SPECIES DIFFERENCES IN CARDIAC VENTRICULAR REPOLARIZATION, AND THEIR IMPLICATIONS FOR HUMAN CARDIAC ELECTROPHYSIOLOGY

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PhD thesis

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Table of Contents

Introduction....2
Sudden cardiac death, ventricular fibrillation, and the repolarization reserve....2
Cardiac electrophysiological experimentation....8
Materials and Methods....10
Results....14
Selective inhibition of ion currents at constant 1000 ms pacing....15
Frequency-dependent changes in APD (variable cycle length pacing)....24
Frequency-dependent changes in APD (extrasystolic stimulation protocol)....24
Ion channel inhibitor effects on human APD restitution....26
Comparison of amiodarone and dofetilide effects on Purkinje-muscle dispersion of repolarization 29
Discussion....32
Constant pacing at 1000 ms cycle length....32
Frequency-dependent changes in APD....38
Electrotonically coupled and uncoupled preparations....40
Conclusion....42
Acknowledgments....44
References....45

Introduction

Sudden cardiac death, ventricular fibrillation, and the repolarization reserve

The annual incidence of sudden cardiac death (SCD) has been reported to approximate 50 to 100 per 100,000 in the general populations of Europe and North America (Wong et al., 2019), accounting for approximately half of the deaths in patients with cardiac conditions (Jazayeri and Emert, 2019). The most common mechanism of SCD is cardiac ventricular fibrillation (VF), which is a complex and still poorly understood phenomenon in the heart.

The action potential

From an electrophysiological standpoint, the action potential (AP) is the basis of cardiac mechanisms. The waveforms of atrial and ventricular action potentials show notable differences, and this work is mainly concerned with the electrophysiology of the ventricles.

In the hearts of large mammals, including that of human, the cardiac ventricular action potential is responsible for impulse conduction throughout the myocardium, and its plateau phase is the basis of the relatively long refractory period of the ventricular myocytes. In all excitable tissues, the shape and, subsequently, the duration of the AP (action potential duration; APD) is determined by the activation and inactivation sequences of the ion currents (Figure 1). In the case of the human ventricular myocardium, the most notable inward, depolarizing currents are the fast and the slowly decaying or late components of the sodium current (I_{Na} and I_{NaLate}), and the L-type calcium current (I_{CaL}). I_{Na} is responsible for the rapid depolarization, and the impulse conduction in the heart, and I_{NaLate} helps to maintain the plateau phase contributing to the relatively long AP duration in the ventricle. I_{CaL} is responsible for the formation of the plateau phase, and for the initiation of muscle contraction through the mechanism of Ca^{2+}-induced Ca^{2+}-release (Stern, 1992). Cardiac potassium currents are associated with repolarization: their activation counteract the depolarization elicited by

I_{Na} and I_{CaL}, leading to the membrane reaching its resting potential at the end of the AP. The most notable repolarizing currents in the human ventricle are the transient outward current (I_{to}), the slow and rapid delayed rectifier potassium currents (I_{Kr} and I_{Ks}), and the inward rectifier potassium current (I_{K1}). The acetylcholine-sensitive potassium current ($I_{K\text{-}Ach}$) and the ultrarapid delayed rectifier potassium current (I_{Kur}) are considered to be functional primarily in the atria of the human heart. The function of the ATP-dependent potassium current ($I_{K\text{-}ATP}$) is manifested under ischemic conditions. The sodium/calcium exchanger transports Ca^{2+} and Na^{+} in and out, to and from the myocytes depending on the actual membrane potential as well as extra and intracellular Na^{+} and Ca^{2+} concentrations. Since 1 Ca^{2+} is exchanged for 3 Na^{+}, it delivers an inward or outward current, primarily depending on the voltage and intracellular concentration of Ca^{2+}.

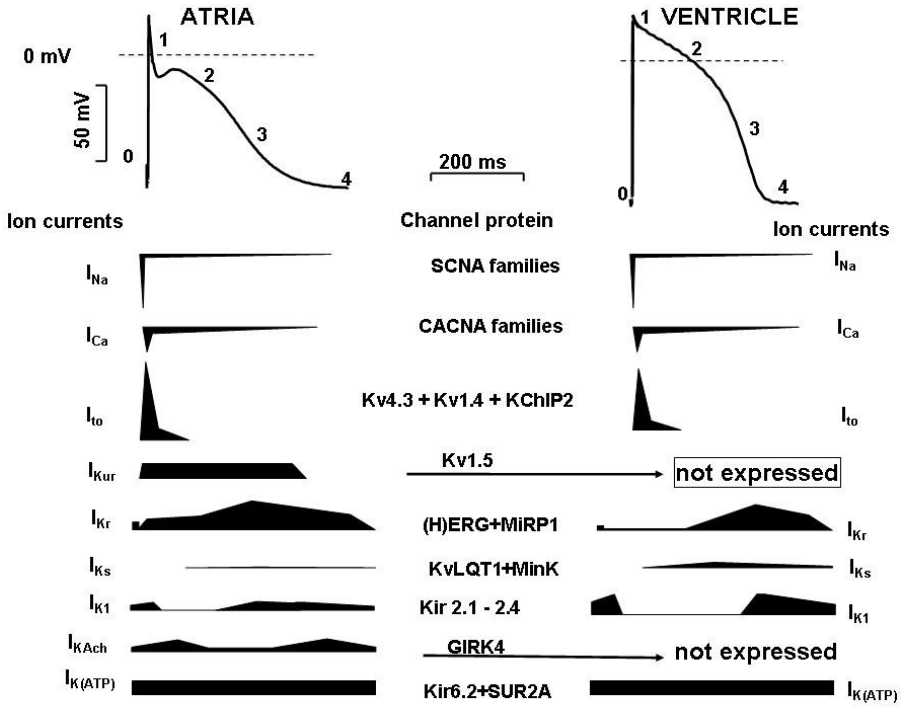

Figure 1 – The ionic currents forming the atrial and ventricular cardiac action potentials. The horizontal line in the current profiles represents zero current level, and the inward currents are below the line, outward currents are above it. (Reproduced with permission; Jost et al., 2007)

The mechanism of ventricular fibrillation due to enhanced dispersion of repolarization

Under healthy conditions, cardiac impulse conduction is fast (1–2 m/s), and the APD of ventricular myocytes is long (200–300 ms). The APD also determines the effective refractory period (ERP), because during most of the duration of the action potential, the myocardial cells are not excitable. Therefore, the relatively long and consistent ERP combined with fast impulse conduction ensures homogeneous depolarization and repolarization waves in the heart. On the other hand, spatial changes in APD and consequent changes in refractoriness lead to increased repolarization inhomogeneity, which may open otherwise inaccessible pathways to impulse conduction (Figure 2; Varró and Baczkó, 2010), because areas with longer APD and ERP block the conduction, but areas with relatively shorter APD and ERP can be excited, leading to the opening of complex, inhomogeneous pathways of impulse conduction. Since this state of cardiac impulse conduction significantly increases the chances of re-entrant arrhythmias, it can be considered as a *substrate* to ventricular fibrillation. However, this vulnerable state of the heart is not sufficient to develop arrhythmias. A *trigger*, most likely an extra beat that can travel through the complex pathway of low-ERP areas of the heart is what starts the re-entrant arrhythmia, possibly escalating to ventricular fibrillation and SCD. In other terms, this extra beat can travel in a zig-zag pattern and can re-enter into areas that have been previously excited, eliciting chaotic rhythm or even fibrillation.

Substrate formation

Therefore, drugs that prolong the APD (e. g., potassium channel inhibitors) and/or decelerate the impulse conduction (inhibitors of I_{Na}) in myocardial cells protect against VF not only by increasing the ERPs of the myocytes and decreasing the chance of rapidly conducted extra beats, but they can also enhance the propensity of ventricular arrhythmias by the same mechanism (Figure 2). Amiodarone, a widely used antiarrhythmic drug is characterized by the combination of these two effects with additional beta-receptor and I_{Ca} blocking activities, and it indeed decreases the spatial

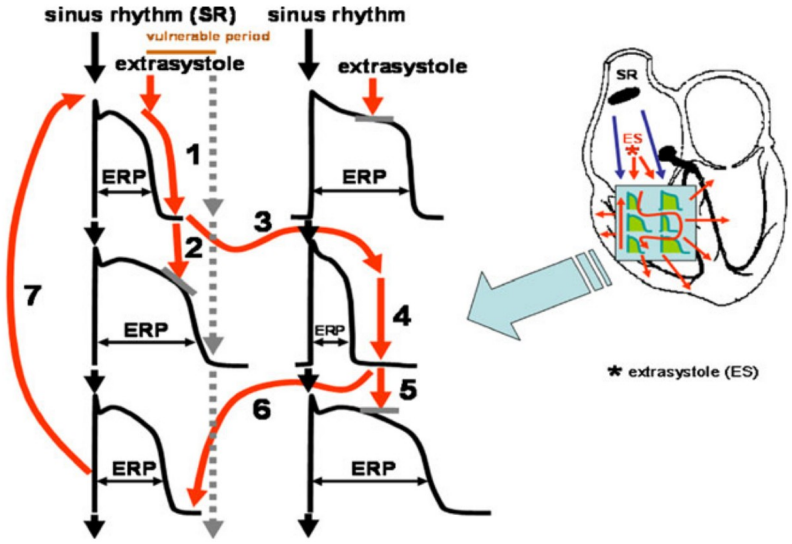

Figure 2 – The propagation of depolarization in the ventricle represented by action potentials. The black arrows represent the propagation of sinus impulses via the physiological conduction pathways. Action potentials with different durations, and therefore, different levels of refractoriness may also allow for an alternate pathway, represented by red arrows. The high dispersion, caused by inhomogeneities in repolarization, allows for an alternate pathway to form (substrate), and an early ectopic impulse (trigger) may travel through this pathway, possibly leading to re-entrant arrhythmia. (Reproduced with permission; Varró and Baczkó, 2010)

APD differences (APD dispersion) transmurally (Cui et al. 1994; Cui et al. 1998). Conversely, drugs that selectively inhibit potassium channels would increase APD and regional APD difference, often leading to an increase in dispersion, consequently increasing the risk of VF formation. *In vivo*, transmural dispersion is derived from QT/QTc or JTc dispersion in the ECG, all of which have been decreased by chronic amiodarone treatment in the clinical setting (Cui et al. 1994; Cui et al. 1998), whilst dofetilide has increased global electrical heterogeneity (Stabenau et al. 2020). Furthermore, such APD prolonging effects are not only common in class III antiarrhythmic drugs, but in non-cardiac drugs as well, such as grepafloxacin, sparfloxacin, astemizole, terfenadine, mesoridazine, cisapride, and levomethadyl; all of which have been withdrawn from the US market due to high

proarrhythmic risk (Roden, 2016). We have also identified the possible proarrhythmic risk of the combined administration of ibuprofen and levofloxacin (Pászti et al., 2019).

Another region characterized by high dispersion is the junction between Purkinje fibers and the ventricular myocardium, also known as the Purkinje-muscle junction. The Purkinje-muscle junction has been described as a contributor to local heterogeneity of ventricular APD (Walton et al., 2014; Martinez et al., 2018). Based on this, if repolarization lengthening in the Purkinje system is markedly stronger than that of the surrounding myocardium at the Purkinje-muscle junction, early afterdepolarization may develop in Purkinje fibers, which under certain conditions can evoke propagating extra beats in the ventricles (Nattel and Quantz, 1988; Varró et al., 1990). While transmural dispersion may be directly measured *ex vivo* and closely estimated *in vivo*, studying the dispersion of repolarization between Purkinje fibers and the myocardium is currently only possible in preparations containing electrotonically well coupled Purkinje fibers and ventricular muscle. Studying the effects of antiarrhytmic agents or agent candidates in such preparations may be beneficial, since decreasing dispersion could be a goal of antiarrhythmic therapy. Screening for drug candidates that do not increase dispersion may also increase cardiac safety. Even though the general electrophysiological effects of dofetilide and amiodarone are well understood, their effect on the dispersion between Purkinje system and the myocardium may only be estimated based on measurements from individual, uncoupled preparations.

The individual susceptibility to the harmful side effects of APD prolonging drugs is determined by inherent, genetic factors, as well as cardiac comorbidities that decrease the activity of repolarizing currents. The effects of APD prolongation can be evaluated in the context of the repolarization reserve (Roden, 1998; Roden, 2006). According to this concept, all available repolarizing currents contribute to a total pool of available repolarization capacity, and if the activation of one current is

decreased, the activity of other currents may increase, at least partially taking over the function of the missing capacity of repolarization. Therefore, under healthy conditions, inhibition of a single potassium current does not lead to significant prolongation in APD and in QTc time. However, even under seemingly healthy conditions, the repolarization reserve may already be exhausted if the activity of a repolarizing current is diminished by some other condition. In the case of the I_{Ks} current, such conditions may include heart failure, cardiac hypertrophy, diabetes, and genetic defects as well (Jost et al., 2007). If the repolarization reserve is exhausted, a slight inhibition of a potassium current may lead to extreme APD prolongation, which dramatically increases the susceptibility of the heart to potentially life-threatening arrhythmias. Therefore, a vulnerable status of cardiac repolarization may arise from any combination of conditions that prolong APD, and thus weaken the repolarization reserve. Nevertheless, this vulnerability without a trigger is unlikely to lead to arrhythmia.

Arrhythmia triggering events and electrical restitution

Extra beats of any origin can be considered as triggering events, possibly leading to VF. Repolarization lengthening in the Purkinje system can also serve as a trigger for arrhythmias; and, in addition, increased dispersion of repolarization itself can also enhance the risk of tachyarrhythmia in the Purkinje-muscle junction as a substrate for arrhythmia (Nogami 2011*a*, 2011*b*).

The timing of these extra beats is critical in terms of arrhythmia risk. The APD/ERP of myocytes is determined by the diastolic interval (DI): the temporal proximity of the preceding beat. The pacing from the sinoatrial node provides a relatively consistent DI between beats; and therefore, the duration of subsequent APs is also relatively consistent.

Extra beats, on the other hand, disrupt this consistency. The APD/ERP of an extrasystole depends on the DI, and as the DIs increase, the APDs/ERPs of the extra beats also increase in human. This process is called electrical restitution (Nolasco and Dahlen, 1968; Boyett and Jewell, 1978; Elharrar and Surawicz, 1983). According to the restitution hypothesis, as DIs increase due to the propagation of an extrasystole, the next following possible extrasystole would encounter a prolonged APD/ERP, therefore a local conduction defect may occur. A steeper/faster restitution curve would favor such an effect, thus it would be considered proarrhythmic. A flattened/slower restitution curve would have the opposite effect.

Cardiac electrophysiological experimentation

The mechanisms and drug-induced alterations of repolarization and restitution have been thoroughly studied in an excessive number of species. Even though the numerous reports contribute to the understanding of repolarization and restitution, drawing direct comparisons between them and estimating their translational value may be rather difficult due to the differences in the cardiac ion channel constitution of each model species.

Some mammalian hearts are more similar to the human heart than others (Varró et al., 2021). While rodents are relatively accessible and can even be genetically modified to explore genetic variability, their electrophysiological properties are deeply different from that of human (Bogeholz et al., 2014).

The resting heart rate of mammals show a strong correlation with their respective bodyweight (Dawson, 2014). The human physiological resting heart rate of 60–70 beats per minute is much slower compared to the 300–500 beats per minute range inherent in rats, while the heart rate of

other commonly utilized animal models are much closer to that of human: dogs, goats, pigs and sheep, 100–150; rabbits and guinea pigs 200–300 beats per minute.

The slower resting heart rate of humans and large animals are also associated with a specific cardiac ventricular action potential morphology, characterized by the spike-and-dome (*notch*) morphology and a long plateau phase, governed by the delicate balance between the open and closed states of calcium and potassium channels. This characteristic is shared across human, dog, and rabbit hearts. The ventricular action potential of the guinea pig does also displays a long plateau phase, but without the notch. Furthermore, when paced at a constant cycle length of 1000 ms, not only the shape, but also the APDs of these species are relatively similar, all falling within the range of 170–280 ms. It should be noted that when paced at their physiological heart rates, the APD of smaller animals may be somewhat shorter. On the other hand, rodent action potentials are much shorter, and are characterized by a triangular morphology with no plateau phase, as their relatively fast heart rate does not allow for lengthy repolarization. This is also reflected in their APD, as it rarely exceeds 90 ms under control conditions.

However, the heart rate may only change the shape and duration of the action potential within a given spectrum. The available spectrum for possible action potential shapes and durations of each species and each individual organism is primarily determined by the ion channel constitution of their hearts. Therefore, the suitability and translational value of each animal model is largely determined by its similarity in cardiac ion channel composition to that of human. In addition, the abundance of the same ion channels and the activation of the respective ion currents can also be different, leading to important differences in repolarization reserve between various species.

One important aim of my PhD thesis is to offer a systematic comparison between the repolarization of the human ventricle and that of the most commonly used model animals in cardiac electrophysiology: the rabbit, the dog, the guinea pig, and the rat. Comparisons were also made in terms of the characteristics of APD restitution in these species, and ion channel inhibitor effects on human restitution curves were also evaluated. Furthermore, the effects dofetilide and amiodarone were also assessed in preparations containing well coupled Purkinje fibers and ventricular muscle in order to uncover additional features of these agents in the context of local dispersion of repolarization.

Materials and Methods

Human General Donor Cardiac Tissue Ethics Statement

Hearts were obtained from general organ donors whose undiseased hearts were explanted to obtain pulmonary and aortic valves for transplant surgery. Before cardiac explantation, organ donors did not receive medication apart from dobutamine, furosemide, and plasma expanders. According to the Hungarian law, the consent of the patients or relatives is not needed to obtain samples from donors. Therefore, consent is waived under local legislation. The investigations conformed to the principles of the Declaration of Helsinki. Experimental protocols were approved by the National Scientific and Research Ethical Review Boards (4991-0/2010-1018EKU [339/PI/010]).

Animals

All experiments 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).

Dogs (Beagle, either sex), rabbits (New Zealand, either sex), guinea pigs (Dunkin Hartley, either sex), rats (Wistar, either sex) were sacrificed (sodium pentobarbital 30 mg/kg, administered intravenously) following sedation (xylazine 1 mg/kg, administered intravenously) and after an intravenous injection of 400 U/kg heparin. Then the heart of each animal was rapidly removed through right lateral thoracotomy, and immediately rinsed in oxygenated modified Locke's solution containing (in mM): NaCl 128.3, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 0.42, $NaHCO_3$ 21.4, and glucose 10. The pH of this solution was set between 7.35 and 7.4 when gassed with the mixture of 95% O_2 and 5% CO_2 at 37 °C.

