Research Scholarship of the Hungarian Academy of Sciences (for N.N.). The GINOP and EFOP projects are co-financed by the European Union and the European Regional Development Fund.

References

Bartos, D.C., Grandi, E., and Ripplinger, C.M. 2015. Ion channels in the heart. Compr. Physiol. **5**(3): 1423–1464. doi:10.1002/cphy.c140069. PMID: 26140724.

Bombardier, C., Laine, L., Reicin, A., Shapiro, D., Burgos-Vargas, R., Davis, B., et al. 2000. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. N. Engl. J. Med. **343**(21): 1520–1528. doi:10.1056/NEJM200011233432103.

Chiba, K., Sugiyama, A., Satoh, Y., Shiina, H., and Hashimoto, K. 2000. Proarrhythmic effects of fluoroquinolone antibacterial agents: in vivo effects as physiologic substrate for torsades. Toxicol. Appl. Pharmacol. **169**(1): 8–16. doi:10.1006/taap.2000.9041.

Douglas, R.J. 2010. Palpitations following regular ibuprofen dosing in a 13-year-old girl: a case report. J. Med. Case Rep. **4**: 76. doi:10.1186/1752-1947-4-76.

Garnett, C., and Johannesen, L. 2016. Commentary on: "Levofloxacin-Induced QTc prolongation depends on the time of drug administration. CPT: Pharmacometrics Syst. Pharmacol. **5**(9): 452–454. doi:10.1002/psp4.12128. PMID:27647678.

Hagiwara, T., Satoh, S., Kasai, Y., and Takasuna, K. 2001. A comparative study of the fluoroquinolone antibacterial agents on the action potential duration in guinea pig ventricular myocardia. Jpn. J. Pharmacol. **87**(3): 231–234. doi:10.1254/jjp.87.231. PMID:11885973.

Holubek, W., Stolbach, A., Nurok, S., Lopez, O., Wetter, A., and Nelson, L. 2007. A report of two deaths from massive ibuprofen ingestion. J. Med. Toxicol. **3**(2): 52–55. doi:10.1007/BF03160908.

Huang, W.-F., Hsiao, F.-Y., Tsai, Y.-W., Wen, Y.-W., and Shih, Y.-T. 2006*a*. Cardiovascular events associated with long-term use of celecoxib, rofecoxib and meloxicam in Taiwan: an observational study. Drug Safety, **29**(3): 261–272. doi:10.2165/00002018-200629030-00009.

Huang, W.-F., Hsiao, F.-Y., Wen, Y.-W., and Tsai, Y.-W. 2006*b*. Cardiovascular events associated with the use of four nonselective NSAIDs (etodolac, nabumetone, ibuprofen, or naproxen) versus a cyclooxygenase-2 inhibitor (celecoxib): a population-based analysis in Taiwanese adults. Clin. Therap. **28**(11): 1827–1836. doi:10.1016/j.clinthera.2006.11.009.

Jost, N., Virág, L., Comtois, P., Ordög, B., Szuts, V., Seprényi, G., et al. 2013. Ionic mechanisms limiting cardiac repolarization reserve in humans compared to dogs. J. Physiol. **591**(17): 4189–4206. doi:10.1113/jphysiol.2013.261198.

Kang, J., Wang, L., Chen, X.L., Triggle, D.J., and Rampe, D. 2001. Interactions of a series of fluoroquinolone antibacterial drugs with the human cardiac K+ channel HERG. Mol. Pharmacol. **59**(1): 122–126. doi:10.1124/mol.59.1.122.

Kohajda, Z., Farkas-Morvay, N., Jost, N., Nagy, N., Geramipour, A., Horváth, A., et al. 2016. The effect of a novel highly selective inhibitor of the sodium/calcium exchanger (NCX) on cardiac arrhythmias in in vitro and in vivo experiments. PLoS ONE, **11**(11): e0166041. doi:10.1371/journal.pone.0166041.

Komatsu, R., Mizuno, H., Ishizaka, T., Ito, A., Jikuzono, T., Kakoi, T., et al. 2019. Exposure-response analysis of drug-induced QT interval prolongation in telemetered monkeys for translational prediction to human. J. Pharmacol. Toxicol. Methods, **99**: 106606. doi:10.1016/j.vascn.2019.106606.

Kristóf, A., Husti, Z., Koncz, I., Kohajda, Z., Szél, T., Juhász, V., et al. 2012. Diclofenac prolongs repolarization in ventricular muscle with impaired repolarization reserve. PLoS ONE, **7**(12): e53255. doi:10.1371/journal.pone.0053255.

Milberg, P., Hilker, E., Ramtin, S., Cakir, Y., Stypmann, J., Engelen, M.A., et al. 2007. Proarrhythmia as a class effect of quinolones: increased dispersion of repolarization and triangulation of action potential predict torsades de pointes. J. Cardiovasc. Electrophysiol. **18**(6): 647–654. doi:10.1111/j.1540-8167.2007.00793.x.

Pratt, C.M., Hertz, R.P., Ellis, B.E., Crowell, S.P., Louv, W., and Moyé, L. 1994. Risk of developing life-threatening ventricular arrhythmia associated with tefenadine in comparison with over-the-counter antihistamines, ibuprofen and clemastine. Am. J. Cardiol. **73**(5): 346–352. doi:10.1016/0002-9149(94)90006-X.

Rainsford, K.D. 2009. Ibuprofen: pharmacology, efficacy and safety. Inflammopharmacology, **17**(6): 275–342. doi:10.1007/s10787-009-0016-x.

Roden, D.M. 2006. Long QT syndrome: reduced repolarization reserve and the genetic link. J. Intern. Med. **259**(1): 59–69. doi:10.1111/j.1365-2796.2005.01589.x.

Sondergaard, K.B., Weeke, P., Wissenberg, M., Schjerning Olsen, A.-M., Fosbol, E.L., Lippert, F.K., et al. 2017. Non-steroidal anti-inflammatory drug use is associated with increased risk of out-of-hospital cardiac arrest: a nationwide case-time-control study. Eur. Heart J. Cardiovasc. Pharmacother. **3**(2): 100–107. doi:10.1093/ehjcvp/pvw041. PMID:28025218.

Varró, A., and Baczkó, I. 2011. Cardiac ventricular repolarization reserve: a principle for understanding drug-related proarrhythmic risk. Br. J. Pharmacol. **164**(1): 14–36. doi:10.1111/j.1476-5381.2011.01367.x.

Varró, A., Baláti, B., Iost, N., Takács, J., Virág, L., Lathrop, D.A., et al. 2000. The role of the delayed rectifier component IKs in dog ventricular muscle and Purkinje fiber repolarization. J. Physiol. **523**(Pt. 1): 67–81. doi:10.1111/j.1469-7793.2000.00067.x.

Virág, L., Jost, N., Papp, R., Koncz, I., Kristóf, A., Kohajda, Z., et al. 2011. Analysis of the contribution of I(to) to repolarization in canine ventricular myocardium. Br. J. Pharmacol. **164**(1): 93–105. doi:10.1111/j.1476-5381.2011.01331.x.

