Cardiac electrophysiological effects of ibuprofen in dog and rabbit ventricular preparations: Possible implication to enhanced proarrhythmic risk

Bence Pászti[a,§], János Prorok[a,c,§], Tibor Magyar[a,], Tamás Árpádffy-Lovas[a,], Balázs Györe[d], Leila Topál[a], Péter Gazdag[a], Jozefina Szlovák[a], Muhammad Naveed[a], Norbert Jost[a,b,c], Norbert Nagy[a,c], András Varró[a,b,c,+], László Virág[a,b,c,*], István Koncz[a,*]

[a]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, Szeged, Hungary;
[b]Department of Pharmacology and Pharmacotherapy, Interdisciplinary Excellence Centre, University of Szeged, Szeged, Hungary
[c]MTA-SZTE Research Group of Cardiovascular Pharmacology, Szeged, Hungary
[d] Department of Oral Surgery, Faculty of Dentistry, University of Szeged, Hungary

[§] Shared first authorship
[*] Shared senior authorship
[+] Corresponding author

Author for correspondence at:
Andras Varró MD, PhD
Department of Pharmacology & Pharmacotherapy
Faculty of Medicine
University of Szeged
Dóm tér 12,
H-6721 Szeged, Hungary

Telephone: +36 62 545 682
Fax: +36 62 545 680
E-mail: varro.andras@med.u-szeged.hu

Abstract

Ibuprofen is a widely used non-steroidal 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%. We conclude that ibuprofen seems to be free from effects on AP parameters at lower concentrations. However, at higher concentrations it may alter repolarization reserve, contributing to the observed proarrhythmic risk in patients.

List of abbreviations

APA: Action potential amplitude

APD_{50}: Action potential duration at 50% of repolarization

APD_{90}: Action potential duration at 90% of repolarization

I_{Ca}: Voltage-dependent calcium current

I_{K1}: Inward rectifier potassium current

I_{Kr}: Rapidly activating delayed rectifier potassium current

I_{Ks}: Slowly activating delayed rectifier potassium current

I_{NaL}: Late sodium current

I_{to}: Transient outward potassium current

RMP: Resting membrane potential

TdP: Torsades de pointes ventricular tachycardia

SEM: Standard error of the mean

V_{max}: Maximal rate of the action potential upstroke

Introduction

Ibuprofen is one of the most widely used non-steroidal anti-inflammatory drugs (NSAIDs) (Rainsford et al., 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 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 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 & 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 action potential duration (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 & 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 (2–3 kg) were terminated by rapid cervical dislocation, and Beagle dogs (10–15 kg) of both sexes were anesthetized and sacrificed using high dose sodium pentobarbital (60 mg/kg iv). The preparations were placed in a tissue bath and allowed to equilibrate for at least 2 hours 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 to 15 MΩ). The microelectrodes were coupled through an Ag-AgCl junction to the input of a high-impedance, capacitance-neutralizing amplifier (Experimetria 2011). Intracellular recordings were displayed on a storage oscilloscope (Hitachi V-555) and led to a computer system (APES) designed for on-line determination of the following parameters: resting membrane potential (RMP), action potential amplitude (APA), action potential duration 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 equilibration period. The effects of ibuprofen and DMSO not exceeding 18.8% were determined at the given concentrations, after the addition of each compound until 30 minutes

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 weights 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 1985). The protocols were approved by the review board of Committee on Animal Research (CAR) 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 minutes 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 Co., Novato, CA, 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, CA, 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 1440A, Molecular Devices Inc., Sunnyvale, CA, USA) under software control (pClamp 10, Molecular Devices Inc., Sunnyvale, CA, USA). The various ion currents were measured as described earlier in detail (Kohajda et al., 2016). The same software was used for off-line analysis.

Statistical analysis

Results are expressed as 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 Tables 1, 2A, and 2B. Variance analysis (ANOVA) for repeated measurements was performed, followed by Bonferroni's post-hoc test for Table 2C. Differences were considered significant when $p < 0.05$.