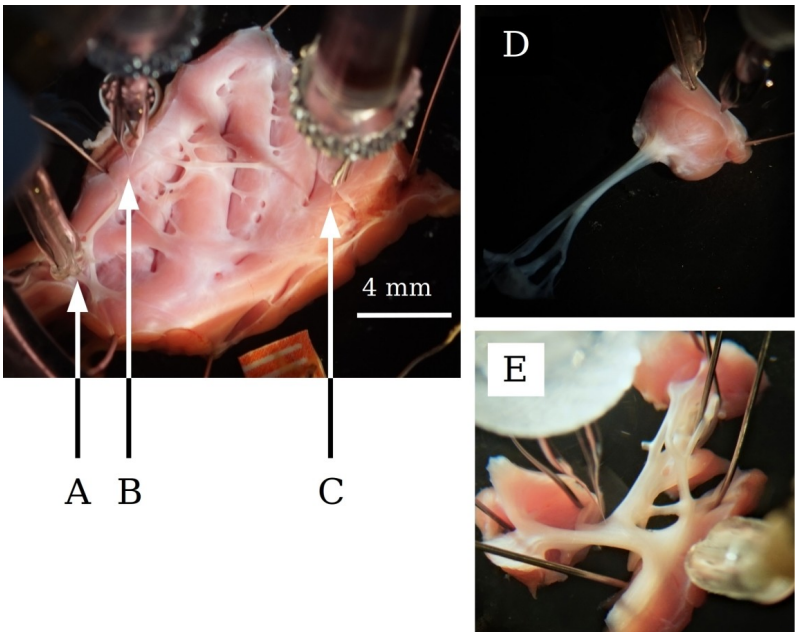

Figure 3 – Photographs of an electrotonically coupled ventricular preparation (A, pacing microelectrode; B, microelectrode impaled in Purkinje fiber; C, microelectrode impaled in ventricular muscle); an uncoupled papillary muscle (D); and an uncoupled Purkinje fiber (E) inside the tissue bath.

Preparations (Figure 3) were individually mounted in a tissue chamber with the volume of 50 ml containing modified Locke's solution, gassed with the mixture of 95% O_2 and 5% CO_2. Stimulation was executed using a pair of platinum electrodes in contact with the preparation using rectangular current pulses of 0.5–2 ms duration. Electrotonically coupled preparations (Figure 3A–C) were paced from a Purkinje fiber, mimicking physiological cardiac conduction. These stimuli were delivered at a constant cycle length of 1000 ms for at least 60 min allowing the preparation to equilibrate before the measurements were initiated. Transmembrane potentials were recorded using conventional glass microelectrodes (simultaneously in the Purkinje fiber and the ventricular muscle regions of coupled preparations), 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) which was coupled to a dual beam oscilloscope. The resting potential (RP), action potential amplitude (APA), maximum upstroke velocity (dV_{max}), and APD measured at 90% of repolarization (APD_{90}) were online monitored and offline recorded using a home-made software (APES) running on a computer equipped with an ADA 3300 analog-to-digital data acquisition board (Real Time Devices, Inc., State College, Pennsylvania) having a maximum sampling frequency of 40 kHz. Stimulation with a constant cycle length of 1000 ms was applied in the course of all experiments, and with a cycle length of 200 ms was applied in select rat experiments. Stimulation with different constant cycle lengths ranging from 300 to 5000 ms was also applied. To determine the recovery kinetics of APD_{90} (APD_{90} restitution), extra test action potentials were elicited by using single test pulses (S2) in a preparation driven at a basic cycle length of 1000 ms. The S1–S2 coupling interval (DI) was increased progressively from the end of the refractory period. The diastolic intervals preceding the test action potential were measured from the point corresponding to 90% of repolarization of the preceding basic beat to the upstroke of the test action potential and were increased progressively. In electrotonically coupled and uncoupled preparations, dispersion of repolarization was inferred from the difference in the APD_{90} value of Purkinje fibers

and ventricular muscles, referred to as ΔAPD_{90}. Attempts were made to maintain the same impalement throughout each experiment. In case an impalement became dislodged, adjustment was attempted, and if the action potential characteristics of the re-established impalement deviated by less than 5% from the previous measurement, the experiment continued (Lengyel et al., 2001; Jost et al., 2005; Orvos et al., 2019). All measurements were carried out at 37°C.

Current	**Compound**	**Concentration**
Rapid delayed rectifier potassium current (I_{Kr})	Dofetilide	50 nM
	Sotalol	1 μM
	E-4031	30 μM
Slow delayed-rectifying potassium current (I_{Ks})	HMR-1556	500 nM
	L735-821	100 nM
Transient outward current (I_{to})	Chromanol-293B	100 μM
Inward rectifier potassium current (I_{K1})	$BaCl_2$	10 μM
Ultrarapid delayed rectifier potassium current (I_{Kur})	XEN-D0101	1 μM
Sodium current (I_{Na})	TTX	2 μM
	Mexiletine	10 μM
L-type calcium current ($I_{Ca\text{-}L}$)	Nisoldipine	1 μM
Sodium–calcium exchanger (NCX)	ORM-10103	3 μM

Table 1 – Compounds and their concentrations used to inhibit various ion currents.

Ion channel inhibition

The various ion channel inhibiting agents and their corresponding concentrations were carefully chosen to achieve a significant degree of inhibition of the given current but maintaining a sufficient degree of selectivity. The drugs and their concentrations for each ion current are shown in Table 1, these drugs were administered directly to the tissue bath during the experiments. In select experiments, dogs were orally treated with amiodarone (50 mg/kg/day, 4 weeks).

Data analysis

The parameters of the action potentials were determined using a home-made software tool (ActionPytential). Statistical analysis was performed in RStudio, and the figures were produced in Veusz. All data are expressed as means ± standard error of the mean (SEM). The "n" number refers to the number of experiments. The homoscedasticity of each variable was checked using Bartlett's test, and the normality of their distribution was checked using the Shapiro-Wilk test.

Data points of restitution curves were fitted by a mono-exponential function in order to calculate the kinetic time constant of the APD_{90} restitution process:

$$APD = APD_{ss} - A^{-DI/\tau}$$

where APD_{ss} is the maximal APD (measured as APD_{90}), A is the amplitude of the exponential function, DI is the diastolic interval, and τ is the time constant.

The effect of each drug at basic cycle length in each species was evaluated by Student's t-test for paired data. In the case of species-wise comparison, the changes in APD were expressed as percentages compared to the control value for each preparation. These percentages were then compared for each species by ANOVA. APD_{90} values of coupled and uncoupled preparations were compared using Student's t-test for independent samples (amiodarone) and for paired samples (dofetilide). The results were considered statistically significant when p was <0.05.

Results

All preparations presented in this work were characterized by baseline parameters within the range that has been historically considered physiological for the preparation type from the given species. The APD was defined as the action potential duration measured at 90% of repolarization (APD_{90} value). Thus, effects referred to as changes in APD were measured as changes in APD_{90}.

Selective inhibition of ion currents at constant 1000 ms pacing

Inhibition of the rapid delayed-rectifying potassium current

Selective inhibition of the rapid delayed-rectifying potassium current (I_{Kr}) by 50 nM dofetilide induced marked and significant prolongation of the APD in human and rabbit ventricular preparations (Figure 4, Table 2). On the other hand, the APD of rat preparations remained unchanged after the application of 50 nM dofetilide.

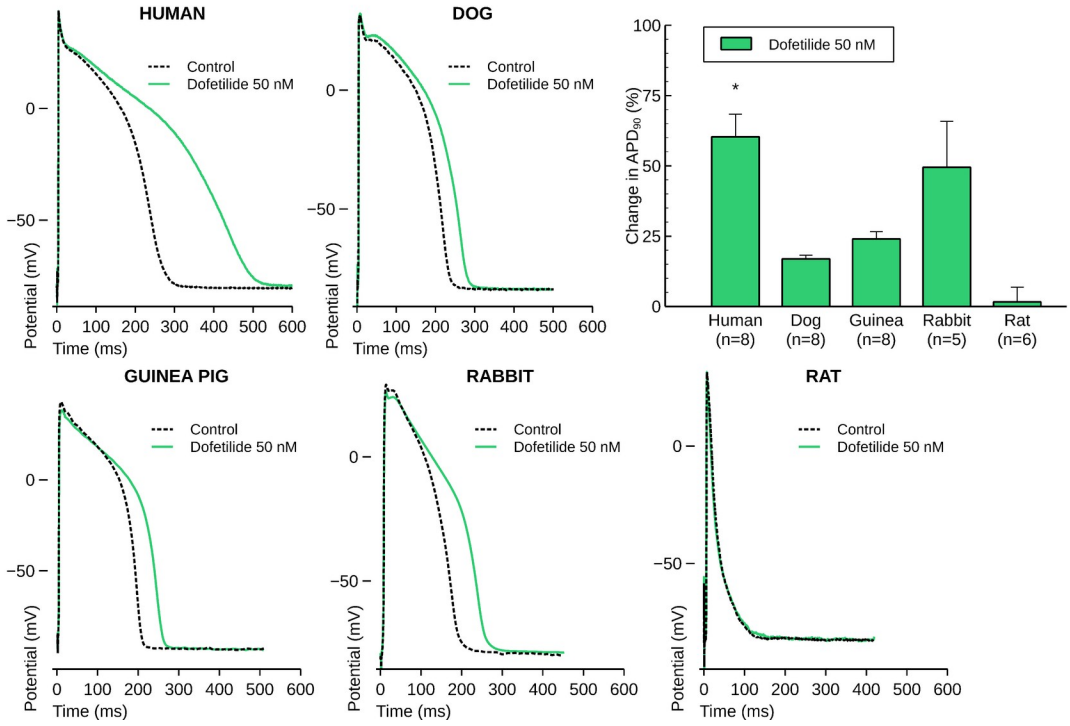

Figure 4 – Representative action potential traces from ventricular preparations showing that dofetilide (50 nM) did not affect rat repolarization, but markedly prolonged action potential duration in human and rabbit; top right corner: bar diagram showing the change in APD expressed as action potential duration at 90% repolarization (APD_{90}) in percentage (%). *p<0.05; ANOVA followed by Bonferroni's post-hoc test; the effect of dofetilide was found to be significantly more pronounced in human compared to all other species.

In dog and guinea pig preparations, moderate but statistically significant APD prolongation was elicited. The extent of prolongation was found to be significantly more pronounced in human preparations when compared to the effect in the other species (p<0.05, Figure 4). Since rats are

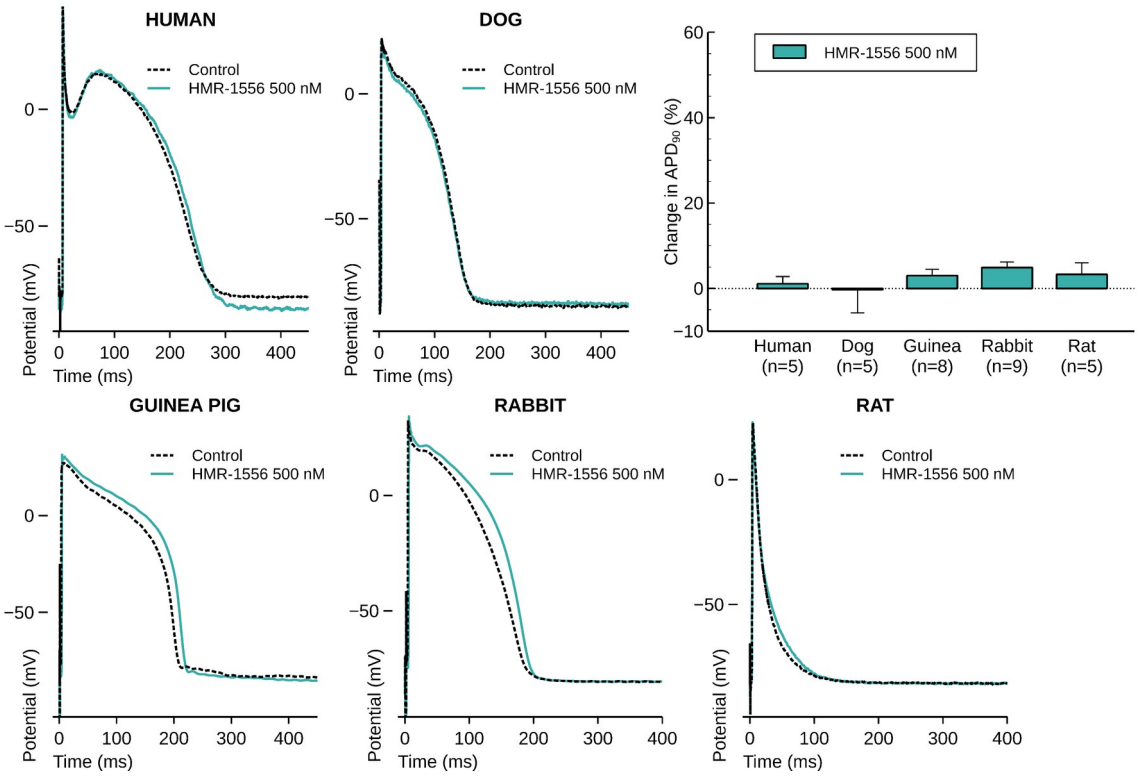

Figure 5 – Representative action potential traces from ventricular preparations showing that HMR-1556 (500 nM) elicited slight prolongation in the repolarization of guinea pig, rabbit and dog, but did not alter it in human and dog; top right corner: bar diagram showing the change in APD expressed as action potential duration at 90% repolarization (APD_{90}) in percentage (%).

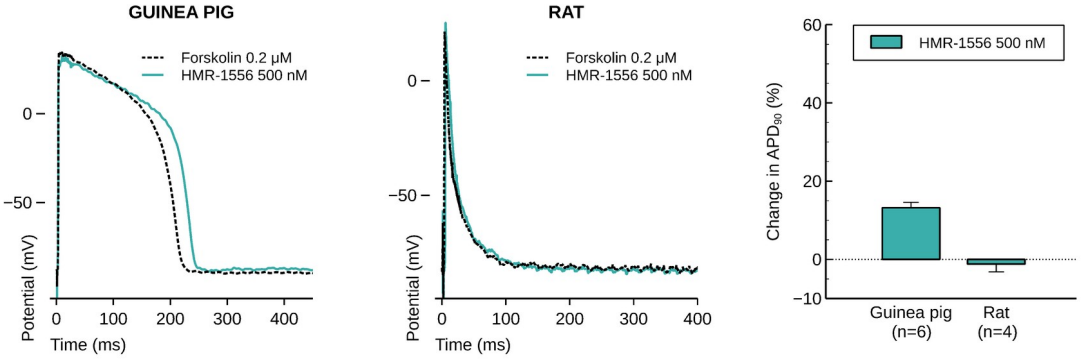

Figure 6 – Representative action potential traces from ventricular preparations showing that HMR-1556 (500 nM) elicited marked prolongation in the repolarization of guinea pig when applied after forskolin (0.2 µM), but no such effect was elicited in rat; right panel: bar diagram showing the change in APD expressed as action potential duration at 90% repolarization (APD_{90}) in percentage (%).

characterized by a considerably faster heart rate compared to the other species, we studied the effect of dofetilide at a fast pacing rate as well. Decreasing the pacing cycle length to 200 ms did not change the lack of prolongation in rat preparations (0.4±1.8%, n=3).

Inhibition of the slow delayed-rectifying potassium current

Inhibiting the slow delayed-rectifying potassium current (I_{Ks}) by 500 nM HMR-1556 elicited slight but statistically significant prolongation of the APD in rabbit ventricular preparations (Table 2), but not in other tissue types (Figure 5). It should be noted that the activity of the I_{Ks} current is considered to be modulated by the tissue level of cAMP. In guinea pig preparations, applying HMR-1556 elicited prolongation only in 3/9 cases (7.6±0.9 ms on average), but had no effect in 5/8 cases (0.2±0.9 ms on average). However, applying HMR-1556 after the cAMP level of guinea pig preparations had been increased by administering 0.2 μM forskolin, the inhibition of I_{Ks} lead to significant APD prolongation (Figure 6). The same effect could not be reproduced in dog, human, and rat preparations. As with dofetilide, HMR-1556 did not change the APD at fast, 200 ms cycle length pacing in rats.

Inhibition of the transient outward current

We applied chromanol-293B in 100 μM concentration to inhibit the transient outward current (I_{to}). Since chromanol-293B also inhibits I_{Ks}, it was administered after the full inhibition of I_{Ks} by 500 nM HMR-1556. Therefore, the effects elicited by chromanol-293B were due to the inhibition of I_{to}.

Inhibition of I_{to} induced moderate and statistically significant APD prolongation in rat, dog, and rabbit preparations, but did not change APD in a significant manner in guinea pig preparations (Table 2, Figure 7). In human ventricular muscle, phase 1 rapid repolarization (*notch* or *spike-and-*

dome morphology) was suppressed after I_{to} inhibition (Figure 7), and the APD was abbreviated in a slight but statistically significant manner (Table 2).

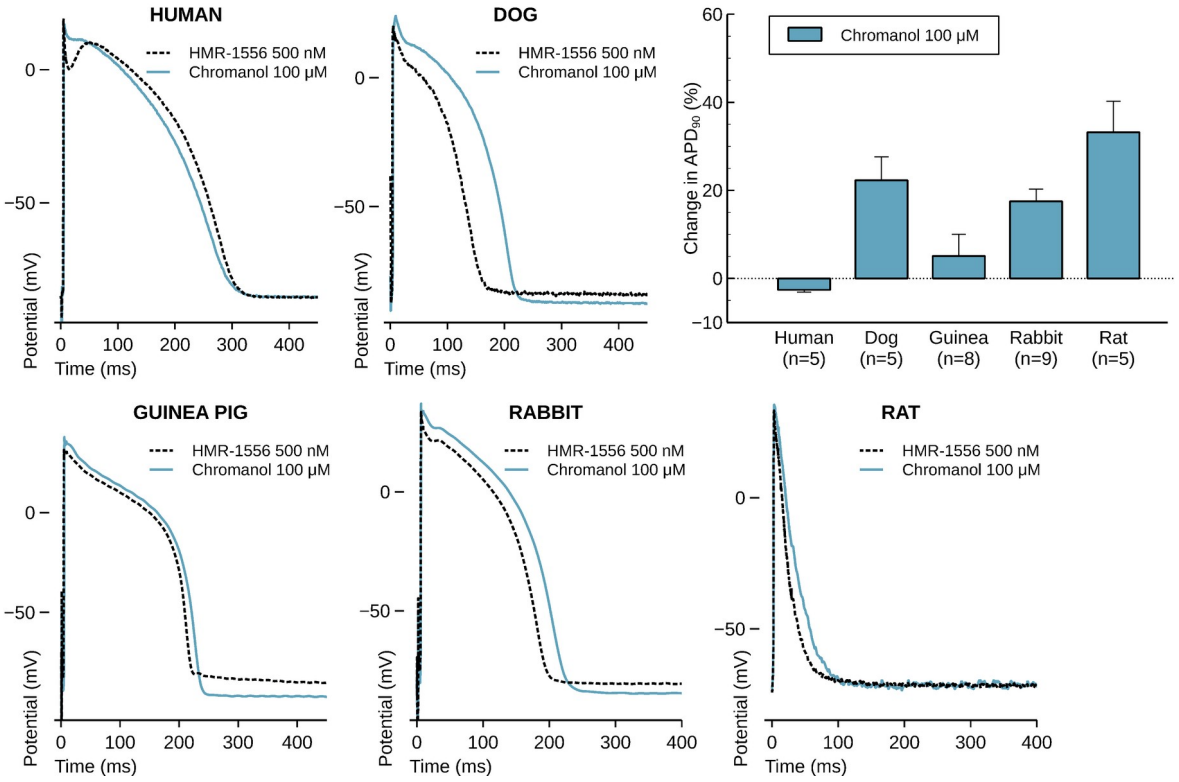

Figure 7 – Representative action potential traces from ventricular preparations showing that chromanol-293B (100 µM) elicited slight APD prolongation in the repolarization of guinea pig, and considerable prolongation in dog, rabbit and rat, but slightly abbreviated APD in human; top right corner: bar diagram showing the change in APD expressed as action potential duration at 90% repolarization (APD_{90}) in percentage (%).