Yang, Z., Wang, H., Zheng, Y., Zhang, Y., Liu, Y., and Li, C. 2008. Possible arrhythmiogenic mechanism produced by ibuprofen. Acta Pharmacol. Sin. **29**(4): 421–429. doi:10.1111/j.1745-7254.2008.00754.x.

Yarishkin, O.V., Hwang, E.M., Kim, D., Yoo, J.C., Kang, S.S., Kim, D.R., et al. 2009. Diclofenac, a non-steroidal anti-inflammatory drug, inhibits L-type Ca channels in neonatal rat ventricular cardiomyocytes. Korean J. Physiol. Pharmacol. **13**(6): 437–442. doi:10.4196/kjpp.2009.13.6.437.

Zicha, S., Moss, I., Allen, B., Varro, A., Papp, J., Dumaine, R., et al. 2003. Molecular basis of species-specific expression of repolarizing K+ currents in the heart. Am. J. Physiol. Heart Circ. Physiol. **285**(4): H1641–H1649. doi:10.1152/ajpheart.00346.2003.

II.

SCIENTIFIC REPORTS
nature research

OPEN

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

Péter Orvos[1,7], Bence Pászti[2,7], Leila Topal[2], Péter Gazdag[2], János Prorok[3], Alexandra Polyák[2,3], Tivadar Kiss[4], Edit Tóth-Molnár[1], Boglárka Csupor-Löffler[5], Ákos Bajtel[4], András Varró[2,3,6], Judit Hohmann[4], László Virág[2,6,8] & Dezső Csupor[4,8]✉

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 (1 µM, 2.5 µM and 5 µM) 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.

Cannabis is the most abused hallucinogenic drug, with an estimated of 150 million consumers worldwide[1]. With the increasingly widespread use of e-cigarettes, the number of people inhaling cannabinoids might even be higher. Moreover, the use of cannabis products for medicinal purposes is increasing globally. The interest for the use of cannabis and cannabis-derived products started following the discovery of the cannabinoid system in the human brain and body and the subsequent reports on new findings on biological activities of cannabinoids on central nervous system and immune functioning. Currently, there are cannabis-based medicines on the market with well-defined medicinal indications, including treatment of nausea and vomiting associated with chemotherapy, anorexia, pain related to cancer, spasticity and pain associated with multiple sclerosis, Dravet and Lennox-Gastaut syndromes. These medicines contain known amounts of CBD and/or THC in pure form or as standardized herbal extract[2]. Besides, the use of CBD-containing products (CBD oil) is very widespread with several, clinically unsupported indications. The intake of cannabinoids, especially CBD, which is enriched in several products, may be higher in case of the consumption of CBD oils than in case of smoking cannabis.

[1]Department of Ophthalmology, Faculty of Medicine, Albert Szent-Györgyi Clinical Center, University of Szeged, Szeged, Hungary. [2]Department of Pharmacology and Pharmacotherapy, University of Szeged, Szeged, Hungary. [3]MTA-SZTE Research Group for Cardiovascular Pharmacology, Hungarian Academy of Sciences, Szeged, Hungary. [4]Department of Pharmacognosy, Faculty of Pharmacy, University of Szeged, Eötvös u. 6, Szeged 672, Hungary. [5]Institute for Translational Medicine, Medical School, University of Pécs, Pecs, Hungary. [6]Department of Pharmacology and Pharmacotherapy, Interdisciplinary Excellence Centre, University of Szeged, Szeged 6720, Hungary. [7]These authors contributed equally: Péter Orvos and Bence Pászti. [8]These authors jointly supervised this work: László Virág and Dezső Csupor. ✉email: csupor.dezso@pharmacognosy.hu

The cardiovascular adverse effects of cannabinoid use have been reported in several case reports, and range from arrhythmias to myocardial infarction and sudden death[3]. According to the results of a cohort study, marijuana smokers have a 4.8-fold increased risk of developing acute myocardial infarction during the first hour of exposure[4]. However, other data do not support the association between cannabis use and cardiovascular events[5]. The most comprehensive study assessed data for 316,397 cannabis users and 20,499,215 non-users, and found that cannabis use is an independent predictor of heart failure[1]. Although the exact mechanisms explaining these observations are unknown, the activities of cannabinoids exerted via the G protein-coupled cannabinoid receptors are supposed to be of key importance. In addition, several studies described the proarrhythmic potency of cannabinoids ranging from ventricular arrhythmias to sudden cardiac death[6–8]. However, the exact association and mechanism of these arrhythmias remain unknown[7]. Besides, certain voltage-gated ion channels like cardiac sodium, calcium[9] and Kv4.3 channels[10] might also be related to the reported cardiovascular effects of cannabinoids, but the exact role of these channels has not been studied yet in detail. One of the most important ion channels in cardiac repolarization is the rapid delayed rectifier potassium channel (I_{Kr}), which plays a critical role in cardiac repolarization, having a pore-forming subunit encoded by the *hERG* (the human Ether-à-go-go-Related Gene) gene. Inhibitors of I_{Kr} (also called hERG ion channel) are known to lengthen the QT interval, and hence might induce life-threatening arrhythmias. Therefore, formal drug development requires an early screening of whether the potential drug candidates bear any activities on the hERG channels[11]. However, drug effects on cardiac repolarization cannot be accurately estimated by measuring hERG channel currents alone[12], since drug responses on native I_{Kr} channel and action potential can be different from those measured in hERG.

In the present study cannabidiol (CBD), tetrahydrocannabinol (THC) and 11-nor-9-carboxy-tetrahydrocannabinol (11-nor-9-carboxy-THC), the main metabolite of THC was assessed for their effects on the hERG channels in an in vitro assay. CBD and THC are the major components of cannabis products for medicinal and recreational use, respectively, and since the latter is quickly metabolized to 11-nor-9-carboxy-THC, these three compounds were chosen to be tested in vitro.

Therefore, the aim of our work was to study the in vitro and in vivo effects of CBD a major cannabinoid on cardiac ventricular action potential, on ECG parameters, on the hERG and on other native cardiac transmembrane channels to provide experimental data for the elucidation of their possible adverse cardiac electrophysiological effects.

Results

As our first test shown in Fig. 1, CBD was found to be an inhibitor of the hERG potassium channel with intermediate potency represented by IC_{50} values of 2.07 ± 0.12 µM (n = 6) at room temperature. The IC_{50} values for the inhibition elicited by THC were higher (10.30 ± 0.55 µM, n = 6 at room temperature). 11-Nor-9-carboxy-THC exhibited only a marginal effect ($IC_{50} = 65.40 \pm 3.82$ µM, n = 4 at room temperature).

The cardiac cellular electrophysiological effect of the most potent cannabis compound CBD was further studied on various transmembrane ionic currents by the whole-cell configuration of the patch clamp technique in native rabbit ventricular myocytes and on action potentials in rabbit and guinea pig right ventricular papillary muscle by the conventional microelectrode technique and on in vivo ECG studies in anaesthetized guinea pigs. Figure 2 shows that CBD lengthens action potential duration (APD_{90}) slightly but significantly at 1 µM and at 2.5 µM. This latter effect depended on the stimulation frequency and vanished at slow pacing rate. At high 10 µM concentration CBD exerted variable effect on repolarization including minimal or no change, shortening and lengthening of APD_{90} resulting statistically not significant alteration of APD. At 1 and 2.5 µM CBD caused triangulation in some experiments but not in others reflected as not significant change in APD_{90}–APD_{25}. Similar results were obtained in guinea pig papillary muscles where 2.5 and 5 µM CBD increased APD_{90} from 186.2 ± 6.1 ms and 179.9 ± 6.0 ms to 192.2 ± 6.8 and to 191.5 ± 8.9 ms, respectively ($p < 0.05$, n = 5).