3. 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 Table 1B and 1C, and Figures 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 (RMP), the action potential amplitude (APA), or the maximal rate of depolarization (V_{max}), but at 200 μM it moderately lengthened the action potential duration measured at 50 and 90% levels (APD_{50} and APD_{90}). The solvent DMSO at the applied concentration did not affect any of the measured action potential parameters (Table 1A and Figures 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 (Figure 2A). At constant cycle length stimulation, ibuprofen at 200 μM concentration elicited significant abbreviation of the action potential duration (APD_{90}), while all other characteristics, including the RP, APA and V_{max}, remained unchanged. As Figure 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 ms 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, Figures 1E, 2C and 2D).

The widely known antibiotic, levofloxacin at 40 μM did not change action potential parameters, including APD_{90} in rabbit papillary muscles at 1 Hz stimulation rate (Table 2B

and Figure 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 Figure 3B).

In order 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 (Figures 4–5). In dog ventricular myocytes, 250 μM ibuprofen did not significantly alter the inward rectifier (I_{K1}) potassium (Figure 4A) and moderately but significantly decreased the transient outward (I_{to}, Figure 4B and 4D) and rapid delayed rectifier (I_{Kr}, Figure 4C and 4E) potassium currents.

Since 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 Figure 5 indicates, 250 μM ibuprofen moderately, but in a statistically significant manner decreased the amplitude of both I_{NaL} and I_{Ca}.

4. 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, Hsiao, Tsai, et al., 2006; Huang, Hsiao, Wen, et al., 2006). 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 the previous experimental study (Kristóf et al., 2012), which indicated that diclofenac decreased repolarisation reserve by inhibiting I_{Ks} and I_{Kr} in dog heart. In this paper it has also been shown that diclofenac also facilitated 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. (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, altered metabolism caused by disease or drug interactions, plasma levels may rise beyond normal. In addition, much higher (260 and 352 μg/ml) 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. (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 to that in rabbit or dog. Therefore, in guinea pig ventricle the ibuprofen-evoked 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 & 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 hERG channel inhibiting (Kang et al., 2001) properties compared to 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 & 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, since ibuprofen is a very commonly used over-the-counter drug, taken every day by several million people without medical control.

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Figure 1. The effects of ibuprofen and 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 ventricular muscle (panel A) and in dog ventricular muscle (at 200 μM, panel B) at a basic cycle length of 1000 ms. However, in dog Purkinje fiber (panel C) 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).

Figure 2. Cycle length dependent changes in action potential duration (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 DMSO at 2.2‰ in dog Purkinje fiber preparations. Values are means ± SEM, asterisks indicate significant changes.

Figure 3. The effects of levofloxacin alone (panel A) and in combination with 100 μM ibuprofen (panel B) on action potentials recorded from rabbit ventricular 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).

Figure 4. Panels A–C show the effects of the solvent DMSO at 1‰ 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. Legend: control, black lines and empty boxes; DMSO, green lines and green boxes; ibuprofen; red lines and circles. 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.

Figure 5. Panels A and B show the effects of the solvent DMSO at 1‰ 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 TTX sensitive current by subtracting current traces recorded in the presence of 20 μM TTX from traces of control, DMSO and ibuprofen recordings.

Table 1. The electrophysiological effects of DMSO (2.2‰) and ibuprofen (50 μM and 200 μM) in canine right ventricular papillary muscle preparations (VM; A, B and C), at basic cycle length of 1000 ms; and ibuprofen (200 μM) in canine Purkinje fibers (PF; D) at basic cycle length of 500 ms; 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. Results are expressed as means ± SEM; *p<0.05, Student's t-test for paired data.

A	**Sample**	**RP (mV)**	**APA (mV)**	**V_{max} (V/s)**	**APD_{50} (ms)**	**APD_{90} (ms)**	**APD_{90} (%)**
Control	Canine VM (6)	-83.3 ± 2.3	106.7 ± 1.5	136.7 ± 14.2	157.8 ± 11.6	203.6 ± 7.6	
DMSO (2.2‰)	Canine VM (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	Canine VM (8)	-83.2 ± 1.6	108.1 ± 1.0	175.2 ± 22.3	187.0 ± 9.3	227.5 ± 9.7	
Ibuprofen (50 μM)	Canine VM (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	Canine VM (6)	-89.0 ± 1.8	110.6 ± 2.4	174.6 ± 20.3	173.8 ± 8	214.1 ± 5.9	
Ibuprofen (200 μM)	Canine VM (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	Canine PF	-89.7	133.5	580.7	163.9	253.4	