Inhibition of the inward rectifier potassium current

$BaCl_2$ in 10 µM concentration was applied for selective inhibition of the inward rectifier potassium current (I_{K1}). This concentration of $BaCl_2$ did not elicit full I_{K1} block, but further elevation of its concentration would have also inhibited I_{to}. In human preparations, I_{K1} inhibition elicited no significant changes in APD. Slight prolongation was observed in dog, rabbit and guinea pig preparations. In contrast, marked prolongation was observed in rat preparations (Figure 8, Table 2).

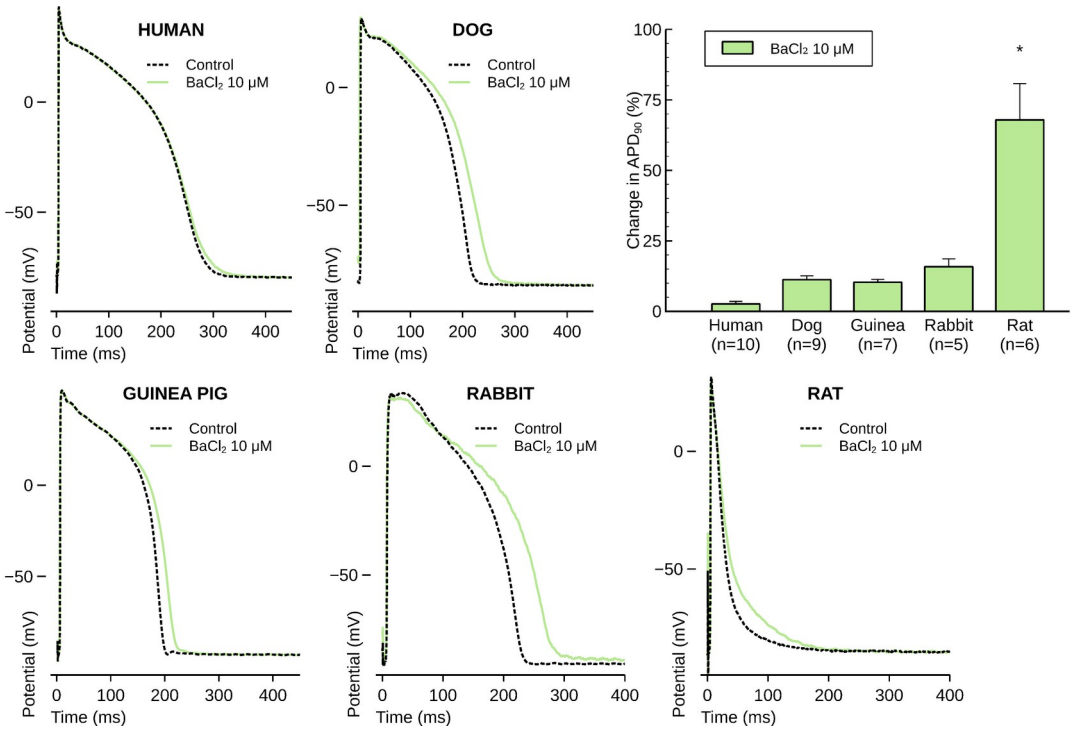

Figure 8 – Representative action potential traces from ventricular preparations showing that $BaCl_2$ (10 µM) significantly prolonged rat repolarization in, but elicited only slight changes in human and guinea pig, and moderate prolongation dog and rabbit; top right corner: bar diagram showing the change in APD expressed as action potential duration at 90% repolarization (APD_{90}) in percentage (%). *p<0.05; ANOVA followed by Bonferroni's post-hoc test; the effect of $BaCl_2$ was found to be significantly more pronounced in rat compared to all other species.

APD_{90} (ms) Species		Dofetilide 50 nM	HMR-1556 500 nM	Chromanol-293B 100 µM	BaCl2 10 µM	XEN-D0101 1 µM
Human	Control	316.4±25.1	271.0±8.8	273.7±7.9	294.6±21.4	300.4±21.0
	Drug	497.5±26.1*	273.7±7.9	266.5±7.5*	302.1±21.5	303.4±21.8
Dog	Control	232.2±6.4	184.2±16.3	182.2±15.6	205.3±3.9	219.2±15.8
	Drug	271.2±6.8*	182.2±15.6	219.6±8.7*	228.5±6.0*	228.7±16.5
Guinea pig	Control	204.7±6.6	193.5±9.4	198.6±7.9	202.8±8.7	203.2±10.8
	Drug	253.3±8.0*	198.6±7.9	206.8±8.2	223.8±10.1	208.6±12.4
Rabbit	Control	174.1±4.3	185.2±8.1	194.3±9.1	221.1±12.1	202.4±2.9
	Drug	259.8±28.9*	194.3±9.1*	227.1±8.1*	255±10.6*	213.2±5.6
Rat	Control	61.1±9.3	60.9±4.5	63.9±5.9	63.6±6.5	50.7±4.4
	Drug	60.4±7.8	63.9±5.9	89.5±10*	103.8±7.4*	68.2±5.8*

Table 2 – Changes in the action potential duration (expressed as APD_{90}) elicited by potassium current inhibiting agents in dog, human, guinea pig, rabbit, and rat preparations. *p<0.05, Student's t-test for paired values.

Inhibition of the ultrarapid delayed rectifier current

Selective inhibition of the ultrarapid delayed rectifier current (I_{Kur}) was performed by applying 1 µM XEN-D101. This chosen concentration of XEN-D101 did not elicit full I_{Kur} block; however, further elevation of its concentration would have inhibited I_{to} as well (Ford et al., 2013). The APD of rat preparations was markedly and significantly prolonged (Figure 9, Table 2). In contrast, APD in all ventricular muscle preparations obtained from the other species remained unchanged after the application of XEN-D101.

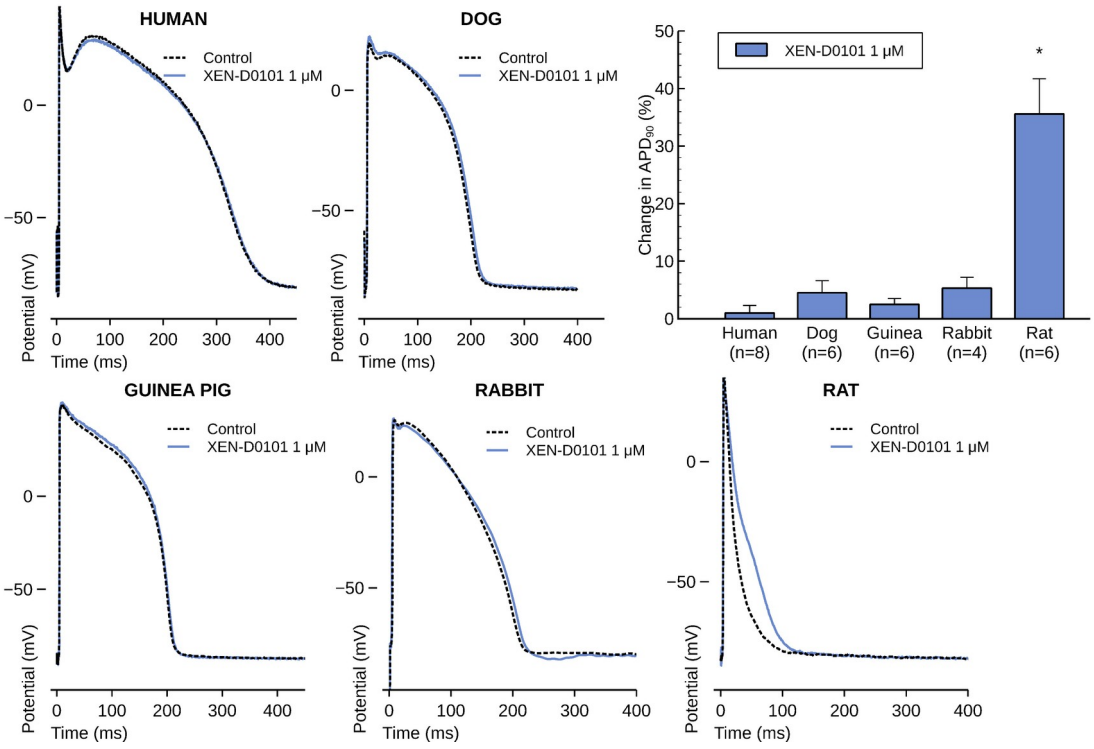

Figure 9 – Representative action potential traces from ventricular preparations showing that XEN-D0101 (1 µM) significantly prolonged rat repolarization, but elicited only slight to no change in human, dog, rabbit and guinea pig; top right corner: bar diagram showing the change in APD expressed as action potential duration at 90% repolarization (APD_{90}) in percentage (%). *$p<0.05$; ANOVA followed by Bonferroni's post-hoc test; the effect of XEN-D0101 was found to be significantly more pronounced in rat compared to all other species.

Inhibition of the sodium current

Tetrodotoxin (TTX) was applied in 2 μM concentration to selectively inhibit the sodium current (I_{Na}). This elicited a significant abbreviation in human ventricular preparations, and slight abbreviation in dog, guinea pig and rat preparations (Figure 10, Table 3). The effect was found to be more pronounced in human preparations compared to the model species ($p<0.05$).

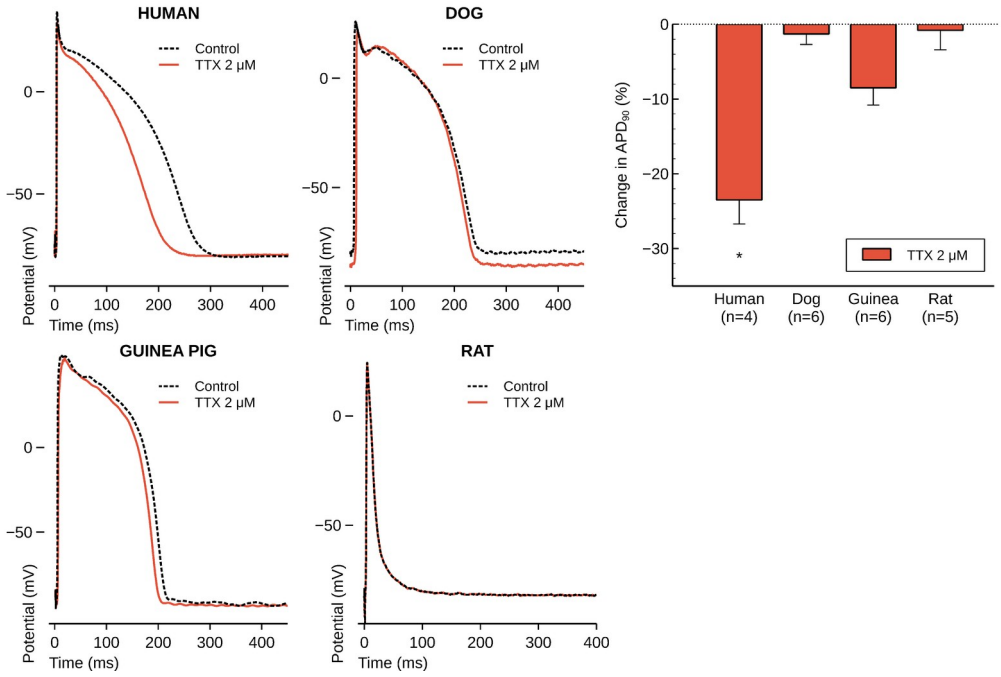

Figure 10 – Representative action potential traces from ventricular preparations showing that tetrodotoxin (TTX; 2 μM) significantly abbreviated human repolarization, but elicited only slight to no change in dog, rabbit, guinea pig, and rat; top right corner: bar diagram showing the change in APD expressed as action potential duration at 90% repolarization (APD_{90}) in percentage (%). *$p<0.05$; ANOVA followed by Bonferroni's post-hoc test; the effect of TTX was found to be significantly more pronounced in human compared to all other species.

Inhibition of the Na^+–Ca^{2+}-exchanger

Inhibition of the Na^+–Ca^{2+}-exchanger (NCX) by ORM-10103 elicited significant abbreviation in rat preparations, and slight abbreviation in guinea pig preparations (Figure 11). On the other hand, ORM-10103 had mixed effects in human and dog preparations: in some cases it elicited

prolongation, but abbreviated APD_{90} in others. The effect in rat preparations was found to be significantly different in rat preparations compared to the other species ($p<0.05$; Table 3).

Inhibition of the L-type calcium current

The $I_{Ca\text{-}L}$ current was inhibited by applying 1 μM nisoldipine, eliciting significant abbreviation in human and guinea pig preparations, and slight abbreviation in dog and rat preparations (Figure 12, Table 3). No significant difference was found in the effect between the species.

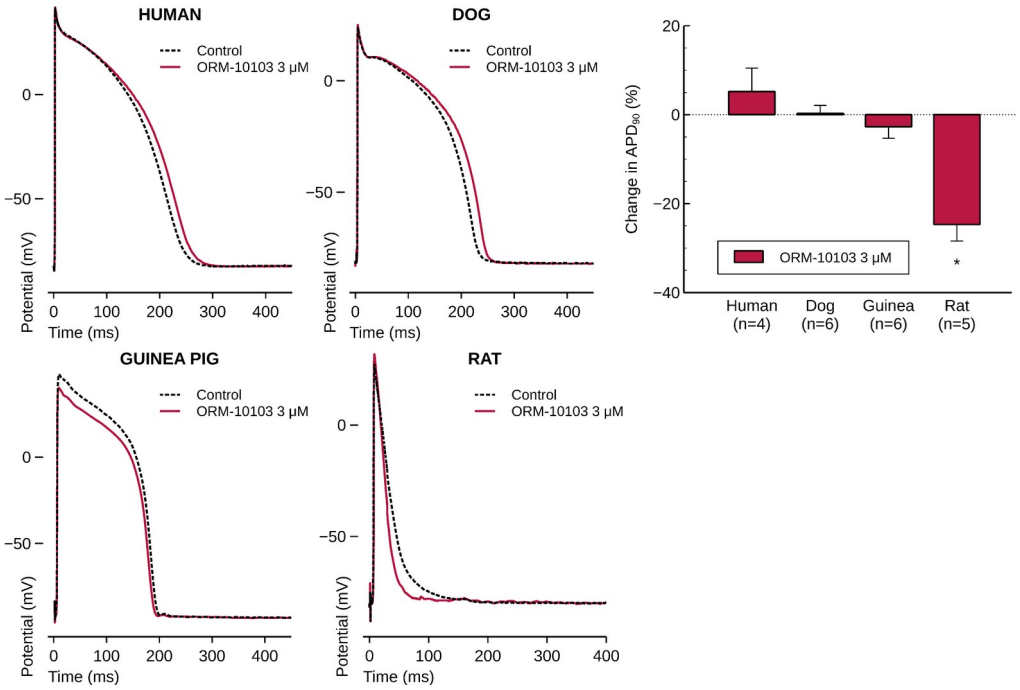

Figure 11 – Representative action potential traces from ventricular preparations showing that ORM-10103 (3 μM) significantly abbreviated rat repolarization, but elicited only slight abbreviation in guinea pig and slight to no prolongation in human and dog preparations; top right corner: bar diagram showing the change in APD expressed as action potential duration at 90% repolarization (APD_{90}) in percentage (%). *$p<0.05$; ANOVA followed by Bonferroni's post-hoc test; the effect of ORM-10103 was found to be significantly more pronounced in rat compared to all other species.

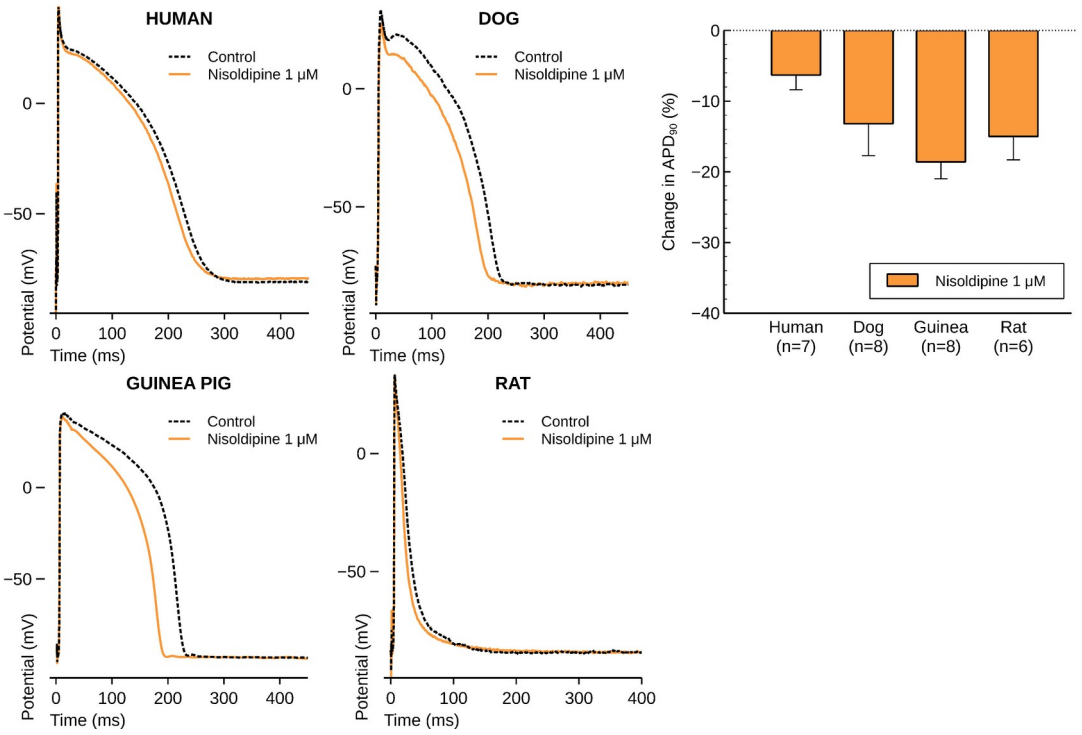

Figure 12 – Representative action potential traces from ventricular preparations showing that nisoldipine (1 μM) elicited similar levels of abbreviation in human, dog, rabbit, guinea pig, and rat preparations; top right corner: bar diagram showing the change in APD expressed as action potential duration at 90% repolarization (APD_{90}) in percentage (%).