In anaesthetized in vivo guinea-pig experiments intravenous administration of 0.3 mg/kg and 1 mg/kg CBD lengthened QTc and QRS intervals in a statistically significant manner without significantly changing other ECG parameter (Fig. 3).

Whole-cell patch clamp experiments on rabbit native cardiac ventricular myocytes revealed significant and voltage-dependent inhibition of the rapid delayed rectifier potassium current (I_{Kr}) (Figs. 4a,c) with an estimated IC_{50} value of 6.5 µM after a 20 mV 1 s long test pulse and measured at −40 mV as deactivating tail current (Fig. 4b).

The observation that high (10 µM) concentration of CBD did not further lengthened APD prompted us to study the possible effect of CBD on inward L-type Ca^{2+} (I_{CaL}) and late inward Na^{+} (I_{NaL}) currents. As Fig. 5a,b show, 10 µM CBD decreased I_{CaL} significantly and in a frequency-dependent manner. In addition, 10 µM CBD also significantly inhibited I_{NaL} by 41.5% at −20 mV (Fig. 5c,d).

Discussion

The main result of our study is that CBD lengthens repolarization at low and does not change it statistically significant manner at higher concentrations. This effect on repolarization in rabbit papillary muscle can be best explained by the multiple ion channel effects of CBD. Accordingly, at lower concentrations (1, 2.5 and 5 µM) I_{Kr} depression results in lengthening of APD_{90}, which is counterbalanced by inward Ca^{2+} and Na^{+} currents inhibition at 10 µM. Similar effect was earlier described by quinidine, an antiarrhythmic drug, with reported proarrhythmic property[13,14]. The I_{Kr} inhibition by CBD, which is consistent with the hERG blockade, is most probably a direct effect on the channel but the effect of CBD on I_{NaL} and on I_{CaL} can be either direct or receptor mediated, as well. It needs more research to be established.

The higher IC_{50} values of THC and 11-nor-9-carboxy-THC than that of CBD in hERG current measurement in our study does not necessarily mean that CBD is more potent in other type of experiments since potency can

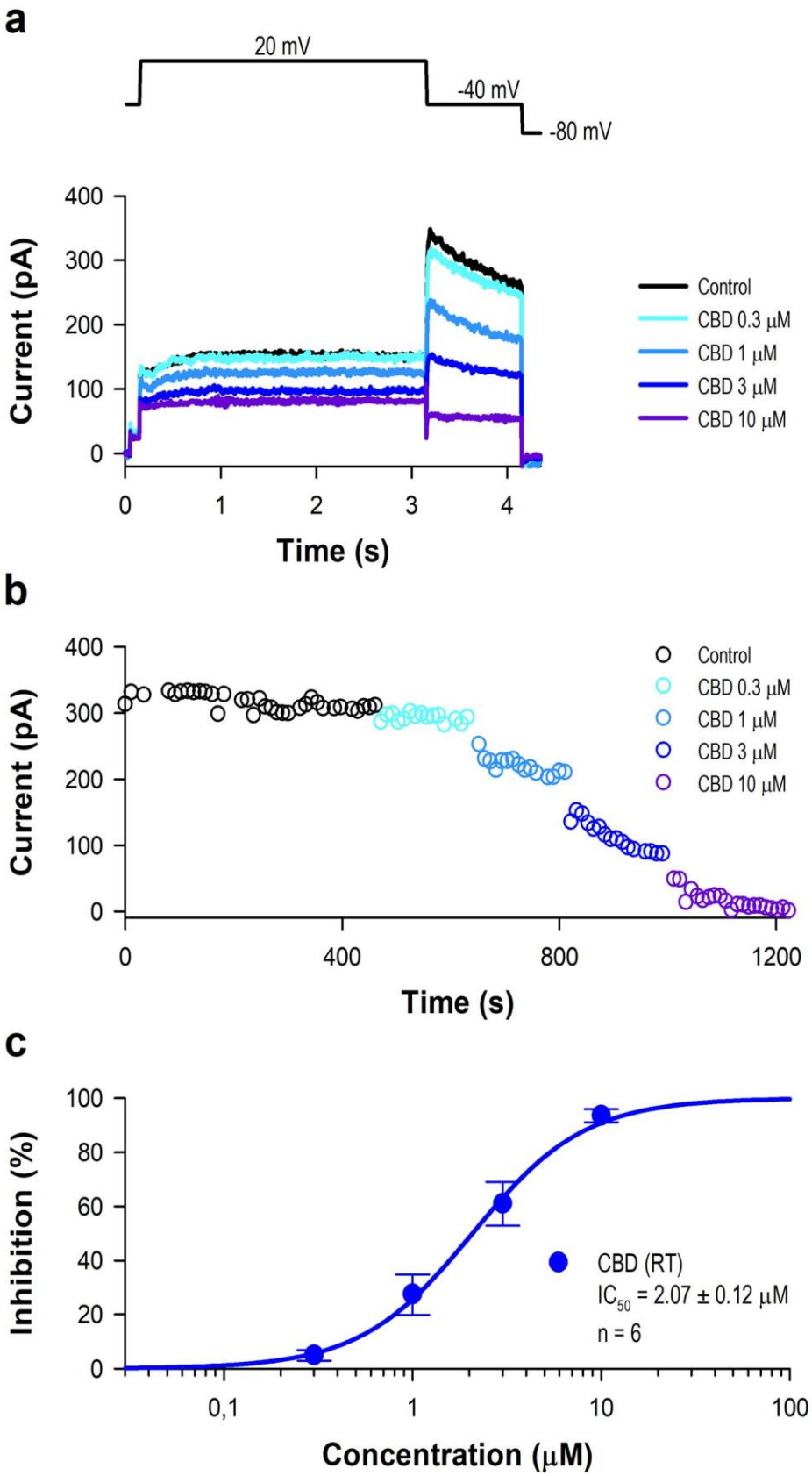


Figure 1. Effect of CBD on hERG current at room temperature. (**a**) Representative current curves obtained from HEK-hERG cells treated with 0.3, 1, 3, and 10 μM CBD. The currents were recorded using the voltage protocol shown at the top of the panel after 3–5 min acute superfusion of the drugs without washout. (**b**) Time-course of the hERG peak tail current amplitude upon the application of different concentrations of CBD. (**c**) Dose–response curves of CBD's inhibitory activity on the hERG channel.