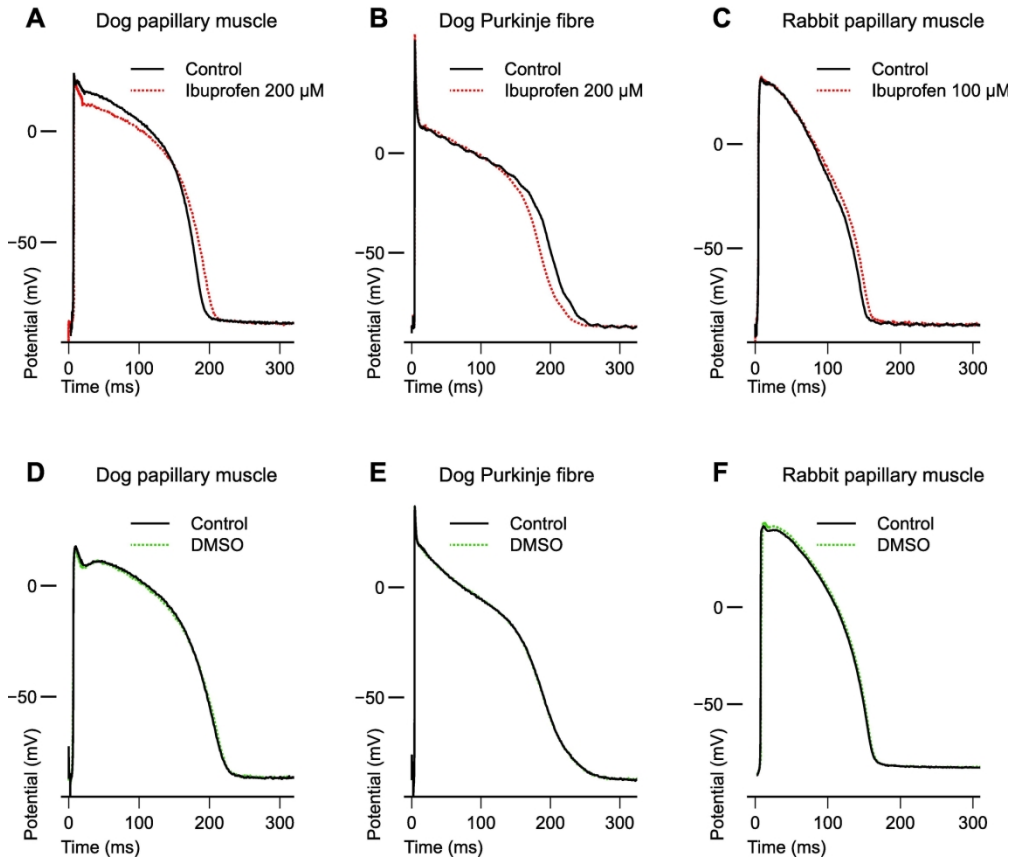
	(7)	± 0.7	± 3.3	± 36.0	± 10.9	± 14.2	
Ibuprofen	Canine PF	-87.3	135.9	621.5	163.7	242.0	-4.5
(200 μM)	(7)	± 1.0	± 3.4	± 93.5	± 11.2	± 13.7*	± 0.7

Table 2. The electrophysiological effects of DMSO (2.2‰), levofloxacin (40 µM) and ibuprofen (100 µM) in rabbit right ventricular papillary muscle preparations at a basic cycle length of 1000 ms; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{50} and APD_{90}, action potential durations at 50% and 90% of repolarization. Results are expressed as means ± SEM; *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).