APD_{90} (ms)		Mexiletine 10 μM	TTX 2 μM	Nisoldipine 1 μM	ORM-10103 3 μM
Human	Control	280±26.4	298.7±28.2	262.6±10.3	233.4±19.7
	Drug	266.9±26.4	227.4±19.0*	245.5±9.4*	242.3±12.8*
Dog	Control	249.4±9.1	208.7±6.8	215.3±14.5	233.4±19.7
	Drug	246.5±8.5	205.6±4.5	184.1±5.7	242.3±12.8
Guinea pig	Control	190.8±19.2	203.7±3.9	234.4±14.3	207.2±10.7
	Drug	179±18*	186.5±6.7*	189.4±9.7*	202.5±13.7
Rat	Control	181.6±17.6	47.9±9.3	44.9±2.0	62.6±5.5
	Drug	160.1±17.5*	47.1±9.0	38.5±3.2*	47.9±5.9*

Table 3 – Changes in the action potential duration (expressed as APD_{90}) elicited by potassium current inhibiting agents in dog, human, guinea pig, rabbit, and rat preparations. *$p<0.05$, Student's t-test for paired values.

Frequency-dependent changes in APD (variable cycle length pacing)

The frequency-dependent changes in APD were compared in human, dog, rabbit, guinea pig and rat preparations in the range of 300–5000 ms cycle lengths, the cycle length was dynamically increased in this protocol. In human and dog, APD consistently increased with the cycle length (Figure 13, left panel). Rabbit and guinea pig preparations showed a different pattern: APD slightly increased up to 1000 ms cycle length, but showed a declining slope at longer cycle lengths. The opposite was found in rat preparations, as APD decreased up to 1000 ms cycle length, and showed a consistent increase with the increase of the cycle length afterwards.

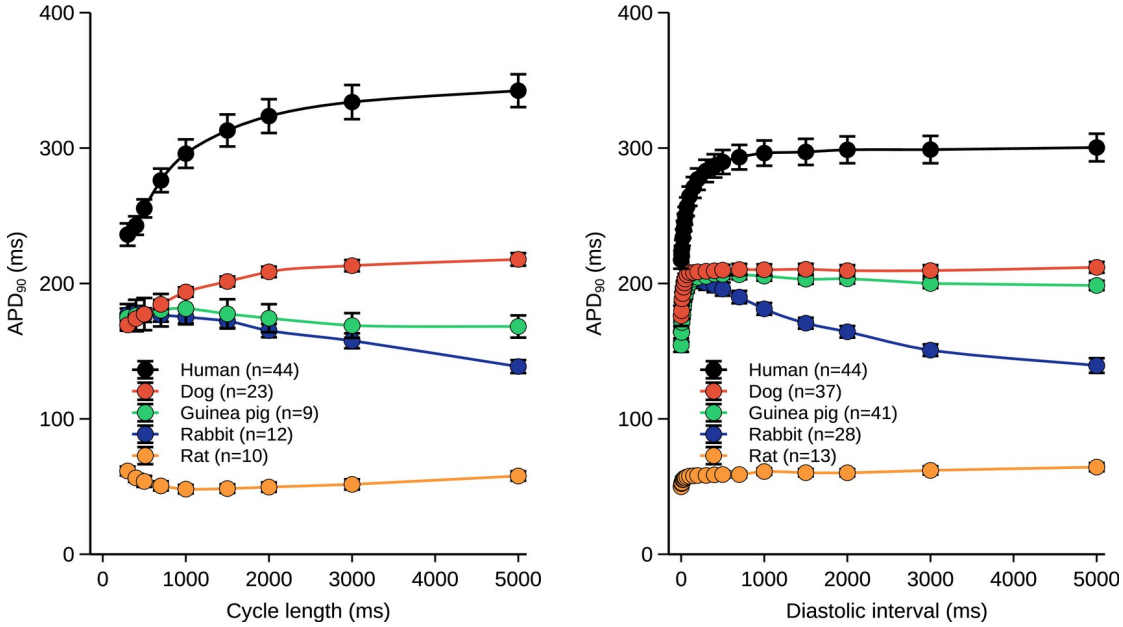

Figure 13 – Action potential duration (APD_{90}) restitution curves (right panel) and the steady-state cycle length dependence of the action potential duration (left panel) in human, dog, guinea pig, rabbit, and rat right ventricular muscle preparations.

Frequency-dependent changes in APD (extrasystolic stimulation protocol)

Frequency dependent APD changes in these species were also evaluated using the standard restitution protocol: constant steady-state (S1–S1) pacing at 1000 ms with abrupt changes of cycle

lengths (S1–S2). The APD values in the corresponding figures (Figures 13–17) were taken from the APs evoked by the S2 stimuli. Human, dog, and guinea pig preparations showed similar APD restitution when comparing their restitution curves up to 5000 ms DI (Figure 13, right panel).

Even though the dog and the guinea pig were characterized by shorter overall APD, their kinetic of rapid increase in APD up to ~500 ms DI, followed by nearly unchanged APD to the end of the restitution curve at 5000 ms was shared with that of human. However, their time constants (τ) showed marked differences: τ=104.7 ms in human, τ=29.1 ms in dog, and τ=37.8 ms in guinea pig. Rabbit preparations showed an overall different restitution curve, as the rapid increase up to 300 ms DI was followed by a prominent abbreviation of APD at longer DI values. Rat preparations showed a slight increase in APD up to 100 ms DI, but from that point, APD remained unchanged. The shape of this restitution curve resembled that of larger animals, but the range between the shortest and longest APD values was seemingly limited (from 49.8 ms±1.0 ms to 64.38±3.0 ms) when compared to those of the guinea pig (from 154 ms±5.0 ms to 205.4±3.4 ms) or the dog (from 176.1 ms±7.5 ms to 211.9±4.0 ms). However, the average relative increase was quite similar between the rat, guinea pig and dog, at 33%, 30%, and 20% respectively.

In the case of human, dog, and guinea pig preparations, subgroups were formed based on the APD_{90} values of the preparations at the basic cycle length of 1000 ms (Figure 14). In human, dog, and rat preparations, marked differences were found both in the electrical restitution curves. In human preparations with APD_{90} shorter than 250 ms, τ was 63.9 ms (n=10), while in preparations with APD_{90} longer than 300 ms, τ was 125.5 ms (n=17). In a similar manner, dog preparations with APD_{90} shorter than 200 ms τ was 19.9 ms (n=13), while in preparations with APD_{90} longer than 210 ms, τ was 53.3 ms (n=14). In the case of rat preparations, τ was 12.7 ms in preparations with APD_{90} shorter than 60 ms, while 194.8 ms in preparations with APD_{90} longer than 60 ms (n=6 and

n=7, respectively). On the other hand, guinea pig preparations showed very similar τ values of 36.2 ms and 37.8 ms both in preparations with APD_{90} shorter than 200 ms (n=17) and in preparations with APD_{90} longer than 210 ms (n=19) respectively.

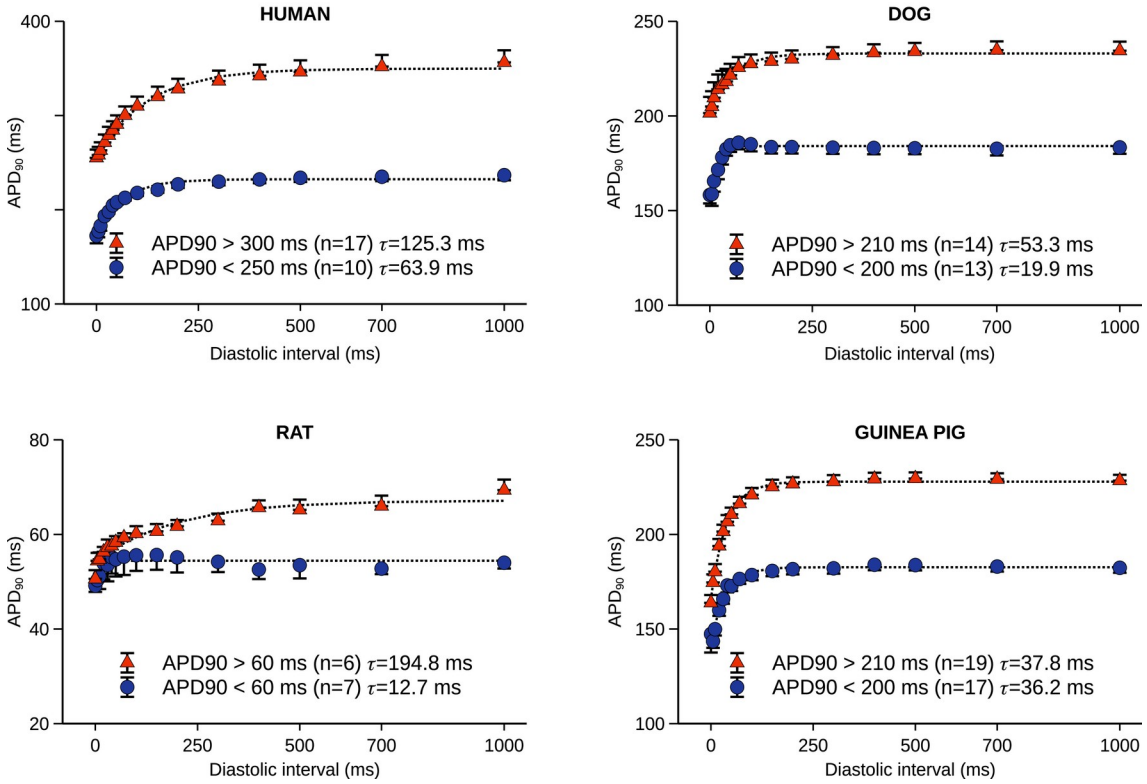

Figure 14 – Preparations with relatively longer action potential durations (APD_{90}) were characterized by slower time constants (τ) in the case of human, dog, and rat, but not in guinea pig. The data points up to 1000 ms diastolic interval were fitted by single exponential function.

Ion channel inhibitor effects on human APD restitution

In further experiments, the effects of several antiarrhythmic drugs were studied on the electrical restitution curves in human undiseased ventricular muscle preparations. Figure 16 shows that selective I_{Kr} inhibitors E-4031 and sotalol increased overall APD and slowed the kinetics of the restitution curve (from τ=82.6 ms to τ=160.3 ms, n=5; and from τ=95.8 ms to τ=152.7 ms, n=5, respectively). Figure 17 illustrates that L-735,821, a specific inhibitor of the I_{Ks} current did not influence APD and electrical restitution curves (τ=113.1 ms vs. τ=111.9 ms, n=7). In further

experiments, the effects of the I_{Na} inhibitor mexiletine and the $I_{Ca\text{-}L}$ inhibitor nisoldipine were studied on human ventricular electrical restitution curves. Figure 18 shows that both mexiletine and nisoldipine shortened APD but only mexiletine slowed restitution kinetics in human ventricular muscle preparations (from τ=98 ms to τ = 133.2 ms, n=6; from τ = 111.1 ms to τ = 113.1 ms, n=6, respectively).

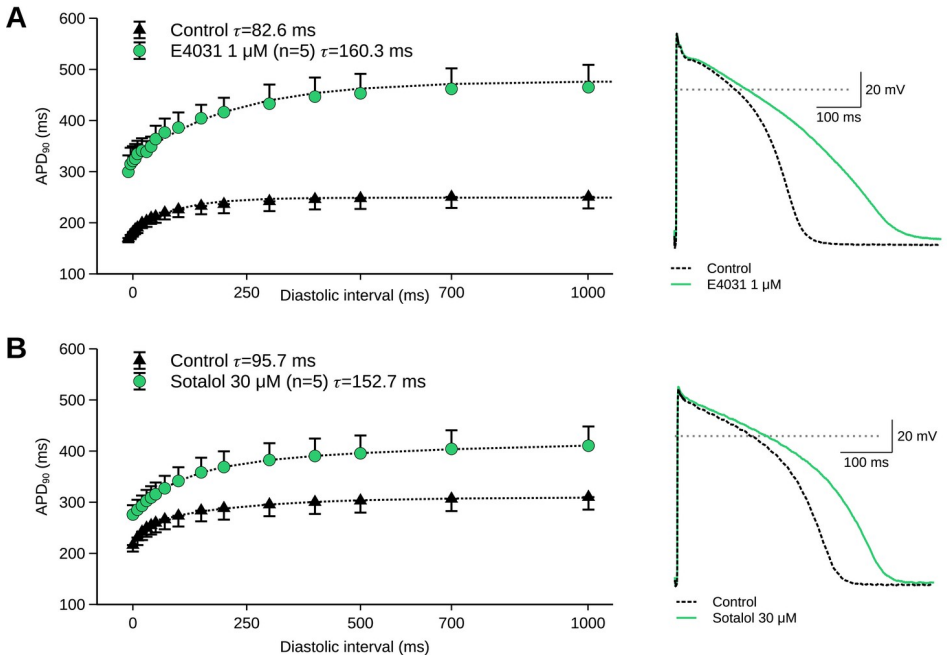

Figure 16 – E-4031 (panel A) and sotalol (panel B) increased the time constants (τ) of human ventricular muscle preparations. The data points up to 1000 ms diastolic interval were fitted by single exponential function. On the right part of the figure original action potential traces are shown before and after drug application at basic cycle length of 1000 ms.

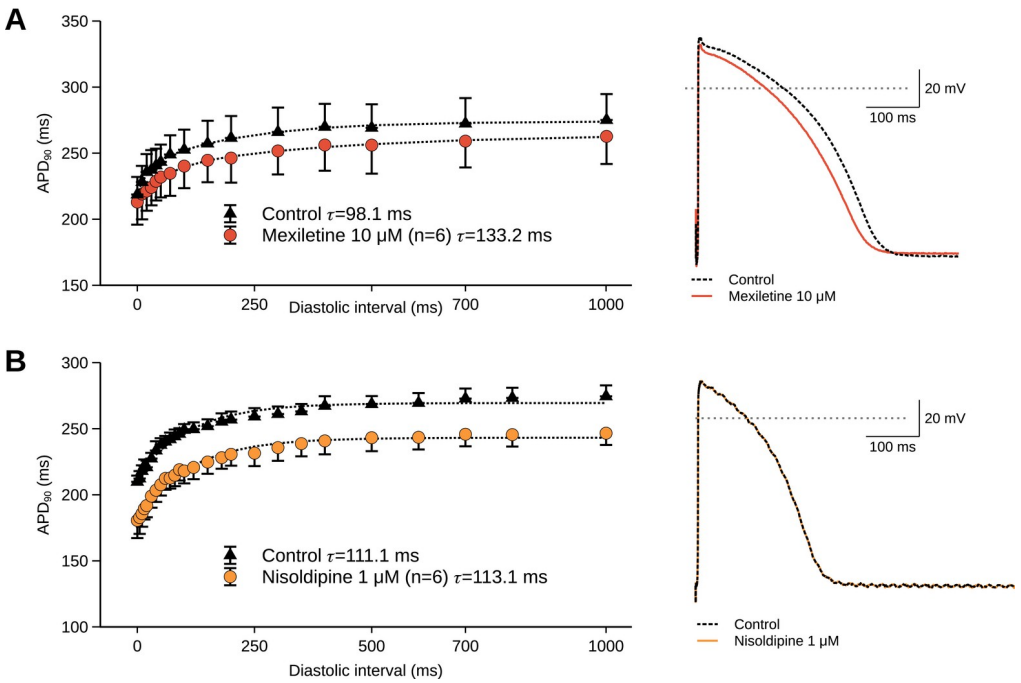

Figure 17 – Mexiletine increased (panel A) and nisoldipine (panel B) did not change the time constants (τ) of human ventricular muscle preparations. The data points up to 1000 ms diastolic interval were fitted by single exponential function. On the right part of the figure original action potential traces are shown before and after drug application at basic cycle length of 1000 ms.

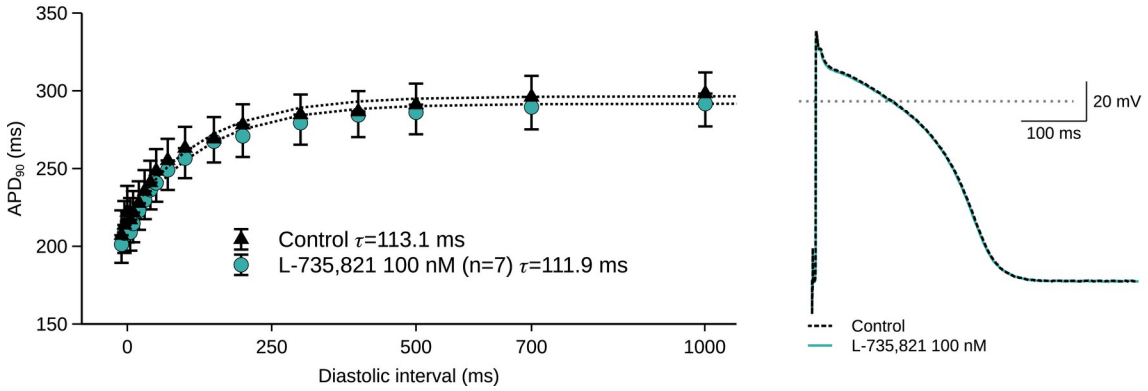

Figure 18 – L735,721 did not change the time constants (τ) of human ventricular muscle preparations. The data points up to 1000 ms diastolic interval were fitted by single exponential function. On the right part of the figure original action potential traces are shown before and after drug application at basic cycle length of 1000 ms.

Comparison of amiodarone and dofetilide effects on Purkinje-muscle dispersion of repolarization

Baseline electrophysiology of electrotonically coupled and uncoupled preparations

In electrotonically coupled control preparations (Figures 19 and 20), most action potential characteristics both of Purkinje fibers and the ventricular muscle were comparable to those of individual (uncoupled; i. e., separate ventricular muscle and Purkinje fiber) preparations (n=21). The experimental settings of coupled and uncoupled preparations are shown in Figure 3. Ventricular APD was slightly longer in coupled preparations compared to the uncoupled ones, while the APD of Purkinje fibers was slightly shorter when coupled with the ventricular muscle. The action potential amplitude, the dV_{max}, and the resting potential values were similar to those of uncoupled (individual) preparations. Baseline dispersion (ΔAPD_{90}) was 39.6 ± 4.0 ms (pooled controls; n=21). Conduction time to Purkinje fibers was shorter in all preparations than that of ventricular muscles, confirming an anterograde wave of depolarization. Since individual, electrotonically uncoupled preparations were not necessarily taken from the same heart and were not in connection, differences in their APD cannot be directly measured, but average values showed an APD difference of 72.8 ms between the two groups of preparations under control circumstances (pooled controls; n=13, 16).