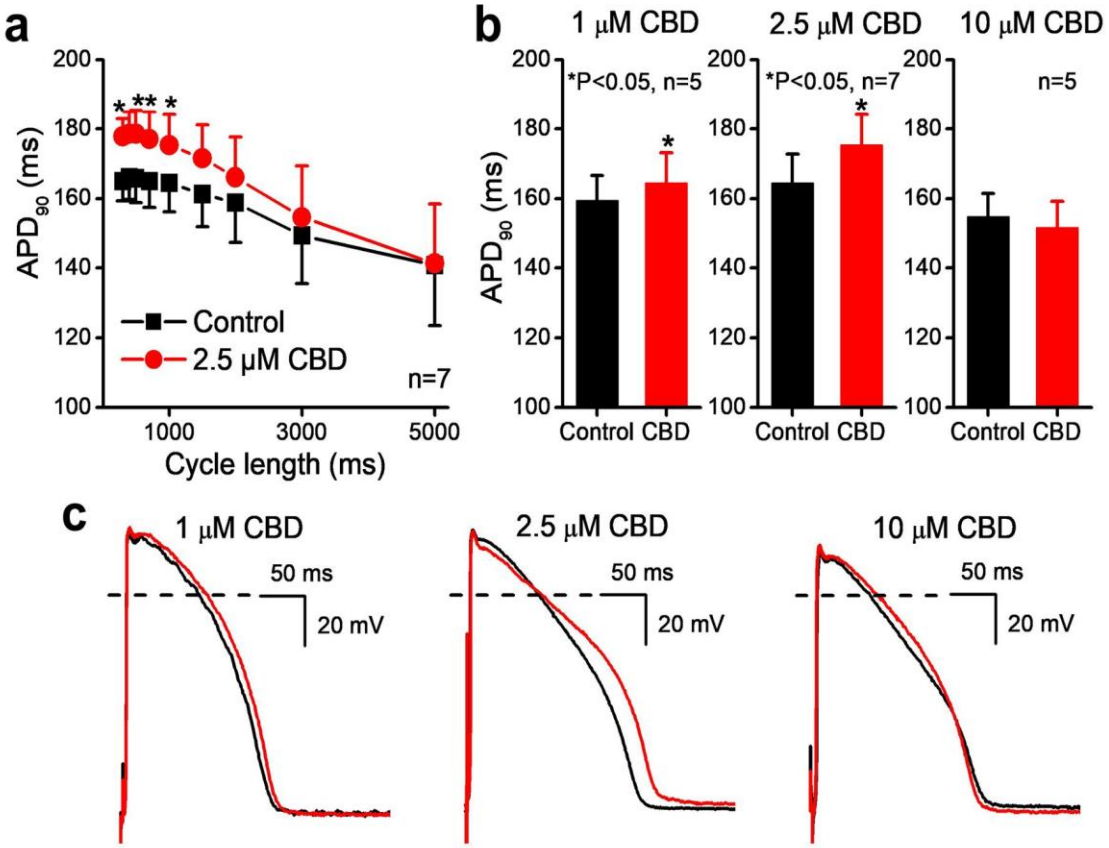
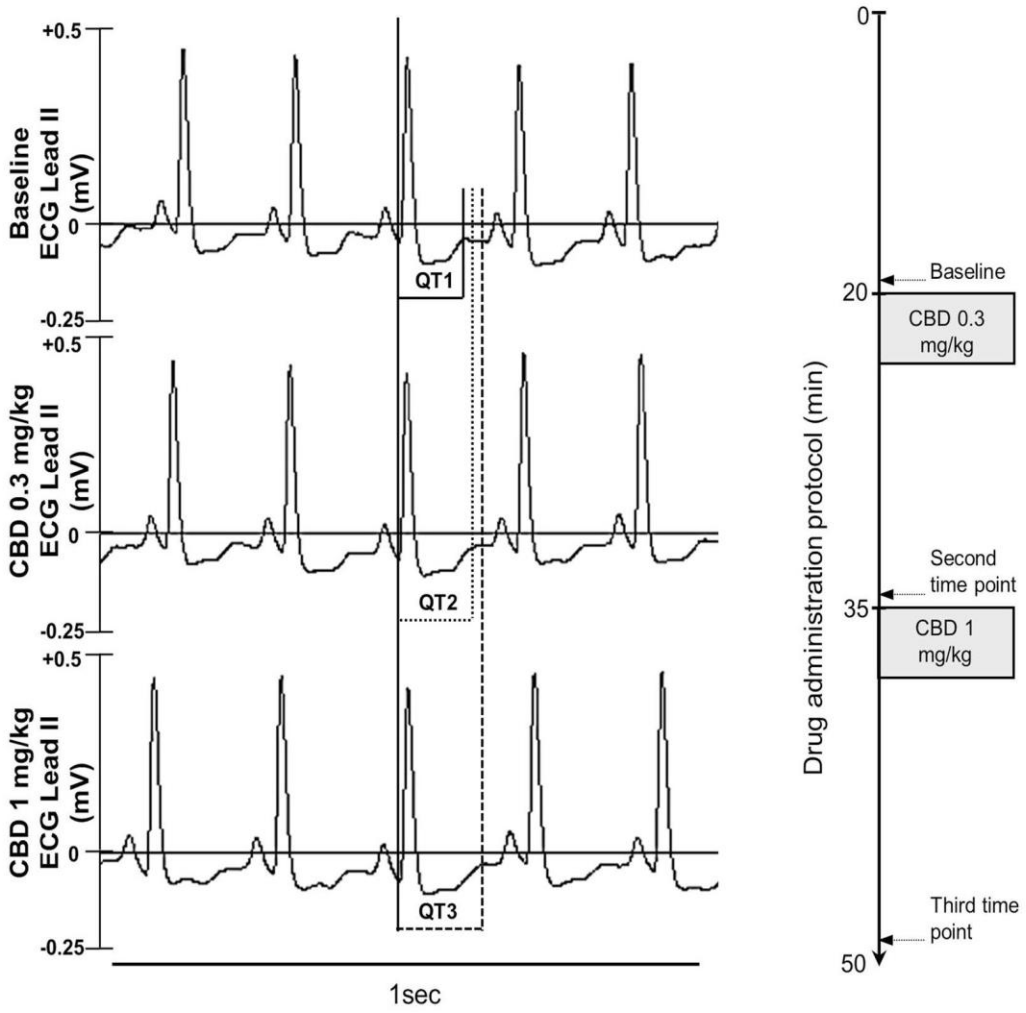


Figure 2. Effect of CBD after 30–50 min acute superfusion of the drug without washout on action potentials recorded from rabbit right ventricular papillary muscle at 37 °C. (**a**) The cycle length-dependent effect of 2.5 μM CBD on the duration of action potentials (APD_{90}). Bar diagrams (**b**) indicates the effects of 1 μM, 2.5 μM and 10 μM CBD on the action potential duration during steady-state at 1000 ms cycle length. Original action potential traces are shown on (**c**) recorded at 1000 ms cycle length in control conditions and in the presence of 1 μM, 2.5 μM and 10 μM CBD.

differ from a target to another[12]. However, in our study due to technical limitations we focused our investigations to examine the effects of CBD in more depth.