A	**Sample**	**RP (mV)**	**APA (mV)**	**V_{max} (V/s)**	**APD_{50} (ms)**	**APD_{90} (ms)**	**APD_{90} (%)**
Control	Rabbit VM (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 (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} (V/s)**	**APD_{50} (ms)**	**APD_{90} (ms)**	**APD_{90} (%)**
Control	Rabbit VM (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 (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 (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 (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 (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



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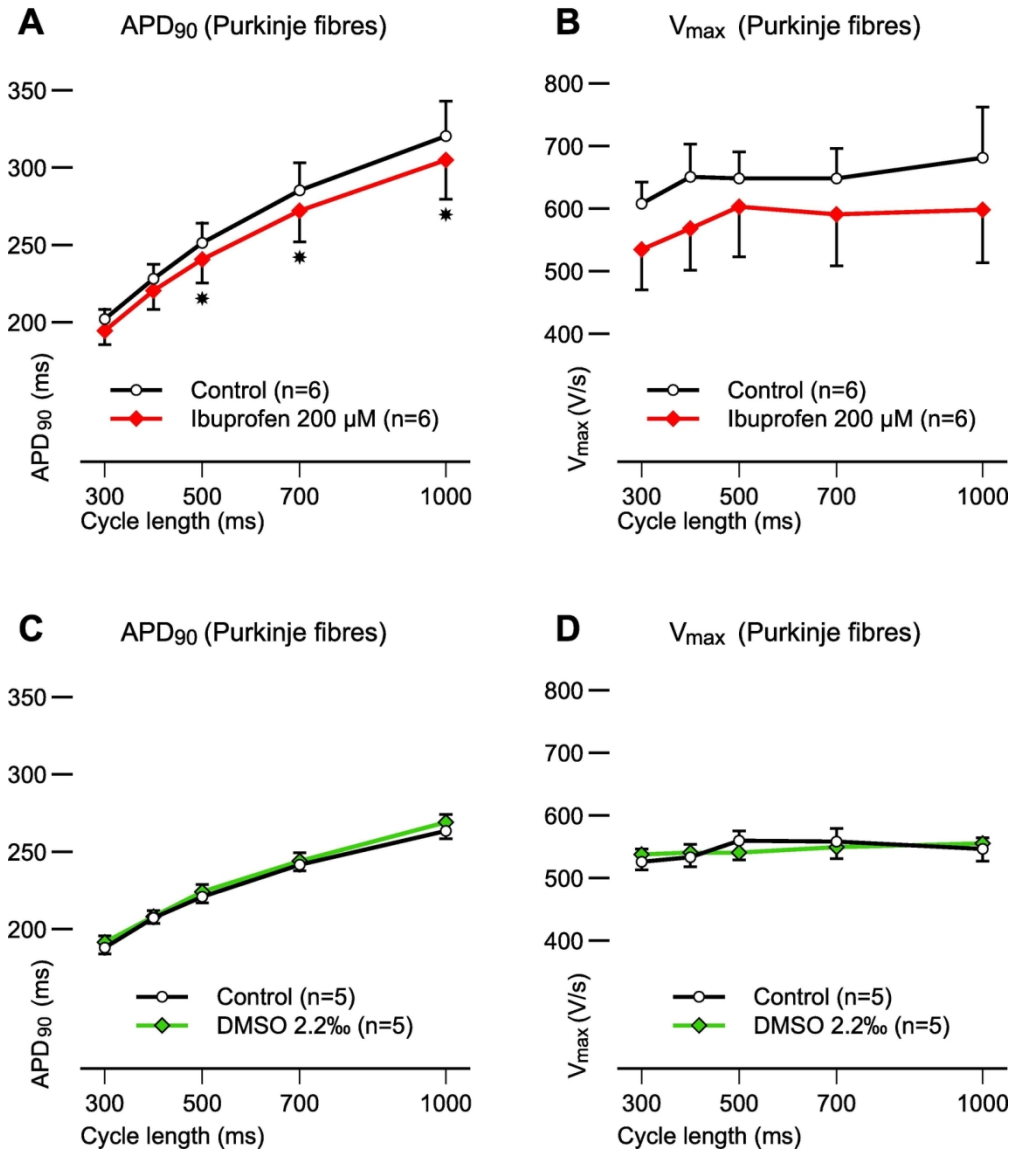

Figure 2. Cycle length dependent changes in action potential duration (APD90, panels A and C) and in maximal rate of depolarization (Vmax, panels B and D) measured under control conditions and in the presence of 200 µM ibuprofen and DMSO at 2.2‰ in dog Purkinje fiber preparations. Values are means ± SEM, asterisks indicate significant changes.