The effects of amiodarone

In the Purkinje fibers of the electrotonically coupled preparations (Figure 3A–C) obtained from animals after chronic amiodarone treatment, the APD_{90} value was increased ($p<0.01$; Figures 19 and 21), while the early phases of repolarization remained unchanged. In the ventricular muscle, prolongation was measured in all stages of repolarization, from APD_{10} to APD_{90} ($p<0.01$). The prolongation of APD in the ventricle was more pronounced, thus ΔAPD_{90} was substantially decreased (to 18.0±5.0 ms from 45.7±5.7 ms in control, $p<0.01$, Figure 20).

In uncoupled Purkinje preparations, APD_{90} was increased ($p<0.05$), the prolongation was more pronounced than in coupled preparations (Figure 18). In uncoupled ventricular preparations, the APD_{90} was also increased ($p<0.01$), although this change was less pronounced compared to the ventricular potentials from coupled preparations. These effects indicate an important difference between coupled and uncoupled preparations in their response to the chronic amiodarone treatment: amiodarone increased APD in uncoupled preparations without altering the local dispersion of repolarization between Purkinje fibers and ventricular muscle. On the contrary, in coupled preparations, amiodarone increased APD in such a manner that resulted in a significant decrease in the local dispersion of repolarization in the Purkinje-muscle junction.

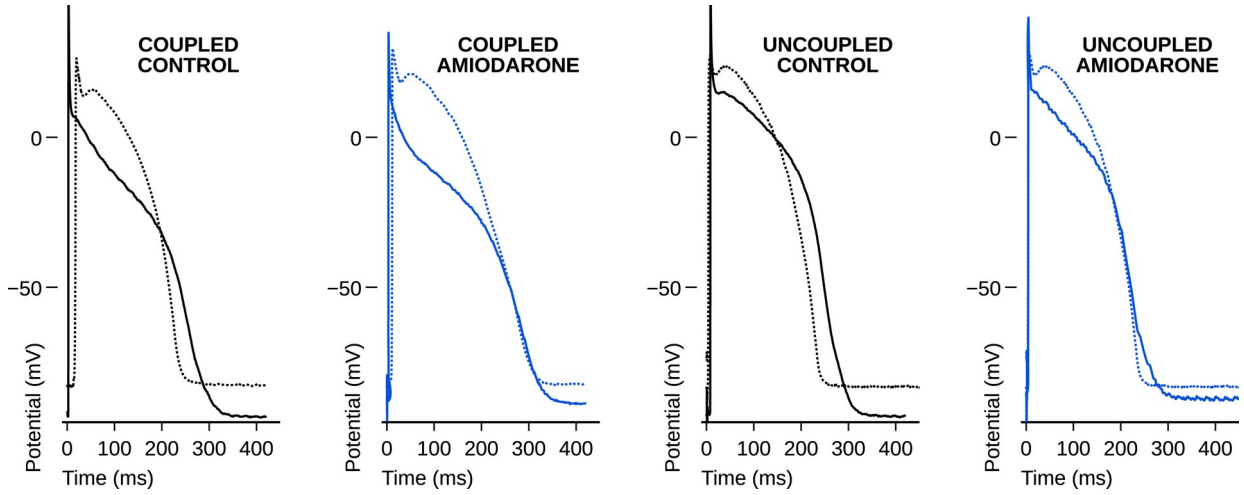

Figure 19 – Chronic amiodarone treatment (50 mg/kg/day) prolonged the action potential duration in coupled and uncoupled preparations from dog hearts. Baseline dispersion was higher in uncoupled preparations Full lines represent Purkinje fiber potentials, dotted lines represent ventricular action potentials.

The effects of dofetilide

In coupled preparations, acutely administered dofetilide (n=8, 50 nM) induced a marked increase in APD_{90} ($p<0.001$) of Purkinje fibers compared to control measurements (Figure 20). APD_{90} was prolonged in ventricular preparations as well ($p<0.01$). The more pronounced prolongation of APD in Purkinje fibers led to an increase in ΔAPD_{90} to 75.2±12.6 ms from 47.0±11.1 ms ($p<0.01$).

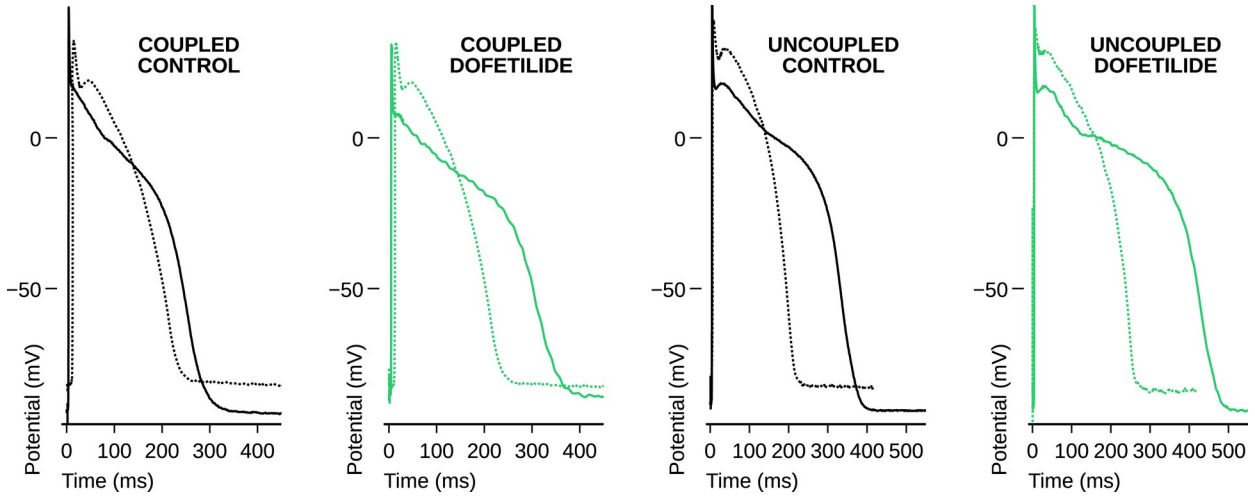

Figure 20 – Dofetilide (50 nM) elicited prolongation both in coupled and in uncoupled preparations from dog hearts. The effect was more pronounced in uncoupled preparations. Baseline dispersion was also higher in uncoupled preparations. Full lines represent Purkinje fiber potentials, dotted lines represent ventricular action potentials.

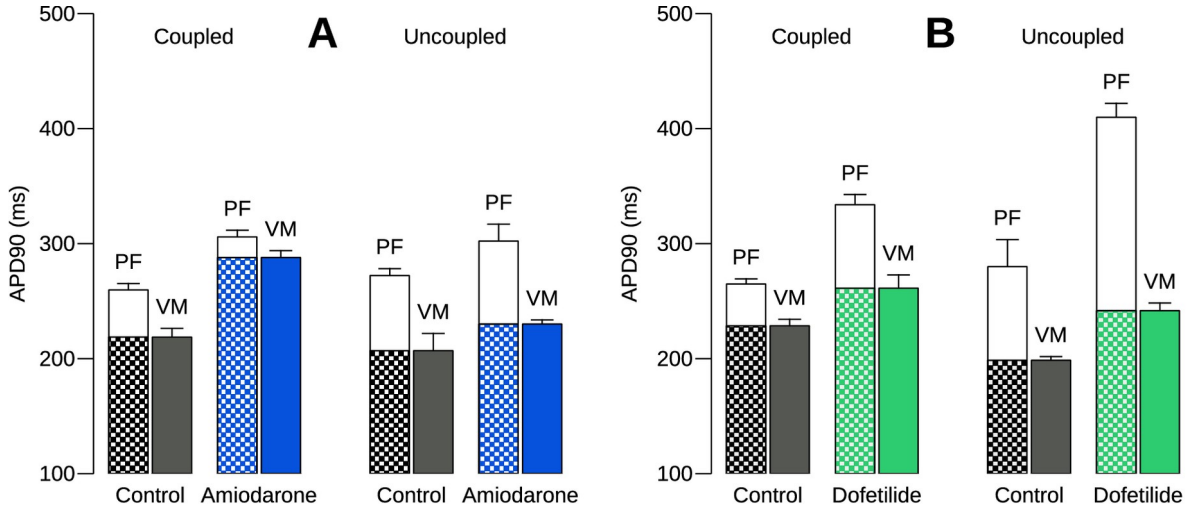

Figure 21 – Comparison of action potential durations in Purkinje fibers (PF) and ventricular muscles (VM) in electrotonically coupled and uncoupled preparations in control conditions, and after chronic amiodarone treatment (50 mg/kg/day) or in the presence of dofetilide (50 nM). White areas in 'PF' bars represent the difference in action potential duration between PF and VM. Bars represent means ± SEM.

In uncoupled Purkinje preparations (n=6–6), APD_{90} was increased after dofetilide treatment ($p<0.001$), but the prolongation was more pronounced compared to the change in coupled preparations (Figures 20 and 21). In uncoupled ventricular preparations, APD_{90} was prolonged after dofetilide treatment ($p<0.01$). The change in APD_{90} in uncoupled ventricular muscle preparations was comparable to that of the coupled preparations, but the APD of uncoupled Purkinje fibers showed a more pronounced prolongation compared to the coupled ones. Therefore, dofetilide elicited a marked increase of local dispersion both in coupled and uncoupled preparations, but the effect was more pronounced in the latter (Figure 21).

Discussion

In this study we compared the cardiac electrophysiological characteristics of ventricular muscle preparations from the heart of commonly used model species with those of human cardiac preparations. Substantial differences were found both in the effects of selective ion channel inhibitors and in terms of APD restitution kinetics. Differences in dispersion of repolarization were also found between coupled and uncoupled ventricular muscle and Purkinje fiber preparations.

Constant pacing at 1000 ms cycle length

The cardiac sodium current consists of a fast component (I_{Na}), and a late component (I_{NaLate}). The former is responsible for phase 0 depolarization and impulse conduction in cardiomyocytes that have a diastolic potential more negative than –60 mV. The latter, I_{NaLate} is characterized by a substantially smaller amplitude, but carries a slowly decaying and/or sustained depolarizing current during phase 2, contributing to the formation of the AP plateau. I_{NaLate} is also more sensitive to sodium channel inhibitors, such as TTX (Carmeliet, 1987; Varró et al., 2021). In the current work, we found that the APD abbreviating effect of TTX was significantly more pronounced in human preparations compared to other species (Figure 8), and the guinea pig showed the highest extent of

abbreviation from the studied animal models. However, in a separate study, we also found that under control conditions the I_{NaLate} current is characterized by a *crescendo*-type current profile, meaning that it reaches its peak near the end of the plateau phase. Conversely, the I_{NaLate} current in human and dog is characterized by a *decrescendo* profile (Horváth et al., 2020).

The I_{Kur} current has been considered to play a complex role in atrial repolarization (Aguilar et al., 2017; Wettwer et al., 2004), but its role in ventricular repolarization is still unclear (Li et al., 1996). Sridhar et al. (2007) have reported that an I_{Kur}-like, 4-AP-sensitive current may be present in dog ventricle as well, but, as this finding is yet to be verified by others, this question remains controversial. Based on our results, the contribution of I_{Kur} to the ventricular repolarization of human and large animals may not be ruled out, but it appears to play a much more important role in rat ventricular repolarization.

In a similar manner, inhibition of the I_{K1} current elicited a significantly more pronounced prolongation in rat APD, confirming APD prolongation reported in rat monophasic action potentials elicited by PA-6 (Skarsfeldt et al., 2016).

The APD prolonging effect of I_{Kr} inhibition has been previously demonstrated, in agreement with our results, using E-4031 and sotalol in human (Jost et al., 2005), sotalol (Varró et al., 1991; Guérard et al., 2008) and dofetilide (Orvos et al., 2019) in rabbit, sotalol (Tande et al., 1990); Varró et al., 1991) and dofetilide (Tande et al., 1990) in guinea pig, and dofetilide in dog (Biliczki et al., 2002). In contrast, it has been reported that dofetilide has no effect on rat repolarization (Tande et al., 1990). In the current study, I_{Kr} inhibition did not affect rat repolarization not only at 1000 ms basic cycle length, but at 200 ms cycle length either. The latter cycle length corresponds to the physiological heart rate of the rat, and rules out the possible accumulation of I_{Kr} due to its

incomplete deactivation at slower stimulation frequency. Despite the available evidence regarding the expression of ERG1 in rat cardiac myocytes, their presence on the cell surface (Pond et al., 2001), and I_{Kr} being measurable by the patch-clamp technique (Yan et al., 2018), our findings suggest that there may be no sufficient amount I_{Kr} current in the rat ventricle during physiological repolarization. Also, the activation of I_{Kr} requires a relatively long positive plateau phase. Therefore, the lack of dofetilide effect in rat can be explained by the rapid repolarization, likely facilitated by the I_{Kur} and the I_{K1} currents, or possibly by some other current yet to be identified.

In human and dog ventricle, I_{Ks} plays an important role in preventing excessive APD prolongation as part of the repolarization reserve when other outward currents, such as I_{Kr}, are blocked, but the extent of its activation during physiological repolarization is limited (Varró et al., 2000; Biliczki et al., 2002; Jost et al., 2005). Furthermore, the activation of I_{Ks} is also dependent on the level of cAMP. In some guinea pig preparations, the residual cAMP may have been enough to keep I_{Ks} active, leading to prolongation after the administration of HMR-1556. Furthermore, HMR-1556 elicited prolongation in every guinea pig preparation after the forskolin-induced increase of cAMP, which was in accordance with previous observations in ventricular muscle preparations from human (Jost et al., 2005), rabbit (Lengyel et al., 2001), and dog (Volders et al., 2003) hearts. In contrast, forskolin in the same concentration failed to evoke HMR-1556-induced APD prolongation in rat preparations, suggesting that, similarly to I_{Kr}, the rapid repolarization in rat ventricle does not allow time for sufficient activation of the I_{Ks} current. Furthermore, since we found that the I_{Kur} current played a prominent role in rat repolarization, and has been found to be activated by increased cAMP in human (Li et al., 1996) and canine (Yue et al., 1999) atria, it is possible that the forskoline-induced further activation of I_{Kur} may have also contributed to the lack of prolongation after I_{Ks} inhibition in rat ventricular preparations.

The I_{to} current is responsible for the rapid repolarization in human, dog, and rabbit, forming the *notch* or *spike and dome* morphology in phase 1 of the action potential, and affects the kinetics of other currents under the plateau phase. Furthermore, I_{to} is also an important component of the repolarization reserve, significantly contributing to the completion of repolarization in the event of I_{Ks} and I_{Kr} inhibition (Virág et al., 2011). It has not been identified in the guinea pig heart (Varró et al., 1993). In our observations, along with the disappearance of the *notch*, human APD was also abbreviated after selective inhibition of the I_{to} current. This suggests that I_{to} may contribute to the regulation of other currents, such as the I_{Kr}, by shifting the voltage in the early phase of the plateau to more negative voltage. Since I_{Kr} has a strong effect on human repolarization (see Figure 2), the shift of voltage to negative direction by I_{to} may explain why some APD shortening was observed following I_{to} inhibition. The inhibition of I_{to} would diminish this voltage shift and consequently more I_{Kr} activation could develop, leading to the shortening of the APD. In contrast, I_{to} seems to remain active not only in phase 1, but throughout phase 2 in rat ventricular muscle, which shows similarity to our observations in rabbit and dog preparations.

The differences we observed in the effects of selective ion channel inhibition in the studied species suggest that the repolarization capacity is constituted by immensely different ion currents, with the most notable differences found between human and rat ventricular preparations. As guinea pigs lack the I_{to} current (Varró et al., 2019), drug effects on this channel would not be visible in this species. Nevertheless, other repolarizing currents seem to be mostly similar between the rabbit and the guinea pig. The dog has been considered to be characterized by a stronger repolarization reserve compared to human, even though the individual ion channels share the most similarities compared to other models. (Jost et al., 2013). When comparing the extents of prolongation elicited by the inhibition individual ion currents, it is interesting to see that APD prolongation in dog preparations never exceeded 30%. On the other hand, I_{Kr} inhibition lead to almost 60% prolongation in human

and 50% prolongation in rabbit preparations, indicating the crucial role of I_{Kr} and/or relative weakness of the repolarization reserve in human and rabbit. Furthermore, I_{K1} inhibition elicited a more pronounced prolongation in dog and rabbit than in human, which may suggest that under control conditions, I_{K1} contributes significantly less to human repolarization, but may compensate a decrease of I_{Kr} in dog, and to some extent, in rabbit as well. The I_{Ks} current is also known to be weaker in human than in dog or in rabbit (Jost et al., 2013). Therefore, when assessing possible QT prolonging properties of drug candidates, the rabbit may be a better model due to its somewhat weaker repolarization reserve but still otherwise similar ion channel constitution. Furthermore, transgenic rabbit models of specific long QT syndromes with reduced repolarization reserve are also emerging, allowing even more nuanced evaluation of proarrhythmic side effects of drug candidates (Major et al., 2016; Baczkó et al., 2020; Hornyik et al., 2020; Hornyik et al., 2020; Castiglione et al., 2021).

Attempting to inhibit I_{Kr} in a rat preparation after the combined inhibition of the I_{Ks}, I_{to}, I_{Kur} currents and amplification of the I_{Na} current at the same time elicited no additional prolongation in a preliminary experiment (Figure 22). The I_{Kr} current is one of the most important repolarizing currents in human, but its inhibition did not affect rat repolarization even after significant weakening of the repolarization reserve. An important consequence of this would be that an I_{Kr} inhibiting drug effect may be overlooked if it was studied only in rat. Conversely, I_{K1}, I_{to}, and I_{Kur} were found to be the strongest repolarizing currents in the rat heart, but inhibiting these currents did not affect repolarization in human, dog and rabbit. These differences suggest that the rat is a rather unsuitable model in the evaluation of drug effects on repolarization, because eliciting similar levels of APD prolongation requires the inhibition of different ion channels, thus decreasing the translational value of findings from rat experiments. Furthermore, it should be noted that despite the combined drug effects, the early phase of repolarization was only slightly prolonged in the same

experiment (Figure 22), suggesting that there may be a strong repolarizing current active during phases 1 or 2 in the rat ventricle that is yet to be identified.