Some previous study with cannabinoids showed effects on various transmembrane ion channels such as inward sodium[9,15], inward calcium[9], outward transient current[16] and human Kv1.5 and Kv4.3 channels[10,17]. Our results in rabbit ventricular myocytes are in good agreement with those reported by Al Kury et al. on inward calcium and sodium currents in rat ventricular myocytes[9]. In a previous study hERG channel inhibition and QT lengthening were also reported in anaesthetized rats[18] by a synthetic cannabinoid compound (JWH-030). This synthetic cannabinoid compound differs from those investigated by us and inhibited hERG channel with a relatively high IC_{50} (88.36 μM). In addition, in rat ventricle hERG/I_{Kr} seems not so important to control repolarization than Kv4.2 and Kv1.5 channels. Therefore, the cannabinoid-evoked QT changes in rat most likely can be attributed to Kv1.5 and Kv4.2 rather than hERG channel inhibition. It is worth to note that in the same study[18] a cannabinoid derivate JWH-030 did not change APD in low but shortened it at high (30 μM) concentration. Therefore, the results of our study is in partial agreement with these earlier reports and the differences are best explained by different preparations, chemical differences of the studied compounds and experimental conditions.

The IC_{50} values reported here together with the C_{max} values of CBD allow the assessment of cardiovascular risks of this compound. Based on the comparison of hERG or I_{Kr} activity, cardiac action potential duration, and QT prolongation against QT effects and reports of arrhythmogenic (torsade de pointes) potential of 100 drugs, a margin of at least 30-fold between hERG IC_{50} and C_{max} was proposed to an acceptable degree of safety from arrhythmogenesis[19]. According to human pharmacokinetic data, the C_{max} values for CBD might reach 0.35 μM and 0.58 μM after CBD smoking (19.2 mg) and oral intake (400 mg), respectively[20]. In our experiments CBD had an inhibitory effect on both the hERG channel and I_{Kr} activity, with an IC_{50} value higher than literary C_{max} values in patients. Considering the IC_{50} values for I_{kr} and hERG channel inhibition in our experiments (6.5 and 2.07 μM, respectively), the ratios of IC_{50} and C_{max} values are in the range of 3.57–18.57. This safety margin (below 30) suggests a potential proarrhythmic risk in human setting. However, previous clinical reports documented no significant QT and QT_c prolongation in patients after CBD administration[21]. Also, in another study it was found that long term Sativex (CBD + THC) treatment evoked T wave changes only 1 out of 146 patients[22]. This might be explained by the effects of CBD on other ion channels than hERG and I_{Kr}.(eg. I_{CaL} and I_{NaL}). However, in patients who have slower drug elimination due to certain diseases or in case of concurrent use of medicines inhibiting the metabolism of CBD, higher C_{max} values may develop and the risk of arrhythmia might be increased[23]. Moreover, when co-administered with pharmacological agents affecting cardiac repolarization, as well as in certain



(n=5)	RR (ms)	HR (bpm)	QT (ms)	QTc (ms)	QRS (ms)
1. Baseline	212.8±12.2	285.5±14.0	130.6±8.2	145.8±3.9	47.6±2.9
2. CBD 0.3 mg/kg	211.2±6.7	286.5±8.0	135.1±5.6	150.9±3.0*	48.1±2.4
3. CBD 1 mg/kg	204.9±5.6	295.7±7.1	139.4±5.3	157.8±3.1*	50.1±1.9*

Figure 3. The volume conducted electrocardiogram (ECG lead II) signals in regular sinus rhythm in a pentobarbital anaesthetized (30 mg/kg i.p. bolus injection) guinea pig at three different time points indicated with dashed arrows: **1.** drug-free baseline, value determined from 40 consecutive beats before drug administration; **2.** value determined from 40 consecutive beats 15 min after the 0.3 mg/kg intravenously (iv) administered cannabidiol (CBD) by 2 min bolus; **3.** value determined from 40 consecutive beats 15 min after the 1 mg/kg iv administered CBD by 2 min bolus. RR interval: the time elapsed between two successive R-waves of the QRS signal on the ECG. *HR* heart rate, *QT interval* the time from the start of the Q wave to the end of the T wave, *QTc interval* heart rate corrected QT interval, calculated with a correction method described earlier[27,28], *QRS interval* the time from the onset to the end of the QRS complex. Table shows the mean ± SE values of the ECG intervals at three different time points. Changes in mean scores over three time points were compared using the repeated measures ANOVA with Bonferroni correction. *$p < 0.05$ was taken as indicative of a statistically significant difference between values.

pathophysiological conditions such as hypokalaemia, or diseases like LQT syndrome, HCM, diabetes mellitus or heart failure where cardiac repolarization reserve or drug metabolism is impaired, CBD may have an additive effect, further increasing the proarrhythmic risk and the possibility of sudden cardiac death. Such additive

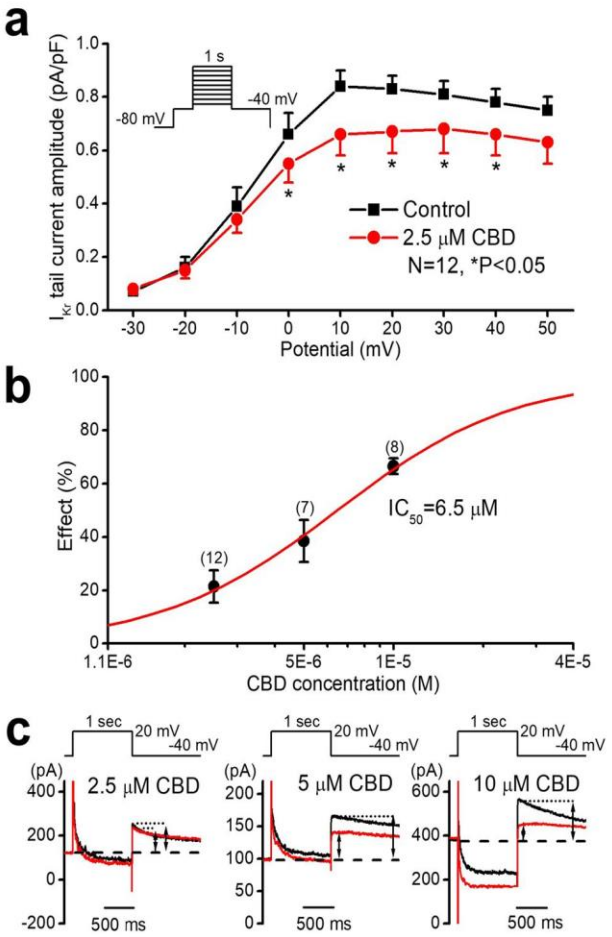


Figure 4. Effect of CBD after 3–5 min acute superfusion of the drug without washout on the rapid delayed rectifier potassium current (I_{Kr}) in rabbit left ventricular myocytes at 37 °C. Current–voltage curves show the inhibition of I_{Kr} by 2.5 μM CBD (**a**). (**b**) Displays CBD concentration–response curve indicating an estimated IC_{50} value of 6.5 μM for I_{Kr} blockade. Original I_{Kr} current traces are shown on (**c**) in control conditions and in the presence of 2.5 μM, 5 μM and 10 μM CBD recorded from rabbit left ventricular myocytes after a 1 s long pulse to 20 mV test potential with pulsing cycle length of 20 s. I_{Kr} deactivating tail current was measured at −40 mV. The vertical axis on the left side of the panels shows the absolute current level. The dashed lines refer to the baseline for I_{Kr} tail current level after the test pulse at −40 mV. The arrows indicate the amplitudes of the I_{Kr} tail currents.

effect was reported in both in experiments[24] and in patients[25]. The cardiovascular effects of CBD may only partly be interpreted on its effects on hERG and I_{Kr} ion channels, the cardiovascular safety of this compound may be influenced by its activities on other ion channels. Further studies are needed to assess the effects of other cannabinoids as well, and the in vivo relevance of these results, with special focus on the benefit-risk assessment of products with different cannabinoid content.