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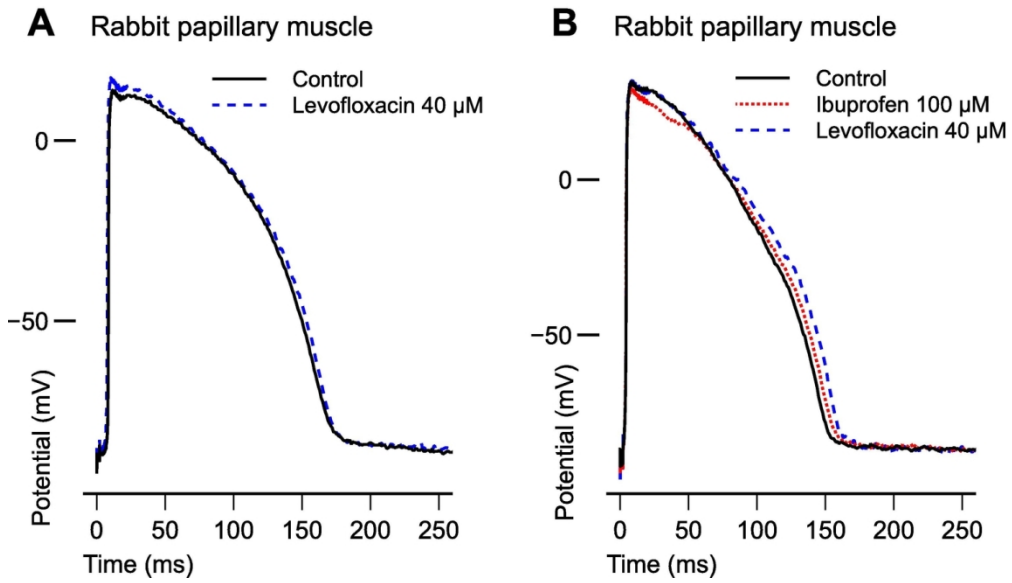

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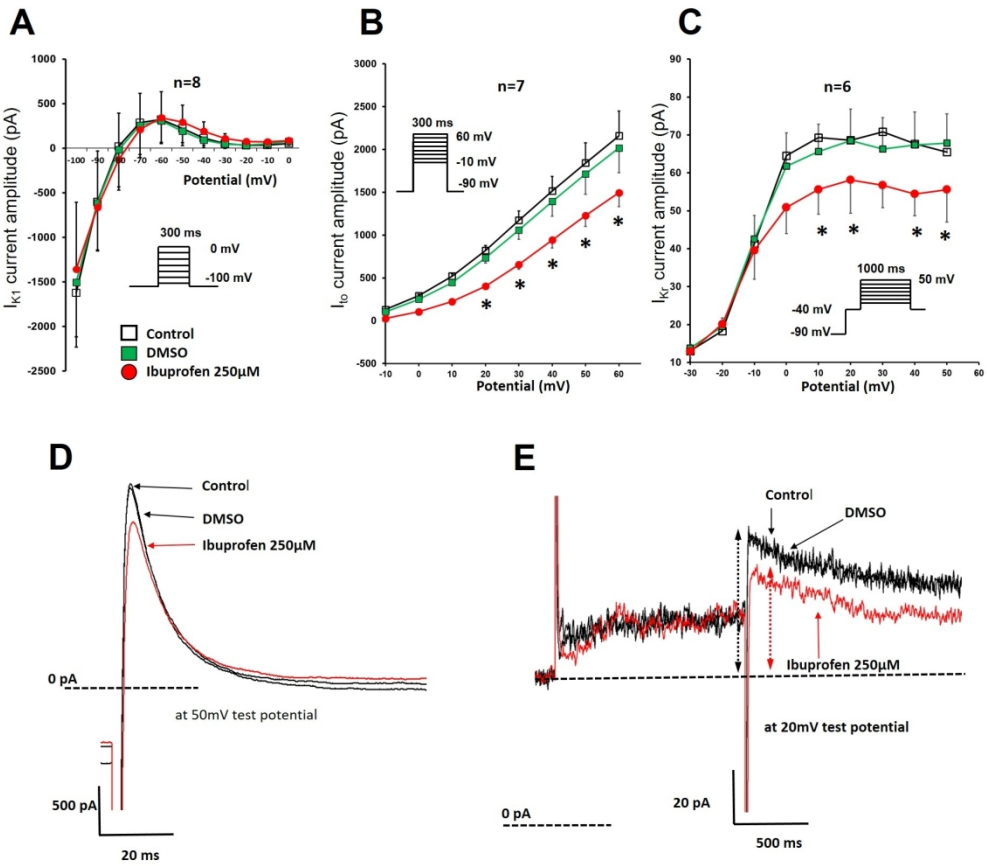