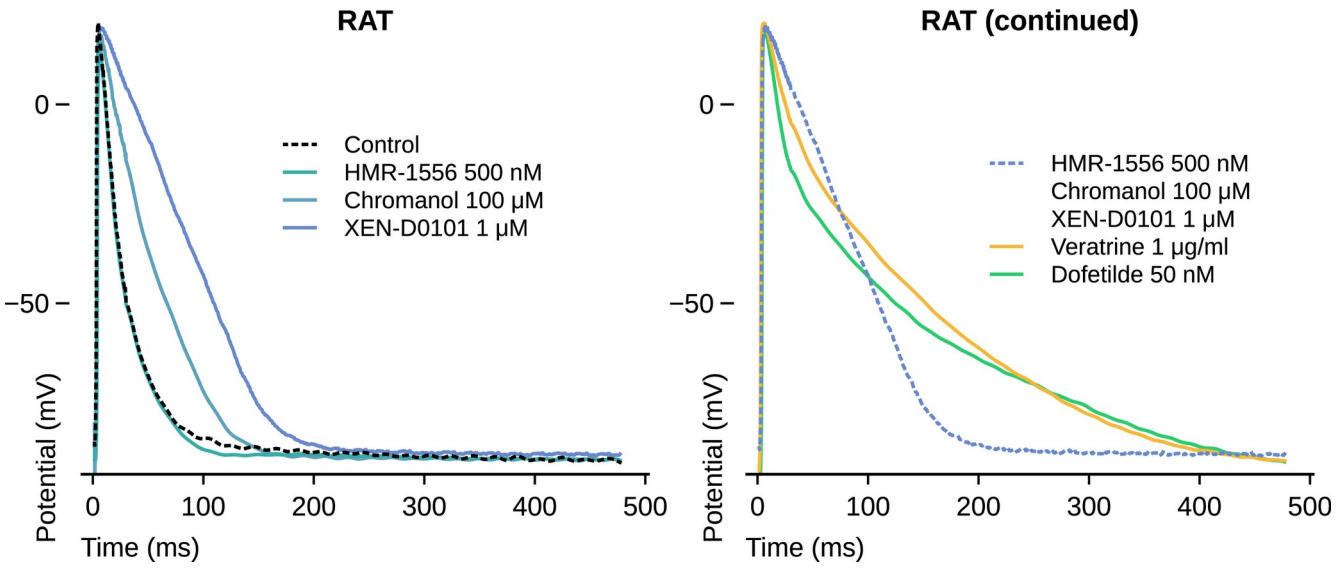

Figure 22 – A preliminary experiment showing that I_{Kr} inhibitor dofetilide did not prolong action potential duration after the combined inhibition of the I_{Ks}, I_{to}, I_{Kur} currents and the amplification of the I_{Na} current in a rat papillary muscle at 1000 ms basic cycle length pacing.

The inhibition of $I_{Ca\text{-}L}$ elicited a similar extent of abbreviation in all species. This strong similarity may be explained by the commonly shared function of this current, as it not only shapes the action potential and contributes to the formation of a plateau phase in large mammals, but it also activates the Ca^{2+} release from the sarcoplasmic reticulum, and thus initiates the contraction of the cardiac muscle (Stern, 1992). In contrast, NCX inhibition consistently abbreviated APD in all rat preparations, but not in those taken from human or dog hearts. This can be explained by the species differences in the ratio of the APD and cardiac duration of the Ca^{2+} transient: even though marked differences exists in APD between species, the length of the Ca^{2+} transient is similar. In rat myocytes, a complete cycle of the Ca^{2+} transient can take more than 200 ms (Moore et al., 1991), more than double the rat APD, and during this time, NCX is actively exchanging 3 extracellular Na^{+} ions to 1 intracellular Ca^{2+} ion, generating a depolarizing net current. Therefore, in rat preparations,

ORM-10103 inhibited a strong depolarizing current, and thus the APD was abbreviated. The slight abbreviation in rabbit preparations may be explained by this mechanism as well. Conversely, in human and dog preparations, NCX inhibition elicited very slight changes in APD, prolongation in most preparations, abbreviation in others. Due to the similar length of the Ca^{2+} transient being combined with an APD longer than the transient itself, NCX may operate both in forward and in reverse mode during the AP. In reverse mode, NCX would act as a repolarizing net current, therefore its inhibition may elicit prolongation in APD.

Frequency-dependent changes in APD

We also studied the changes in APD during a variable cycle length protocol as well as a restitution protocol featuring extrasystolic pacing. We found that human ventricular APD restitution differs from those reported in other species. Even though dog APD restitution curve shared similarities with that of human, its faster time constant is an important difference in terms of restitution kinetics.

The cellular and subcellular mechanisms of APD restitution have been studied extensively. However, they are still subjects of debate (Boyett and Jewell, 1978; Elharrar and Surawicz, 1983; Hsieh et al., 2013; Osadchii, 2017a; Osadchii, 2017b; Shattock et al., 2017; Zaniboni, 2019). Frequency-dependent APD changes including APD restitution, in case the cycle length or DI ranges are sufficiently long, can be characterized by multiple exponential fits (Elharrar and Surawicz, 1983; Varró et al., 1985). The rapid exponential components of these fits are generally attributed to deactivation and recovery from inactivation properties of various ion channels activated during the previous baseline beats, as well as intracellular and extracellular ion concentration changes, which directly or indirectly alter electrogenic pumps and exchangers, often called collectively as “short term memory” (Elharrar and Surawicz, 1983; Toal et al., 2009). Changes in the expression of ion

channels can cause the so-called long-term memory (Obreztchikova et al., 2006), which was not investigated in our experiments.

According to the hypothesis of Zaza (Zaza and Varró, 2006; Zaza, 2010) and later by others (Bányász et al., 2009; Virág et al., 2009; Bárándi et al., 2010), it has been argued that frequency dependent APD changes, including electrical restitution, were determined by the APD at the basic cycle length. Our experiments supported this concept in the case of human, dog, and rat preparations, where shorter baseline APDs were associated with faster time constants. However, restitution kinetics were near identical between the subgroups with long and short baseline APDs in guinea pig preparations. Since one of the most important differences between guinea pig ventricle and the ventricle of the other three species is the absence or presence of the I_{to} current, it could be argued that the relation between the APD at basic cycle length and the kinetics of restitution are, at least in part, governed by the I_{to} current. We also found that the inhibition of I_{Kr} and I_{Na} slowed restitution kinetics, but I_{Ks} inhibition did not affect it in human preparations. This is also in agreement with earlier work in dog Purkinje fibers, where several drugs with I_{Na} and $I_{Ca\text{-}L}$ inhibition properties slowed APD restitution (Elharrar et al., 1984).

Therefore, the density of individual ion currents seem to be able to affect restitution kinetics. A conceivable implication of this would be that changes in the density of some ion currents lead to changes not only in baseline APD, but in restitution kinetics as well, possibly according to their specific time constants in gating, as well as their voltage-current dynamics. In addition, in the case of human ventricular preparations, shortening the baseline APD by $I_{Ca\text{-}L}$ inhibition did not affect the kinetics of APD restitution, indicating that changes in baseline APD or inhibition of some currents may not necessarily affect restitution kinetics. Therefore, in addition to the baseline APD, other factors, such as transmembrane ion currents, can influence restitution. The latter raises the

possibility that ion channel modifier drugs slowing restitution kinetics may have antiarrhythmic properties by affecting electrical restitution, which may be considered in future drug development projects.

Electrotonically coupled and uncoupled preparations

Action potentials recorded from canine transitional cells in the Purkinje-muscle junctions have been previously described to have longer APD than the surrounding ventricular myocardium and slower maximal rate of depolarization than Purkinje fibers, but the presence of these transitional cells is limited to the immediate surroundings of the junction (Martinez-Palomo et al., 1970), suggesting that the slightly longer APD we observed in ventricular preparations and slightly shorter APD in Purkinje fibers in electrotonically coupled preparations may be attributable to the electrotonic interaction between the two tissue types. This interaction is not present in dissected, individual preparations, which leads to slight differences in baseline APD, and, more importantly, to an exaggerated difference in the drug-induced changes of APD. Therefore, when calculating dispersion from individual, uncoupled preparations, dispersion is likely to be overestimated.

The effect of dofetilide in uncoupled cardiac preparations is well documented (Gwilt et al., 1991; Knilans et al., 1991; Bányász et al., 2009), but such measurements have not been reported in *ex vivo* coupled preparations, and therefore, direct comparison of dofetilide effects between coupled and uncoupled preparations was not yet possible. In coupled preparations, dofetilide increased ΔAPD_{90} by causing a much greater prolongation in Purkinje fibers than in ventricular muscle. This difference in mean APD_{90} values was further increased in uncoupled preparations, likely due to the lack of electrotonic coupling.

The different changes in dispersion may be partially explained by the different effects of each drug on ion channels: dofetilide is considered a selective inhibitor of I_{Kr}, while amiodarone also inhibits inward currents, such as I_{NaLate}, I_{CaL} (Follmer et al., 1987; Kodama et al., 1996; Nishimura et al., 1989), and outward currents, such as I_{Ks}, apart from I_{Kr} (Balser et al., 1991; Bertran et al., 1998; Kodama et al., 1996; Sato et al., 1994; Varró et al., 1996). Since I_{NaLate} is considered to be more prominent in Purkinje fibers than in ventricular myocytes (Baláti et al., 1998; Haufe et al., 2005), the inhibition of I_{NaLate} by amiodarone would limit the prolongation of APD in Purkinje fibers more than in the ventricular muscle, resulting in a not as pronounced dispersion of repolarization.

The extent of AP prolongation elicited by each drug also varied between the electronically coupled and uncoupled preparations. The undisturbed electrotonic coupling between the Purkinje fibers and the subendocardial muscle may explain the more pronounced prolongation in uncoupled Purkinje fibers, compared to changes measured when coupled with the myocardium, since this interaction may lead to a slight decrease in the measured APD of Purkinje fibers and slight increase in that of the ventricular muscle, partially evening out the distinct difference in APDs measured when the conduction system and the myocardium are dissected. Accordingly, the extent of prolongation in Purkinje fibers elicited by dofetilide may partially be modulated by the neighboring ventricular muscle, and the prolongation in ventricular muscle elicited by amiodarone may be, in a similar manner, potentiated by the interaction with Purkinje fibers. Thus, our data from coupled and uncoupled preparations suggests that the electrotonic interaction between Purkinje fibers and subendocardial myocardium not only affects the baseline electrophysiology of these tissues when measured using the conventional microelectrode technique, but also the measured effects elicited by antiarrhythmic agents.

Therefore, amiodarone *ex vivo* decreased not only transmural dispersion, previously demonstrated on canine (Sicouri et al., 1997) and human (Drouin, et al., 1998) preparations, but also in the Purkinje-muscle junction. In the clinical setting QT/QTc prolongation increase is associated with an increased risk of VF. However, drugs that not only increase QT/QTc, but also decrease the dispersion of QT/QTc or JT are accompanied by a lower proarrhythmic risk (e. g. amiodarone), while agents that prolong QT/QTc without decrease in dispersion (e. g. quinidine) have a higher risk of arrhythmic events (Cui et al. 1994, Antzelevitch et al., 1998). It has also been reported that dofetilide increases electrical heterogeneity in the human heart (Stabenau et al., 2020), and it is associated with a higher risk of causing torsades than amiodarone (Brendorp et al., 2002). Amiodarone has also been shown to decrease dispersion of APD in monophasic action potential measurements (Osaka et al. 2011).

Therefore, the decrease in dispersion between the cardiac conductive system and myocardium may also be a beneficial action of antiarrhythmic agents, similarly to the reduction of transmural dispersion, by decreasing the diversity in refractoriness between adjacent cardiac regions, consequently decreasing the risk of extra beats propagating by unidirectional block.

Conclusion

The formation of potentially life-threatening arrhythmias requires a trigger event that occurs during a vulnerable period in the heart. Such vulnerable periods may form due to the prolongation of APD, caused by intrinsic or extrinsic defects in repolarization. In order gather relevant data on the APD prolonging effects of drugs or drug candidates, our choice of models should appropriately represent human repolarization, and when interpreting the results obtained from model animals, the limitations of the given model, such as the degree of electrotonic coupling or the differences in ion currents compared to human, should be taken into account. The dog has been considered a

reasonably satisfactory model of human cardiac electrophysiology, regarding its ion channel constitution (Nánási et al., 2021). Our findings are in accordance with this notion. The guinea pig and the rabbit are more accessible models compared to the dog, and also seem more similar to human repolarization when compared to that of the rat. In addition, we also found that testing drug effects on electrotonically uncoupled Purkinje fibers and ventricular muscle may lead to the overestimation of APD prolonging effects in the former, but underestimation in the latter compared to drug effects on the same tissue types in their interconnected state. Ideally, testing novel agents on at least two models at the same time may significantly increase the translational value of basic research in cardiac electrophysiology, and taking the species differences presented in this work into account may provide further insight in expected drug effects on human APD.

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Electrical Restitution and Its Modifications by Antiarrhythmic Drugs in Undiseased Human Ventricular Muscle

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Introduction: Re-entry is a basic mechanism of ventricular fibrillation, which can be elicited by extrasystolic activity, but the timing of an extrasystole can be critical. The action potential duration (APD) of an extrasystole depends on the proximity of the preceding beat, and the relation between its timing and its APD is called electrical restitution. The aim of the present work was to study and compare the effect of several antiarrhythmic drugs on restitution in preparations from undiseased human ventricular muscle, and other mammalian species.

Methods: Action potentials were recorded in preparations obtained from rat, guinea pig, rabbit, and dog hearts; and from undiseased human donor hearts using the conventional microelectrode technique. Preparations were stimulated with different basic cycle lengths (BCLs) ranging from 300 to 5,000 ms. To study restitution, single test pulses were applied at every 20th beat while the preparation was driven at 1,000 ms BCL.

Results: Marked differences were found between the animal and human preparations regarding restitution and steady-state frequency dependent curves. In human ventricular muscle, restitution kinetics were slower in preparations with large phase 1 repolarization with shorter APDs at 1000 ms BCL compared to preparations with small phase 1. Preparations having APD longer than 300 ms at 1000 ms BCL had slower restitution kinetics than those having APD shorter than 250 ms. The selective I_{Kr} inhibitors E-4031 and sotalol increased overall APD and slowed the restitution kinetics, while I_{Ks} inhibition did not influence APD and electrical restitution. Mexiletine and nisoldipine shortened APD, but only mexiletine slowed restitution kinetics.

Discussion: Frequency dependent APD changes, including electrical restitution, were partly determined by the APD at the BCL. Small phase 1 associated with slower restitution suggests a role of I_{to} in restitution. APD prolonging drugs slowed restitution, while mexiletine, a known inhibitor of I_{Na}, shortened basic APD but also slowed restitution.

These results indicate that although basic APD has an important role in restitution, other transmembrane currents, such as I_{Na} or I_{to}, can also affect restitution kinetics. This raises the possibility that ion channel modifier drugs slowing restitution kinetics may have antiarrhythmic properties by altering restitution.

Keywords: arrhythmia, action potential, electrical restitution, human ventricle, cardiac electrophysiogy

INTRODUCTION

Cardiovascular diseases are the leading causes of mortality in Western countries including the USA, Germany, France, and the UK. In approximately 50% of the cases the cause of death in cardiac patients is sudden cardiac death due to ventricular fibrillation (Jazayeri and Emert, 2019). The underlying mechanisms of ventricular fibrillation are complex, often multi-factorial, and are still not fully understood, therefore, they are subjects of current investigations. In general, arrhythmias can be explained by impaired impulse conduction and/or abnormal automaticity within the heart. The cellular cause of impulse conduction defects can have a distinct anatomical cause exhibiting a fixed pathway, usually determined by ischemic or fibrotic injury (Nguyen et al., 2017; Himel et al., 2019). Alternatively, the re-entry pathway can form without such injuries, due to enhanced dispersion of repolarization and consequently enhanced dispersion of refractoriness (Himel et al., 2019). The latter determines the ability of the ventricular muscle to be re-excited following a previous beat. In case the differences in action potential duration (APD), and consequently the effective refractory period (ERP) are enhanced, i.e. dispersion of APD or repolarization is augmented, the propagation of an early extra beat can be delayed or blocked in the direction that has myocytes with longer APDs, but conducted normally to the direction that has myocytes with shorter APDs. Therefore, in such an area, the extra beat can travel in a zig-zag pattern and can re-enter into areas that have been previously excited, eliciting chaotic rhythm or even fibrillation. Accordingly, the timing of an extrasystole is critical for arrhythmogenesis (Akar et al., 2002; Tran et al., 2007; Zaniboni, 2019). It has been known for a long time that the APD/ERP of an extrasystole depends on the proximity of the preceding beat, called diastolic interval; and as the diastolic intervals increase, the APDs/ERPs of the extra beats also increase. This process is called electrical restitution and had been described long ago (Nolasco and Dahlen, 1968; Boyett and Jewell, 1978; Elharrar and Surawicz, 1983), but its importance in arrhythmia research gained particular attention again in the past two decades (Gilmour, 2002; Franz, 2003; Kalb et al., 2004; Gilmour, 2009; Orini et al., 2016; Osadchii, 2017a; Osadchii, 2017b; Shattock et al., 2017; Orini et al., 2019; Osadchii, 2019). According to the restitution hypothesis, as diastolic intervals increase due to propagation of an extrasystole, the next following possible extrasystole would encounter prolonged APD/ERP and local conduction defect can occur. A steeper or faster restitution curve would favor such an effect and would be considered proarrhythmic; flattened or slower electrical restitution would have the opposite effect (Garfinkel et al., 2000; Qu et al., 2014; Shattock et al., 2017; Osadchii, 2017a; Osadchii, 2017b). Several studies in different preparations investigated the effects of antiarrhythmic drugs on the cardiac electric restitution properties (Varró et al., 1985; Hsieh et al., 2013; Osadchii, 2017a; Osadchii, 2017b; Shattock et al., 2017). These studies yielded different results depending on the protocols (dynamic or standard), on the basic stimulation frequencies, on the preparations (ventricular muscle or Purkinje fibers), and on the species (guinea-pig, rabbit, rat, or dog) used in their experimental approaches (Elharrar and Surawicz, 1983; Kalb et al., 2004; Orini et al., 2016; Shattock et al., 2017; Osadchii, 2019). The species used may have special significance, since, as **Figure 2** shows, there are marked differences between restitution curves measured in ventricular papillary muscle from different species (in rat, guinea-pig, rabbit, dog or human preparations) with the same experimental restitution pacing protocol and basic stimulation frequency. Therefore, the aim of the present work was to study the effect of several antiarrhythmic drugs on undiseased human ventricular muscle to better understand the possible implications of drug effects on electrical restitution, and understand these effects in human arrhythmogenesis.

Abbreviations: APA, action potential amplitude; APD, action potential duration; APD50, APD measured at 50% of repolarization; APD90, APD measured at 90% of repolarization; ERP, effective refractory period; Vmax, maximum upstroke velocity; RP, resting membrane potential.

METHODS

Human General Donor Cardiac Tissue Ethics Statement

Hearts were obtained from general organ donors whose undiseased hearts were explanted to obtain pulmonary and aortic valves for transplant surgery. Before cardiac explantation, organ donors did not receive medication apart from dobutamine, furosemide, and plasma expanders. According to the Hungarian law to obtain samples from donors, the consent of the patients or relatives is not needed. Therefore, consent is waived under local legislation. The investigations conformed to the principles of the Declaration of Helsinki. Experimental protocols were approved by the National Scientific and Research Ethical Review Boards (4991-0/2010-1018EKU [339/PI/010]).