Methods

The hERG channel current was measured by planar technology in HEK 293 cell line by the whole-cell configuration using an automated patch clamp system (Patchliner, Nanion Technologies GmbH., Munich, Germany) at room temperature as described previously[12]. The following solutions were used during automated patch-clamp recording (compositions in mM): internal solution: KCl 50, NaCl 10, KF 60, EGTA 20, HEPES 10, pH 7.2 (KOH); external solution: NaCl 140, KCl 4, glucose-monohydrate 5, $MgCl_2$ 1, $CaCl_2$ 3, HEPES 10, pH 7.4 (NaOH). The voltage protocol for hERG ion channel started with a short (100 ms) −40 mV step to establish the baseline region. A depolarizing step was applied to the test potential of 20 mV for 3 s, and then the cell was repolarized to −40 mV (1 s) to evoke outward tail current. The peak tail current was corrected the leak current defined during the first period to −40 mV. Holding potential was −80 mV. The pulse frequency was approximately 0.1 Hz. Recording started in external solution. After this control period, increasing concentrations of the test compound were applied, in order to record a complete concentration–response curve.

The action potential measurements were carried out in rabbit and guinea pig right ventricular papillary muscles by the conventional microelectrode techniques at 37 °C as described in detail earlier[12,26]. Isolated muscle preparations obtained from the right ventricle were individually mounted in a tissue chamber while superfused with oxygenated modified Locke's solution containing (in mM): NaCl 128.3, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 0.42,

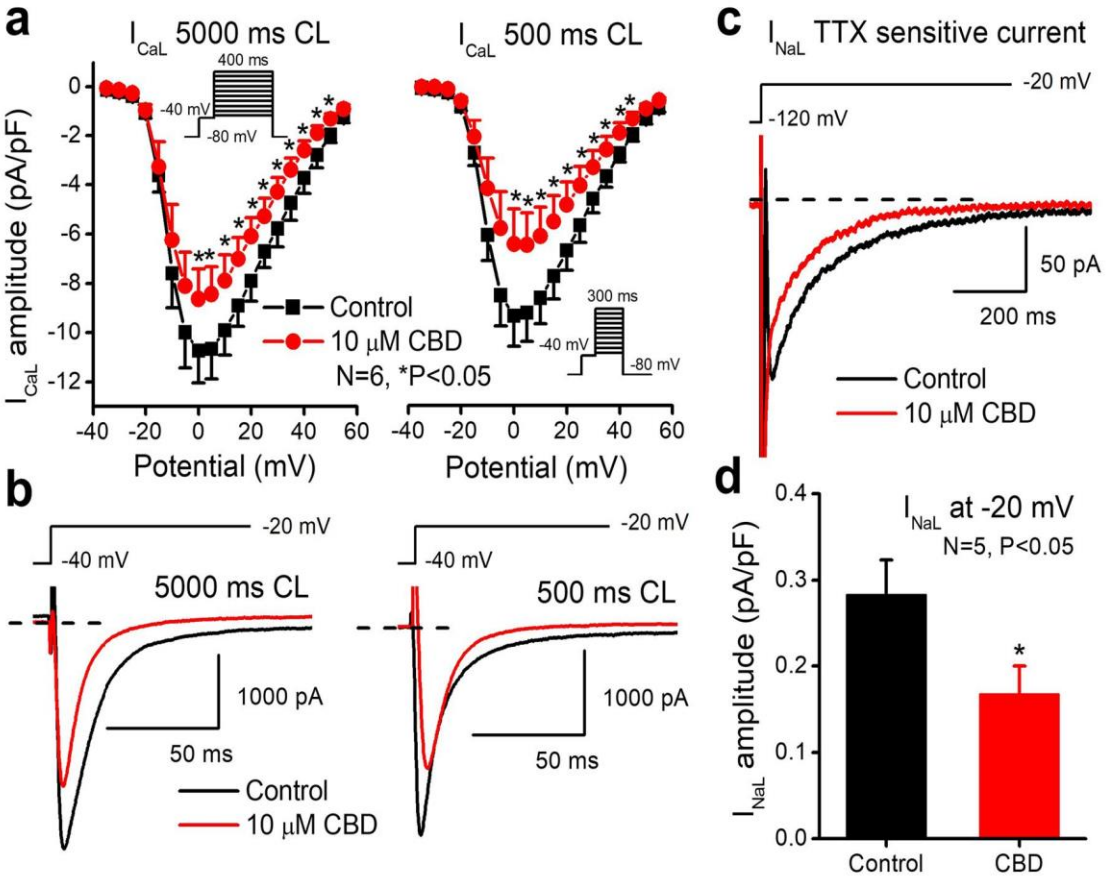


Figure 5. Effect of CBD after 3–5 min acute superfusion of the drug without washout on L-type calcium (I_{CaL}) and on the late sodium (I_{NaL}) currents in rabbit left ventricular myocytes at 37 °C. On (**a**) current–voltage curves show the inhibition of I_{CaL} by 10 μM CBD at 5000 ms (left) and at 500 ms (right) cycle lengths. Original I_{CaL} current traces are shown on (**b**) in control conditions and in the presence of 10 μM CBD recorded from rabbit left ventricular myocytes at 5000 ms (left) and at 500 ms (right) cycle lengths at 0 mV test potential. TTX sensitive current (I_{NaL}) traces (**c**) and a bar diagram (**d**) show the inhibition of I_{NaL} by 10 μM CBD measured at −20 mV in rabbit left ventricular myocytes.

$NaHCO_3$ 21.4 and glucose 10 (pH 7.35–7.4) and stimulated through a pair of platinum electrodes with constant cycle length of 1000 ms. In case of cycle length-dependent measurements stimulation with different constant cycle lengths ranging from 300 to 5000 ms were also applied. Transmembrane potentials were recorded using conventional glass microelectrodes, filled with 3 M KCl and having tip resistances of 5–20 MΩ, connected to the input of a high impedance electrometer (Experimetria, type 309, Budapest, Hungary). The analog action potential signals were digitized with analogue-to-digital converters (ADA 3300, Real Time Devices Inc., State College, PA, USA) under software control (APES home-made software).