Figure 4. Panels A–C show the effects of the solvent DMSO at 1‰ and ibuprofen at 250 µM on the potassium currents IK1, Ito, and IKr respectively in ventricular myocytes; the insets show the applied voltage protocols. Legend: control, black lines and empty boxes; DMSO, green lines and green boxes; ibuprofen; red lines and circles. 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 Ito and IKr 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 IKr tail currents at -40 mV.

315x279mm (150 x 150 DPI)

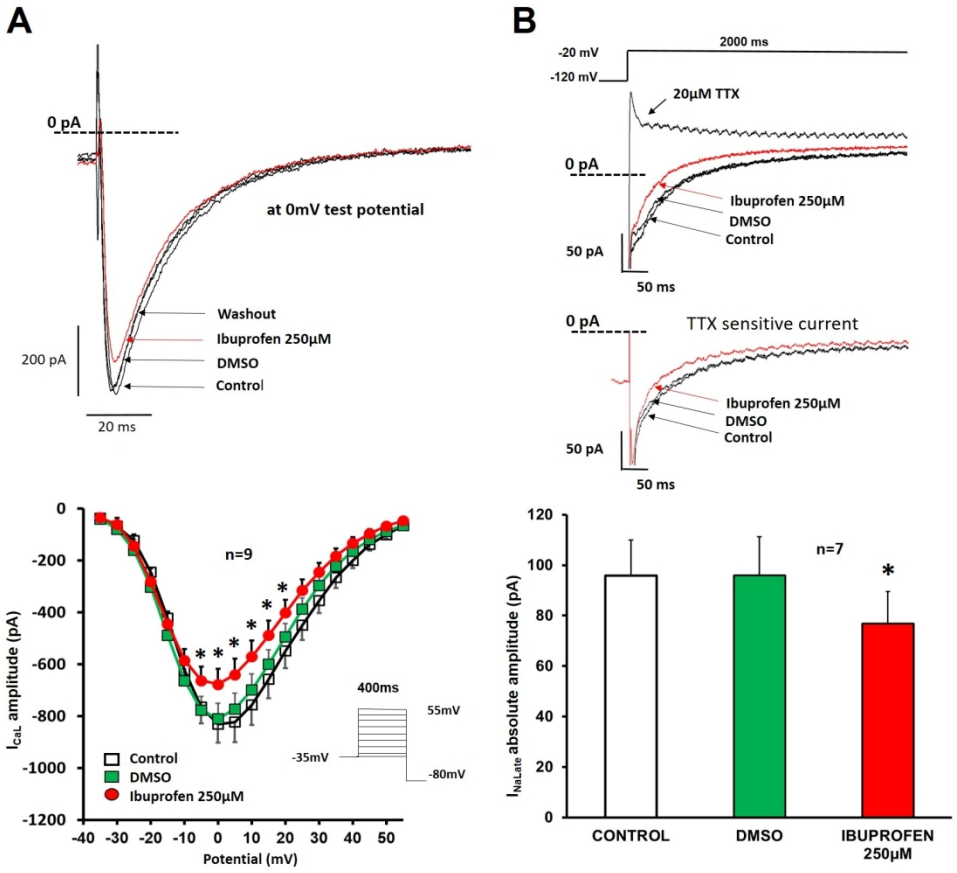

Figure 5. Panels A and B show the effects of the solvent DMSO at 1‰ and ibuprofen at 250 µM on the L-type calcium current (ICa) and the late sodium current (INaL) respectively in ventricular myocytes; the insets show the applied voltage protocols. Values are means ± SEM, asterisks indicate $p<0.05$, 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 INaL and ICa currents respectively, recorded in control conditions and in the presence of DMSO and after the application of 250 µM ibuprofen. INaL was defined as TTX sensitive current by subtracting current traces recorded in the presence of 20 µM TTX from traces of control, DMSO and ibuprofen recordings.

313x278mm (150 x 150 DPI)