Animals

All experiments 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).

Conventional Microelectrode Technique

Action potentials were recorded in right ventricular trabeculae or papillary muscle preparations obtained from rat, guinea pig, rabbit, dog hearts, and from undiseased human donor hearts using the conventional microelectrode technique.

Rats (either sex, 200–400 g), guinea-pigs (either sex, 400–600 g), rabbits (either sex, 2.5–3.5 kg) and dogs (either sex, 10–15 kg) were anesthetized by sodium pentobarbitone (30 mg/kg i.p. for rat and guinea pig, i.v. for rabbit and dog) following sedation (xylazine 1 mg/kg). The animals also received intravenous injection of 400 U/kg heparin. In case of human donor hearts, immediately after explantation, each heart was perfused with cardioplegic solution and kept cold (4–6°C) for 2–4 hours before dissection.

Preparations were individually mounted in a tissue chamber with a volume of 50 ml. During experiments, modified Locke's solution was used, containing (in mM): NaCl, 128.3; KCl, 4; $CaCl_2$, 1.8; $MgCl_2$, 0.42; $NaHCO_3$, 21.4; and glucose, 10. The pH of this solution was set between 7.35 and 7.4 when gassed with the mixture of 95% O_2 and 5% CO_2 at 37°C. Each preparation was stimulated through a pair of platinum electrodes in contact with the preparation using rectangular current pulses of 1 to 3 ms duration at twice of the threshold strength at a constant basic cycle length of 1000 ms (S1). These stimuli were delivered for at least 60 min allowing the preparation to equilibrate before the measurements were initiated. 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) which was coupled to a dual beam oscilloscope.

The resting potential (RP), action potential amplitude (APA), maximum upstroke velocity (V_{max}), and APD measured at 50% and 90% of repolarization (APD_{50} and APD_{90}, respectively) were determined off-line using an in-house developed software (APES) running on a computer equipped with an ADA 3300 analog-to-digital data acquisition board (Real Time Devices, Inc., State College, Pennsylvania) having a maximum sampling frequency of 40 kHz.

The following types of stimulations were applied in the course of the experiments: stimulation with a constant cycle length of 1000 ms; stimulation with different constant cycle lengths ranging from 300 to 5000 ms. To determine the recovery kinetics of APD_{90} (APD_{90} restitution), extra test action potentials were elicited by using single test pulses (S2) in a preparation driven at a basic cycle length of 1000 ms. The S1–S2 coupling interval was increased progressively from the end of the refractory period. The effective refractory period was defined as the longest S1–S2 interval at which S2 failed to elicit a propagated response. The diastolic intervals preceding the test action potential were measured from the point corresponding to 90% of repolarization of the preceding basic beat to the upstroke of the test action potential and were increased progressively.

Attempts were made to maintain the same impalement throughout each experiment. In case an impalement became dislodged, adjustment was attempted, and if the action potential characteristics of the re-established impalement deviated by less than 5% from the previous measurement, the experiment continued. All measurements were performed at 37°C.

Data Analysis

All data are expressed as means ± SEM. The "n" number refers to the number of experiments (i.e. the number of ventricular muscle preparations). Data points of restitution curves were fitted by a mono-exponential function in order to calculate the kinetic time constant of the APD_{90} restitution process:

$$APD = APD_{ss} - A * \exp(-DI/\tau)$$

where APD_{ss} is the maximal action potential duration (APD_{90}), A is the amplitude of the exponential function, DI is the diastolic interval, and τ is the time constant.

RESULTS

In **Figure 1**, frequency dependent APD changes are shown in different species including human following various constant steady-state (S1–S1) and abrupt changes of cycle lengths (S1–S2). The figure shows that there are marked differences both in the electrical restitution and steady-state frequency dependent curves. The nature and mechanism of the frequency dependent APD changes (Carmeliet, 1977; Elharrar and Surawicz, 1983; Obreztchikova et al., 2006; Qu et al., 2014; Ni et al., 2019) including electrical restitution are not fully resolved yet. A recent study of Schattock et al. (2017) suggested that the slope of the restitution curve depends on the APD of the basic heart rate. Therefore, as **Figure 2** shows, human ventricular electrical restitution curves separated according to their action potential durations at basic cycle length of 1000 ms. In preparations with APD_{90} shorter than 250 ms, the time constant (τ) was 63.9 ± 6.0 ms (n = 10), while in preparations with APD_{90} longer than 300 ms τ was 125.5 ± 9.1 ms (n = 17). **Figure 2** indicates that electrical restitution kinetics are slower as action potential durations increase, suggesting that restitution kinetics, at least partly, indeed depend on intrinsic behavior of the repolarization process. In addition, as shown in **Figure 3**, human ventricular APD restitution curves have somewhat slower restitution kinetics where the basic action potentials showed prominent phase 1 repolarization during the plateau phase (τ = 126.1 ± 8.1 ms, n = 16) compared to those that had no strong phase 1 repolarization (τ = 98.5 ± 10.0 ms, n = 10), suggesting a possible role of I_{to} in the restitution process. In this respect, it is worth to note that APDs in preparations having prominent phase 1 repolarization were shorter than those having no or small phase 1 repolarization. Also, rabbit restitution curves and steady-state frequency-dependent APD have a declining slope

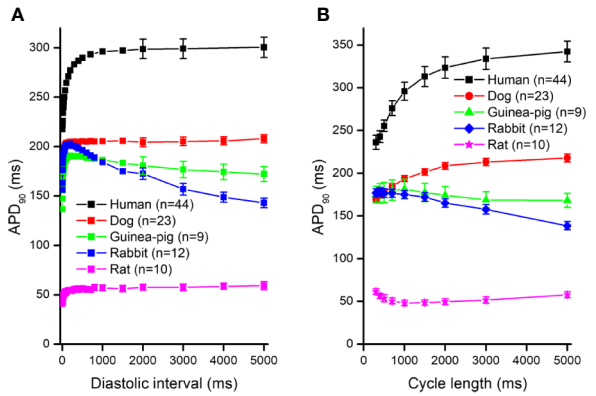

FIGURE 1 | Action potential duration (APD_{90}) restitution curves (panel **A**) and the steady-state cycle length dependence of the action potential duration (panel **B**) in human, dog, guinea pig, rabbit, and rat right ventricular muscle preparations. For the sake of clarity, the SEM values were indicated in case of diastolic intervals 2000 – 5000 ms in (panel **A**).

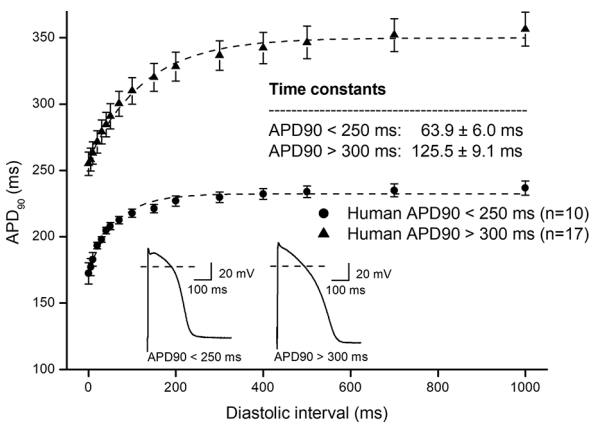

FIGURE 2 | Comparing human ventricular electrical restitution curves based on the action potential duration. Human APD_{90} restitution curves were separated into short APD (APD_{90} < 250 ms) and long APD (APD_{90} > 300 ms) groups. The data points up to 1000 ms diastolic interval were fitted by single exponential function. The inset shows the kinetical time constants for the two groups.

at diastolic intervals and cycle lengths longer than 1000 ms. Since in rabbit I_{to} is characterized by slow recovery (Fermini et al., 1992; Sánchez-Chapula et al., 1994), these results also suggest a possible role of I_{to} in the cycle length dependent APD changes including restitution.

In further experiments, the effects of several antiarrhythmic drugs were studied on the electrical restitution curves in human undiseased ventricular muscle preparations. **Figure 4** shows that the selective rapid delayed rectifier potassium current (I_{Kr}) inhibitor E-4031 and sotalol increased overall APD and slowed the kinetics of the restitution curve (from τ = 82.6 ± 5.5 ms to τ = 160.3 ± 11.1 ms, n = 5; and from τ = 95.8 ± 10.7 ms to τ = 152.7 ± 8.7 ms, n = 5, respectively). **Figure 5** illustrates that L-

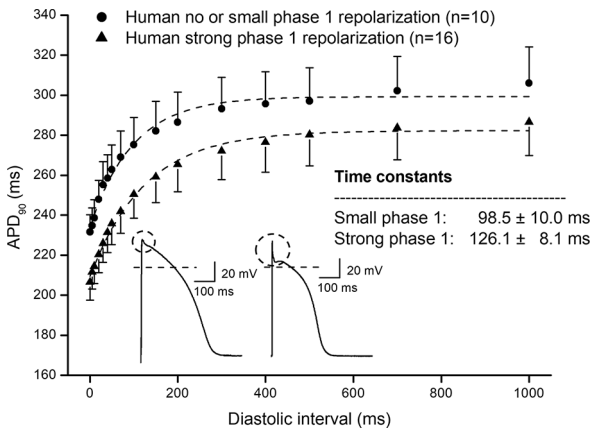

FIGURE 3 | Comparing human ventricular action potential duration restitution curves based on the amplitude of phase 1 repolarization. Human APD_{90} restitution curves were separated into two groups, one showed prominent phase 1 repolarization and another one had no or small phase 1 repolarization. The data points up to 1000 ms diastolic interval were fitted by single exponential function. The inset shows the kinetical time constants for the two groups.

735,821, a specific inhibitor of the slow delayed rectifier potassium current (I_{Ks}) does not influence APD and electrical restitution curves (τ = 113.1 ± 8.4 ms vs. τ = 111.9 ± 7.3 ms, n = 7). In further experiments, the effects of the inward sodium current (I_{Na}) inhibitor mexiletine and the inward L-type calcium current blocker nisoldipine were studied on human ventricular electrical restitution curves. **Figure 6** shows that both mexiletine and nisoldipine shortened APD but only mexiletine slowed restitution kinetics in human ventricular muscle preparations (from τ = 98.1 ± 10.9 ms to τ = 133.2 ± 13.1 ms, n = 6; from τ = 111.1 ± 9.2 ms to τ = 113.1 ± 7.4 ms, n = 6, respectively).

DISCUSSION

In this study, the electrical restitution of APD and its possible influence by several antiarrhythmic drugs in human ventricular muscle was investigated. Notwithstanding plentiful data in different animal experiments, according to our best knowledge, there is no systemic study on electrical restitution available in undiseased human ventricular muscle with the conventional microelectrode technique.

The main novel findings in the present work are as follows;

1. Human ventricular APD restitution differs from those reported in other species.
2. In spite of the marked species differences in the ventricular restitution curve, in human ventricle, similar to those reported earlier in other mammalian species (Shattock et al., 2017), longer repolarization is associated with slower restitution kinetics.
3. However, human ventricle exhibiting prominent phase 1 repolarization, presumably due to high level of I_{to}

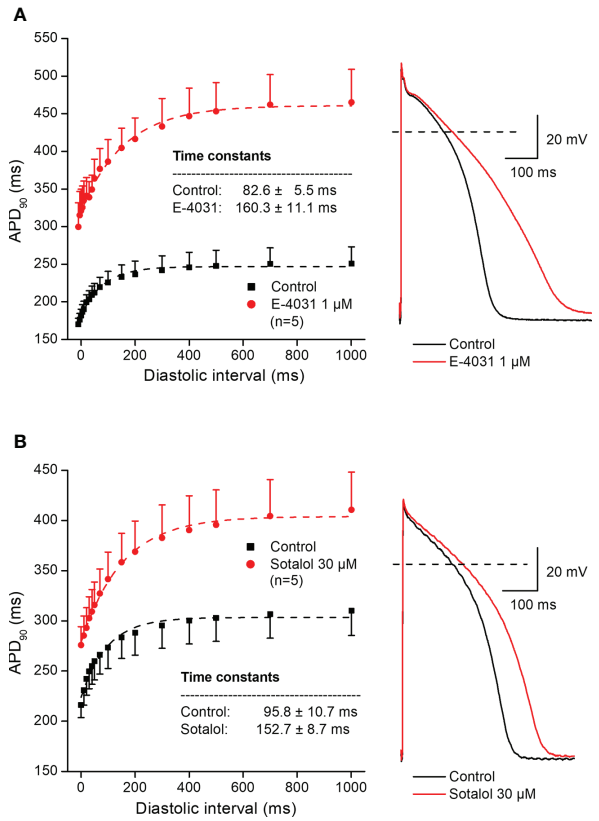

FIGURE 4 | Effect of two selective rapid delayed rectifier inhibitor antiarrhythmic drugs – E-4031 (panel **A**) and sotalol (panel **B**) – on the human electrical restitution curve. The data points up to 1000 ms diastolic interval were fitted by single exponential function. The inset shows the kinetical time constants in control conditions and after drug application. On the right part of the figure original action potential traces are shown before and after drug application at basic cycle length of 1000 ms.

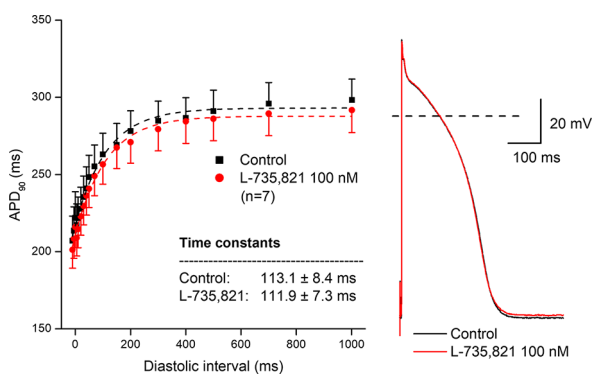

FIGURE 5 | Lack of effect of the selective slow delayed rectifier inhibitor L-735,821 on the human electrical restitution curve. The data points up to 1000 ms diastolic interval were fitted by single exponential function. The inset shows the kinetical time constants in control conditions and after application of L-735,821. On the right part of the figure original action potential traces are shown before and after drug application at basic cycle length of 1000 ms.

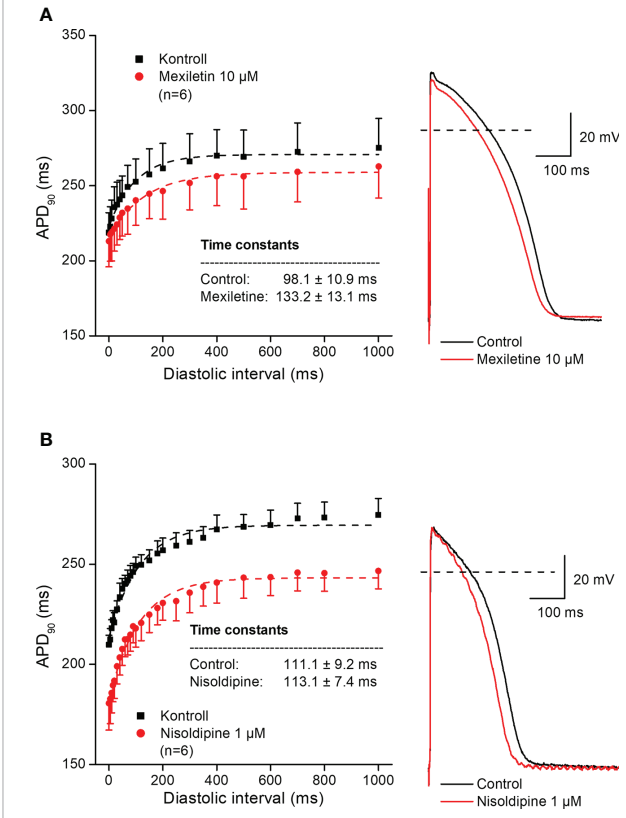

FIGURE 6 | Effect of the sodium channel inhibitor mexiletine (panel **A**) and the L-type calcium current blocker nisoldipine (panel **B**) on the human electrical restitution curve. The data points up to 1000 ms diastolic interval were fitted by single exponential function. The inset shows the kinetical time constants in control conditions and after drug application. On the right part of the figure original action potential traces are shown before and after drug application at basic cycle length of 1000 ms.

expression, was associated with shorter APD but slower restitution kinetics.

4. Drugs that inhibit I_{Kr} and I_{Na} slow restitution kinetics of APD restitution curve but drugs inhibiting I_{Ks} do not influence electrical APD restitution curves in human ventricular muscle.

APD restitution is an important process in the adaptation of the action potential to abrupt changes in cycle length and has been postulated playing an important role in the susceptibility to re-entrant arrhythmias, such as ventricular fibrillation (Garfinkel et al., 2000; Gilmour, 2002; Toal et al., 2009; Qu et al., 2014; Orini et al., 2016; Osadchii, 2017a; Osadchii, 2017b). Accordingly, it is generally agreed that slower restitution kinetics and a less steep restitution slope would result in antiarrhythmic effects, while steeper and faster restitution would be proarrhythmic (Garfinkel et al., 2000; Gilmour, 2002; Qu et al., 2014; Osadchii, 2017a; Osadchii, 2017b; Shattock et al., 2017; Zaniboni, 2019). As diastolic intervals are increasing due to propagation of an extra beat, a next short coupled extra beat would encounter longer

APD or ERP and, as a result of this, local conduction block can develop. A steeper restitution curve would facilitate this possibility with potential proarrhythmic consequences, but a flattened restitution curve would have the opposite effect.

Repolarization of cardiac ventricular muscle has been known for long to be dependent on species and stimulation frequency (Carmeliet, 1977; Boyett and Jewell, 1978). The cellular and subcellular mechanisms of APD restitution have been studied extensively. However, they are still subjects of debate (Boyett and Jewell, 1978; Elharrar and Surawicz, 1983; Hsieh et al., 2013; Osadchii, 2017a; Osadchii, 2017b; Shattock et al., 2017; Zaniboni, 2019). Frequency dependent APD changes including APD restitution in case the cycle length or diastolic interval ranges are sufficiently long can be characterized by multiple exponential fits (Elharrar and Surawicz, 1983; Varró et al., 1985). The rapid exponential components of these fits are generally attributed to deactivation and recovery from inactivation properties of various ion channels activated during the previous baseline beats, as well as intracellular and extracellular ion concentration changes, which directly or indirectly alter electrogenic pumps and exchangers, often called collectively as "short term memory" (Elharrar and Surawicz, 1983; Toal et al., 2009). Changes in the expression of ion channels can cause the so-called long-term memory (Obreztchikova et al., 2006), which was not investigated in our experiments.