Transmembrane ion currents in native rabbit ventricular myocytes were measured by the whole-cell configuration of the patch clamp technique at 37 °C (Axopatch 200B, Molecular Devices Inc., Sunnyvale, CA, USA) as described in detail earlier[12]. Rapid delayed rectifier potassium current (I_{Kr}), was recorded in HEPES-buffered Tyrode's solution containing (in mM) NaCl 144, NaH_2PO_4 0.33, KCl 4.0, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose 5.5 and HEPES 5.0, at pH of 7.4. The composition of the pipette solution (in mM) was the following: KOH 110, KCl 40, K_2ATP 5, $MgCl_2$ 5, EGTA 5, and HEPES 10 (pH was adjusted to 7.2 by aspartic acid). 1 μM nisoldipine and 0.5 μM HMR-1556 (the selective blocker of the slow delayed rectifier K^+ current—I_{Ks}) were added to the external solution to eliminate I_{CaL} and I_{Ks}, respectively. I_{Kr} was determined as tail current at −40 mV after the end of 1 s long depolarizing pulses ranging from −30 to +50 mV with pulsing cycle length of 20 s. The L-type calcium current (I_{CaL}) was recorded in HEPES-buffered Tyrode's solution supplemented with 3 mM 4-aminopyridine. A special solution was used to fill the micropipettes (composition in mM: CsCl 125, TEACl 20, MgATP 5, EGTA 10, HEPES 10, pH was adjusted to 7.2 by CsOH). I_{CaL} current was evoked by 400 ms long depolarizing voltage pulses to various test potentials ranging from −35 to +55 mV with pulsing cycle length of 5 s. The holding potential was −80 mV. A short prepulse to −40 mV served to inactivate Na^+ current. The sodium current was activated by 2 s long depolarizing voltage pulses to −20 mV from the holding potential of −120 mV with pulsing cycle length of 5 s. After 5–7 min incubation with CBD the external solution was replaced by that containing 20 μM TTX. TTX at this concentration completely blocks the late sodium current (I_{NaL}). The external solution was HEPES-buffered Tyrode's solution supplemented with 1 μM nisoldipine, 0.5 μM HMR-1556 and 0.1 μM dofetilide in order to block I_{CaL}, I_{Ks} and I_{Kr} currents. The composition of the pipette solution (in mM) was: KOH 110, KCl 40, K_2ATP 5, $MgCl_2$ 5, EGTA 5, HEPES 10 (pH was adjusted to 7.2 by aspartic acid).

ECG recordings were taken from adult guinea-pigs of both sexes (600–800 g) anaesthetized by intraperitoneal 30 mg/kg pentobarbital and I–III leads were recorded after 15 min of cumulative intravenous administration of CBD into the jugular vein[27].

Statistics. All data are expressed as means ± SEM. Statistical analysis was performed with Student's *t* test for paired data. The results were considered statistically significant when p was < 0.05.

Animal ethics statement. All experiments performed in rabbit and guinea pig ventricular preparations were carried out in compliance with the Guide for the Care and Use of Laboratory Animals (USA NIH publication NO 85-23, revised 1996) and conformed to the Directive 2010/63/EU of the European Parliament. The protocols have been approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Szeged, Hungary (approval number: I-74-24-2017) and by the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development (authority approval number XIII/3331/2017).

Received: 5 February 2020; Accepted: 10 September 2020
Published online: 30 September 2020

References

1. Kalla, A., Krishnamoorthy, P. M., Gopalakrishnan, A. & Figueredo, V. M. Cannabis use predicts risks of heart failure and cerebrovascular accidents: Results from the National Inpatient Sample. *J. Cardiovasc. Med. (Hagerstown)* **19**, 480–484 (2018).
2. Fraguas-Sánchez, A. I. & Torres-Suárez, A. I. Medical use of cannabinoids. *Drugs* **78**, 1665–1703 (2018).
3. Pacher, P., Steffens, S., Hasko, G., Schindler, T. H. & Kunos, G. Cardiovascular effects of marijuana and synthetic cannabinoids: The good, the bad, and the ugly. *Nat. Rev. Cardiol.* **15**, 151–166 (2018).
4. Mittleman, M. A., Lewis, R. A., Maclure, M., Sherwood, J. B. & Muller, J. E. Triggering myocardial infarction by marijuana. *Circulation* **103**, 2805–2809 (2001).
5. Singh, S. M., Koh, M., Fang, J. & Ko, D. T. The risk of cardiovascular events on cannabis' highest day. *Can. J. Cardiol.* **35**, 1589–1591 (2019).
6. Courts, J., Maskill, V., Gray, A. & Glue, P. Signs and symptoms associated with synthetic cannabinoid toxicity: Systematic review. *Australas. Psychiatry* **24**, 598–601 (2016).
7. Ozturk, H. M., Yetkin, E. & Ozturk, S. Synthetic cannabinoids and cardiac arrhythmia risk: Review of the literature. *Cardiovasc. Toxicol.* **19**, 191–197 (2019).
8. Manolis, T. A., Manolis, A. A. & Manolis, A. S. Cannabis associated 'high' cardiovascular morbidity and mortality: Marijuana smoke like tobacco smoke? A Deja Vu/Deja Vecu Story?. *Mini Rev. Med. Chem.* **19**, 870–879 (2019).
9. Al Kury, L. T. *et al.* Effects of the endogenous cannabinoid anandamide on voltage-dependent sodium and calcium channels in rat ventricular myocytes. *Br. J. Pharmacol.* **171**, 3485–3498 (2014).
10. Amoros, I. *et al.* Endocannabinoids and cannabinoid analogues block human cardiac Kv4.3 channels in a receptor-independent manner. *J. Mol. Cell. Cardiol.* **48**, 201–210 (2010).
11. Sanguinetti, M. C. & Tristani-Firouzi, M. hERG potassium channels and cardiac arrhythmia. *Nature* **440**, 463–469 (2006).
12. Orvos, P. *et al.* Evaluation of possible proarrhythmic potency: Comparison of the effect of dofetilide, cisapride, sotalol, terfenadine, and verapamil on hERG and native IKr currents and on cardiac action potential. *Toxicol. Sci.* **168**, 365–380 (2019).
13. Roden, D. M. & Hoffman, B. F. Action potential prolongation and induction of abnormal automaticity by low quinidine concentrations in canine Purkinje fibers. Relationship to potassium and cycle length. *Circ. Res.* **56**, 857–867 (1985).
14. Varro, A., Elharrar, V. & Surawicz, B. Effect of antiarrhythmic drugs on the premature action potential duration in canine cardiac Purkinje fibers. *J. Pharmacol. Exp. Ther.* **233**, 304–311 (1985).
15. Ghovanloo, M.-R. *et al.* Inhibitory effects of cannabidiol on voltage-dependent sodium currents. *J. Biol. Chem.* **293**, 16546–16558 (2018).
16. Li, Q. *et al.* Effects of anandamide on potassium channels in rat ventricular myocytes: A suppression of I(to) and augmentation of K(ATP) channels. *Am. J. Physiol. Cell Physiol.* **302**, C924–C930 (2012).
17. Barana, A. *et al.* Endocannabinoids and cannabinoid analogues block cardiac hKv1.5 channels in a cannabinoid receptor-independent manner. *Cardiovasc. Res.* **85**, 56–67 (2010).
18. Yun, J. *et al.* Synthetic cannabinoid, JWH-030, induces QT prolongation through hERG channel inhibition. *Toxicol. Res. (Camb)* **5**, 1663–1671 (2016).
19. Redfern, W. *et al.* Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: Evidence for a provisional safety margin in drug development. *Cardiovasc. Res.* **58**, 32–45 (2003).
20. Millar, S. A., Stone, N. L., Yates, A. S. & O'Sullivan, S. E. A systematic review on the pharmacokinetics of cannabidiol in humans. *Front. Pharmacol.* **9**, 1365 (2018).
21. Sellers, E. M. *et al.* A multiple-dose, randomized, double-blind, placebo-controlled, parallel-group QT/QTc study to evaluate the electrophysiologic effects of THC/CBD spray. *Clin. Pharmacol. Drug Dev.* **2**, 285–294 (2013).
22. Serpell, M. G., Notcutt, W. & Collin, C. Sativex long-term use: An open-label trial in patients with spasticity due to multiple sclerosis. *J. Neurol.* **260**, 285–295 (2013).
23. Iffland, K. & Grotenhermen, F. An update on safety and side effects of cannabidiol: A review of clinical data and relevant animal studies. *Cannabis Cannabinoid Res.* **2**, 139–154 (2017).
24. Lengyel, C., Varro, A., Tabori, K., Papp, J. G. & Baczko, I. Combined pharmacological block of I(Kr) and I(Ks) increases short-term QT interval variability and provokes torsades de pointes. *Br. J. Pharmacol.* **151**, 941–951 (2007).
25. Wisniowska, B., Tylutki, Z., Wyszogrodzka, G. & Polak, S. Drug-drug interactions and QT prolongation as a commonly assessed cardiac effect—comprehensive overview of clinical trials. *BMC Pharmacol. Toxicol.* **17**, 12 (2016).
26. Lengyel, C. *et al.* Pharmacological block of the slow component of the outward delayed rectifier current (I(Ks)) fails to lengthen rabbit ventricular muscle QT(c) and action potential duration. *Br. J. Pharmacol.* **132**, 101–110 (2001).
27. Farkas, A. S. *et al.* The role of the Na^+/Ca^{2+} exchanger, I Na and I CaL in the genesis of dofetilide-induced torsades de pointes in isolated, AV-blocked rabbit hearts. *Br. J. Pharmacol.* **156**, 920–932 (2009).
28. Carlsson, L., Abrahamsson, C., Andersson, B., Duker, G. & Schiller-Linhardt, G. Proarrhythmic effects of the class III agent almokalant: Importance of infusion rate, QT dispersion, and early afterdepolarisations. *Cardiovasc. Res.* **27**, 2186–2193 (1993).

Acknowledgements
The authors thank Dora Bokor, PharmD, for proofreading the manuscript. Financial support from the Economic Development and Innovation Operative Programme GINOP-2.3.2-15-2016-00012, the National Research Development and Innovation Office (NKFIH K 119992), the Ministry of Human Capacities Hungary (20391 3/2018/FEKUSTRAT and EFOP-3.6.2-16-2017-00006), and from the Hungarian Academy of Sciences are gratefully acknowledged.

Author contributions
D.C., B.C.-L., L.V. and A.V. conceived the experiment, P.O., B.P., L.T., A.P., Á.B., P.G., J.P. and E.T.-M. conducted the experiment(s), P.O., L.V. and A.V. analysed the results, D.C., T.K., J.H., L.V. and A.V. prepared the manuscript. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to D.C.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Terms and Conditions

Springer Nature journal content, brought to you courtesy of Springer Nature Customer Service Center GmbH ("Springer Nature").

Springer Nature supports a reasonable amount of sharing of research papers by authors, subscribers and authorised users ("Users"), for small-scale personal, non-commercial use provided that all copyright, trade and service marks and other proprietary notices are maintained. By accessing, sharing, receiving or otherwise using the Springer Nature journal content you agree to these terms of use ("Terms"). For these purposes, Springer Nature considers academic use (by researchers and students) to be non-commercial.

These Terms are supplementary and will apply in addition to any applicable website terms and conditions, a relevant site licence or a personal subscription. These Terms will prevail over any conflict or ambiguity with regards to the relevant terms, a site licence or a personal subscription (to the extent of the conflict or ambiguity only). For Creative Commons-licensed articles, the terms of the Creative Commons license used will apply.

We collect and use personal data to provide access to the Springer Nature journal content. We may also use these personal data internally within ResearchGate and Springer Nature and as agreed share it, in an anonymised way, for purposes of tracking, analysis and reporting. We will not otherwise disclose your personal data outside the ResearchGate or the Springer Nature group of companies unless we have your permission as detailed in the Privacy Policy.

While Users may use the Springer Nature journal content for small scale, personal non-commercial use, it is important to note that Users may not:

1. use such content for the purpose of providing other users with access on a regular or large scale basis or as a means to circumvent access control;
2. use such content where to do so would be considered a criminal or statutory offence in any jurisdiction, or gives rise to civil liability, or is otherwise unlawful;
3. falsely or misleadingly imply or suggest endorsement, approval , sponsorship, or association unless explicitly agreed to by Springer Nature in writing;
4. use bots or other automated methods to access the content or redirect messages
5. override any security feature or exclusionary protocol; or
6. share the content in order to create substitute for Springer Nature products or services or a systematic database of Springer Nature journal content.

In line with the restriction against commercial use, Springer Nature does not permit the creation of a product or service that creates revenue, royalties, rent or income from our content or its inclusion as part of a paid for service or for other commercial gain. Springer Nature journal content cannot be used for inter-library loans and librarians may not upload Springer Nature journal content on a large scale into their, or any other, institutional repository.

These terms of use are reviewed regularly and may be amended at any time. Springer Nature is not obligated to publish any information or content on this website and may remove it or features or functionality at our sole discretion, at any time with or without notice. Springer Nature may revoke this licence to you at any time and remove access to any copies of the Springer Nature journal content which have been saved.

To the fullest extent permitted by law, Springer Nature makes no warranties, representations or guarantees to Users, either express or implied with respect to the Springer nature journal content and all parties disclaim and waive any implied warranties or warranties imposed by law, including merchantability or fitness for any particular purpose.

Please note that these rights do not automatically extend to content, data or other material published by Springer Nature that may be licensed from third parties.

If you would like to use or distribute our Springer Nature journal content to a wider audience or on a regular basis or in any other manner not expressly permitted by these Terms, please contact Springer Nature at

onlineservice@springernature.com

1.sz. melléklet

Társszerzői lemondó nyilatkozat

Co-author certification

Alulírott Dr. Csupor Dezső, mint az alábbi közlemény felelős szerzője kijelentem, hogy Dr. Pászti Bence József doktorjelölt a „The possible proarrhythmic effects of some non-antiarrhythmic drugs" című PhD értekezésének tézispontjaiban bemutatott – közösen publikált – tudományos eredmények elérésében a pályázónak meghatározó szerepe volt, ezért ezeket a téziseket más a PhD fokozat megszerzését célzó minősítési eljárásban nem használta fel, illetve nem kívánja felhasználni.

A pályázó tézispontjaiban érintett, közösen publikált közlemény:

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.
Impact factor: 3.998 (2019)

A felsorolt publikációban Dr. Pászti Bence József doktorjelölt végezte a kannabidiol szívelektrofiziológiai vizsgálatát nyúl és tengerimalac kamrai papilláris izomrostokon a konvencionális mikroelektród technika segítségével. A mikroelektród technikával végzett mérésekhez történő előkészület, a mérések beállítása, azok elvégzése és az eredmények kiértékelése teljes egészében a jelölt saját munkái. A közlemény ezen részét nem kívánjuk felhasználni más Ph.D. értékezés megírásához.

Szeged, 2021. április 29.

.....

Dr. Csupor Dezső
egyetemi docens
(nevezett publikáció felelős szerzője)