In a recent study by Shattock et al. (2017) it was suggested that APD restitution kinetics were determined by the length of the APD of the basic beat. This speculation was based on guinea pig and rabbit experiments in Langendorff preparation measuring monophasic APD with a Franz catheter, or with the sharp microelectrode technique in single isolated guinea-pig myocytes applying the dynamic restitution protocol (Shattock et al., 2017). In this study, a wide range of drugs that all prolong APD by different modes of actions (such as clofilium, Bay K 8644, veratridine, catecholamines; and interventions such as low extracellular Ca^{2+} and transverse aortic constriction induced heart failure) slowed the kinetics or flattened the restitution curves. Based on these results, in agreement with the hypothesis of Zaza (Zaza and Varró, 2006; Zaza, 2010) and later by others (Bányász et al., 2009; Virág et al., 2009; Bárándi et al., 2010), it was argued that frequency dependent APD changes, including electrical restitution, were determined by the APD at the basic cycle length. The results of the present study partly support this idea, since all of the drugs studied with an APD prolonging effect slowed the restitution curve. Also, longer intrinsic APD was associated with slower restitution in human ventricular muscle. However, mexiletine and nisoldipine shortened basic APD but slowed or did not change the restitution curve. In addition, in the present study, the human ventricular muscle preparations with strong phase 1 repolarization showed slower restitution kinetics with shorter APD at the basic cycle lengths than those that showed no prominent phase 1 repolarization. Rabbit ventricular APD restitution curves showed a declining slope at diastolic intervals longer than 1000 ms. In human ventricular muscle, I_{to} recovers relatively rapidly, with a time constant of 10 ms (our own unpublished observation), but in rabbit the recovery of I_{to} is slower with time constant more than 1 s (Fermini et al., 1992; Sánchez-Chapula et al., 1994). These results suggest that although basic APD has important role in determining restitution slope and kinetics, other transmembrane currents, such as I_{Na} or I_{to}, can also play a role in restitution kinetics. This is also in agreement with earlier work in dog Purkinje fibers, where several drugs with I_{Na} and $I_{Ca\text{-}L}$ inhibition properties slowed APD restitution (Elharrar et al., 1984). It is also important to note that the basic stimulation frequency, which was 5 times higher in the study of Shattock et al. (2017), can partly explain the differences between their and our present works.

In our study, we used only undiseased donor cardiac ventricular preparations and did not study diseased tissue and atrial muscle. To the best of our knowledge there are no reported *in vitro* drug studies available with the latter preparations. Since APD restitution can be important phenomenon in the mechanism of atrial fibrillation this may be a limitation of our present investigations as such it would be worth to study in the future.

In conclusion, it should be recognized that important species differences exist in the ventricular restitution process including human. Our results indicate that the mechanism of the electrical restitution, at least in undiseased human ventricle, seems complex; and to understand it properly, further studies are needed. Based on our results, in addition to the basic APD, other factors, such as transmembrane ion currents, can influence restitution. The latter raises the possibility that ion channel modifier drugs slowing restitution kinetics may have antiarrhythmic properties by affecting electrical restitution, which may be considered in future drug development projects.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

Conception and design of the experiments: AV, LV. Collection, analysis and interpretation of data: TÁ-L, CL, BB, MB, NN, JT. Drafting the article and revising it critically for intellectual content: IB, NJ, AV, LV.

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II

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ARTICLE

Different effects of amiodarone and dofetilide on the dispersion of repolarization between well-coupled ventricular and Purkinje fibers[1]

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Tamás Árpádffy-Lovas, Zoltán Husti, István Baczkó, András Varró, and László Virág

Abstract: Increased transmural dispersion of repolarization is an established contributing factor to ventricular tachyarrhythmias. In this study, we evaluated the effect of chronic amiodarone treatment and acute administration of dofetilide in canine cardiac preparations containing electrotonically coupled Purkinje fibers (PFs) and ventricular muscle (VM) and compared the effects to those in uncoupled PF and VM preparations using the conventional microelectrode technique. Dispersion between PFs and VM was inferred from the difference in the respective action potential durations (APDs). In coupled preparations, amiodarone decreased the difference in APDs between PFs and VM, thus decreasing dispersion. In the same preparations, dofetilide increased the dispersion by causing a more pronounced prolongation in PFs. This prolongation was even more emphasized in uncoupled PF preparations, while the effect in VM was the same. In uncoupled preparations, amiodarone elicited no change on the difference in APDs. In conclusion, amiodarone decreased the dispersion between PFs and VM, while dofetilide increased it. The measured difference in APD between cardiac regions may be the affected by electrotonic coupling; thus, studying PFs and VM separately may lead to an over- or underestimation of dispersion.

Key words: dispersion of repolarization, chronic amiodarone, dofetilide, electrotonic coupling, cardiac Purkinje fibers.

Résumé : Il est établi que l'augmentation de la dispersion de la repolarisation à travers la paroi constitue un facteur contributif aux tachyarythmies ventriculaires. Dans cette étude, nous avons évalué l'effet de l'administration d'amiodarone à long terme et de l'administration aiguë de dofétilide dans des préparations canines de cœur contenant des fibres de Pukinje (FP) et du muscle ventriculaire (MV) couplés électrotoniquement, ainsi que comparé les effets à ceux de préparations de FP et de MV découplés, et ce, à l'aide de la technique de microélectrode classique. La dispersion entre les FP et le MV était déduite de la différence entre les durées des potentiels d'action (DPA) respectives. Dans les préparations couplées, l'amiodarone entraînait une diminution de la différence entre la DPA des FP et du MV, et donc une diminution de la dispersion. Dans les mêmes préparations, le dofétilide entraînait une augmentation de la dispersion par une prolongation plus marquée dans les FP. Cette prolongation était encore plus prononcée dans les préparations de FP découplées, tandis que l'effet était le même dans le MV. Dans les préparations découplées, l'amiodarone n'entraînait pas de changement dans la différence entre les DPA. En conclusion, l'amiodarone entraînait une diminution de la dispersion entre les FP et le MV, tandis que le dofétilide entraînait une augmentation de celle-ci. La différence mesurée entre la DPA des régions cardiaques pourrait être affectée par le couplage électrotonique; l'étude des FP et du MV séparément pourrait donc mener à une sous- ou une surestimation de la dispersion. [Traduit par la Rédaction]

Mots-clés : dispersion de la repolarisation, amiodarone à long terme, dofétilide, couplage électrotonique, fibres de Purkinje cardiaques.

Introduction

Dispersion of repolarization affects various mechanisms in cardiac arrhythmogenesis. Increased transmural dispersion is an established contributing factor to ventricular tachyarrhythmias (VTs), such as Torsades de Pointes arrhythmias (Antzelevitch et al. 1998). Transmural dispersion has been reduced by amiodarone ex vivo in canine wedge preparations (Sicouri et al. 1997) as well as human transmural slice preparations (Drouin et al. 1998). In vivo, transmural dispersion is derived from QT/QTc or JTc dispersion in the ECG, which have all been decreased by chronic amiodarone in a clinical setting (Cui et al. 1994, 1998), whilst dofetilide has increased global electrical heterogeneity (Stabenau et al. 2020).

Another region characterized by high dispersion is the junction between Purkinje fibers (PFs) and the ventricular myocardium (VM), also known as the Purkinje–muscle junction (PMJ). The PMJ has been described as a contributor to local heterogeneity

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of ventricular action potential duration (APD) (Walton et al. 2014; Martinez et al. 2018). Based on this, if PF repolarization lengthening is markedly stronger than that of the surrounding VM at the PMJ, early afterdepolarization (EAD) may develop in PFs, which under certain conditions can evoke propagating extra beats in VM (Nattel and Quantz 1988; Varró et al. 1990). As such, PF repolarization lengthening can serve as a trigger for arrhythmias; in addition, increased dispersion of repolarization itself can also enhance the risk of tachyarrhythmia in the PMJ as a substrate for arrhythmia (Nogami 2011*a*, 2011*b*). While transmural dispersion may be directly measured ex vivo and closely estimated in vivo, studying the dispersion of repolarization between PFs and VM is currently only possible in preparations containing electrotonically well coupled PFs and VM. Studying the effects of antiarrhytmic agents or agent candidates in such preparations may be beneficial, since decreasing dispersion could be a valid goal. Screening for drug candidates that do not increase dispersion may also increase cardiac safety. Even though the general electrophysiological effects of dofetilide (selective I_{Kr} inhibitor) and amiodarone (complex mechanism) are well understood, their effect on the dispersion between PsF and VM may only be estimated based on measurements from individual, uncoupled preparations. The aim of this study was to assess the effects of these two widely used antiarrhythmic drugs with established class III actions in preparations containing well-coupled PFs and VM to uncover additional features of these agents in the context of local dispersion.

Materials and methods

Animals

All experiments 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 No. I-74-24-2017) and by the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development (authority approval No. XIII/3331/2017).

Conventional microelectrode technique

Action potentials were recorded in preparations containing both electrotonically coupled subendocardial VM and PFs and in preparations in which VM and PF had been cut out separately (uncoupled preparations) obtained from the left ventricle and right ventricle of dogs using conventional microelectrode technique. Beagle dogs, either untreated or orally treated with amiodarone (50 mg · kg^{-1} · day^{-1}, 4 weeks), of either sex weighing 10–15 kg were sacrificed (sodium pentobarbital, 30 mg/kg administered intravenously) after an intravenous injection of 400 U/kg heparin. Then the heart of each animal was rapidly removed through a right lateral thoracotomy. The heart was immediately rinsed in oxygenated modified Locke's solution containing (in millimoles per litre): NaCl 128.3, KCl 4, $CaCl_2$ 1.8, $MgCl_2$ 0.42, $NaHCO_3$ 21.4, and glucose 10. The pH of this solution was set between 7.35 and 7.4 when gassed with the mixture of 95% O_2 and 5% CO_2 at 37 °C.

Preparations, containing free-running PFs and VM 25–35 mm in diameter and 2–4 mm in thickness (electrotonically coupled preparations from the left ventricle) (Figs. 1A–1C) and individual PFs (Fig. 1E) with small muscle endings and individual papillary VM (Figs. 1D, electrotonically uncoupled preparations from both of the left and right ventricle) were obtained and individually mounted in a tissue chamber with a volume of 50 mL. Electrotonically coupled preparations were paced from a PF, mimicking physiological cardiac conduction. Stimulation was executed using a pair of platinum electrodes in contact with the preparation using rectangular current pulses of 0.5–2 ms duration. These stimuli were delivered at a constant cycle length of 1000 ms for at least 60 min allowing the preparation to equilibrate before the measurements were initiated. Transmembrane potentials were simultaneously recorded from PF and subendocardial VM using conventional glass microelectrodes (Fig. 1) filled with 3 mol/L KCl and having tip resistances of 5–20 MΩ connected to the input of a high-impedance electrometer (Experimetria, type 309, Budapest, Hungary), which was coupled to a dual-beam oscilloscope. The resting potential (RP), action potential amplitude (APA), maximum upstroke velocity (V_{max}), and APD measured at 50% and 90% of repolarization (APD_{50} and APD_{90}, respectively) were online monitored and offline recorded using a home-made software (APES) running on a computer equipped with an ADA 3300 analog-to-digital data acquisition board (Real Time Devices, Inc., State College, Pennsylvania) having a maximum sampling frequency of 40 kHz. Dispersion of repolarization was inferred from the difference of APD_{90} values of PFs and VM, referred to as ΔAPD_{90}. Stimulation with a constant cycle length of 1000 ms was applied in the course of all experiments. Attempts were made to maintain the same impalement throughout each experiment. In case an impalement became dislodged, adjustment was attempted, and if the action potential characteristics of the reestablished impalement deviated by less than 5% from the previous measurement, the experiment continued (Lengyel et al. 2001; Jost et al. 2005; Orvos et al. 2015, 2019). All measurements were carried out at 37 °C.

Statistical analysis

All data are expressed as means ± SEM. The "*n*" number refers to the number of experiments. Depending on the type of comparison, Student's *t* test was used either for independent samples (amiodarone) or for paired samples (dofetilide). The results were considered statistically significant when p was <0.05.

Results

Comparison of baseline electrophysiology of electrotonically coupled and uncoupled preparations

In electrotonically coupled control preparations (Tables 1 and 2; Figs. 2A and 3A) most action potential characteristics both of PF and VM were comparable to those of individual PF and VM (uncoupled) preparations (n = 21) (Tables 3 and 4; Figs. 2C and 3C). VM APD was slightly longer in coupled preparations compared to the uncoupled preparations (Tables 3 and 4; Figs. 2C and 3C), while PF APD was slightly shorter in coupled preparations. APA, V_{max}, and RP were similar to those of uncoupled (individual) preparations. Baseline dispersion (ΔAPD_{90}) was 39.6 ± 4.0 ms (pooled controls, n = 21) in coupled control groups of the drug studies. Conduction time (CT) to PFs was shorter in all preparations than that of VM, confirming an anterograde wave of depolarization. Since individual, electrotonically uncoupled PF and VM preparations were not necessarily taken from the same heart and were not in connection, differences in their APD cannot be directly measured, but average values showed an APD difference of 72.8 ms between the two groups of preparations under control circumstances (pooled controls, n = 13 and 16).

Effects of amiodarone

Electrotonically coupled preparations obtained from animals after chronic amiodarone treatment (n = 11) did not show statistically significant changes in the RP, APA, and V_{max}. Amiodarone treatment increased APD_{90} and APD_{75} values of PF potentials ($p <$ 0.01) (Table 1; Fig. 2B) while eliciting no effect on the early phases of repolarization. In VM, prolongation was measured in all stages of repolarization, from APD_{10} to APD_{90} ($p < 0.01$). The prolongation of AP duration in VM was more pronounced than in PFs; thus, ΔAPD_{90} decreased substantially (18.0 ± 5.0 ms vs. 45.7 ± 5.7 ms, $p < 0.01$). APA and V_{max} of VM remained unchanged compared to the control.

Fig. 1. Photographs of an electrotonically coupled ventricular preparation. (A) Pacing microelectrode; (B) microelectrode impaled in a Purkinje fiber; (C) microelectrode impaled in a ventricular muscle); (D) an uncoupled papillary muscle; (E) an uncoupled Purkinje fiber inside the tissue bath. [Colour online.]

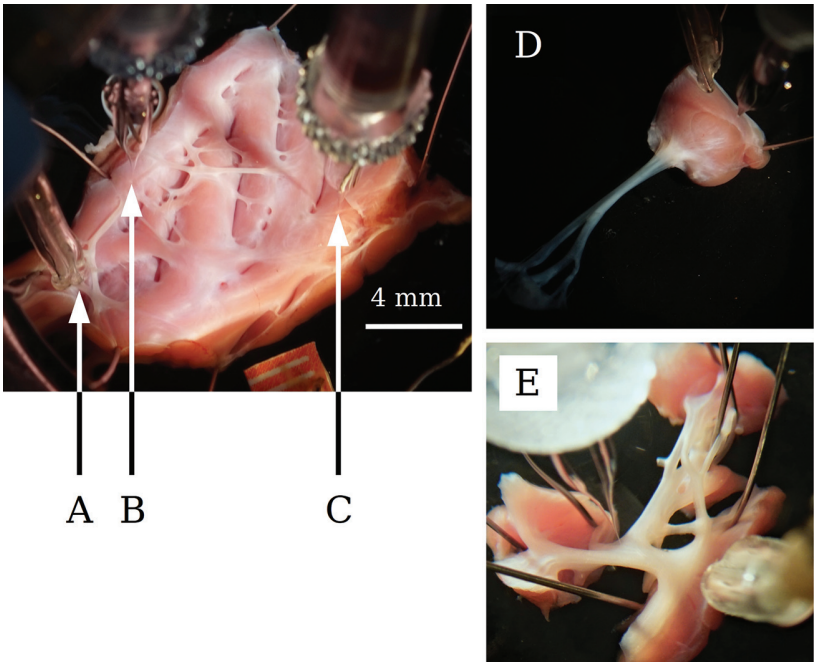

Table 1. The electrophysiological effects of 50 mg·kg^{-1}·day^{-1} amiodarone in electrotonically coupled ("C") Purkinje fiber (PF) and ventriculat muscle (VM) preparations at a basic cycle length of 1000 ms.

	Sample	CT (ms)	RP (mV)	APA (mV)	V_{max} (V/s)	APD_{90} (ms)	APD_{50} (ms)	APD_{90} difference (ms)
Control	PF-C (14)	5.2±0.7	−88.4±3.2	119.2±2.6	515.6±43.2	259.8±5.5	170.9±5.7	41.0±5.6
Amiodarone (50 mg·kg^{-1}·day^{-1})	PF-C (11)	4.0±0.6	−84.6±2	120.7±2.8	516.2±67.2	305.9±5.8$^{\#\#\#}$	184.8±10.9	18.0±5$^{\#\#}$
Control	VM-C (14)	13.5±1.2	−85.8±1.6	110.1±2.9	201.3±27.9	218.8±7.6	165.0±5.5	
Amiodarone (50 mg·kg^{-1}·day^{-1})	VM-C (11)	12.7±1.6	−87.8±2.7	106.8±3.8	150.4±46.1	288.0±5.9$^{\#\#\#}$	218.2±7.6$^{\#\#\#}$	

Note: CT, conduction time; RP, resting potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{90} and APD_{50}, action potential durations at 90% and 50% of repolarization. Results are means ± SEM. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, Student's *t* test for unpaired data.

Table 2. The electrophysiological effects of 50 nmol/L dofetilide in electrotonically coupled ("C") Purkinje fiber (PF) and ventriculat muscle (VM) preparations at a basic cycle length of 1000 ms.

	Sample	CT (ms)	RP (mV)	APA (mV)	V_{max} (V/s)	APD_{90} (ms)	APD_{50} (ms)	APD_{90} difference (ms)
Control	PF-C (7)	6.5±0.9	−90.5±3.8	123.1±4.7	369.2±55	265.0±4.4	203.4±4.4	37.0±4.3
Dofetilide (50 nmol/L)	PF-C (7)	7.1±1.2	−87.4±3.3	122.2±5.5	350.4±51.1	333.9±8.8***	244.6±8.7***	67.2±10.3**
Control	VM-C (7)	15.4±0.8	−82.8±3.7	110.6±4.5	169.9±18.5	228.6±5.7	175.9±5.6	
Dofetilide (50 nmol/L)	VM-C (7)	16.1±1.6	−80.9±3.2	108.6±2.8	178.4±33	261.3±11.5*	188.9±11	

Note: CT, conduction time; RP, resting potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{90} and APD_{50}, action potential durations at 90% and 50% of repolarization. Results are means ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, Student's *t* test for paired data.

In uncoupled PF preparations, APD_{90} and APD_{75} were increased ($p < 0.05$); the prolongation was more pronounced than in coupled preparations (Table 3; Fig. 2D). In uncoupled VM preparations, APD_{90} and APD_{50} were also increased ($p < 0.01$), although this change was less pronounced compared to the VM measurements from coupled preparations. These effects reflected an important difference between coupled and uncoupled preparations in response to chronic amiodarone treatment. Accordingly, in uncoupled preparations, amiodarone increased APD without significantly changing dispersion of repolarization between PF and VM, measured as ΔAPD_{90}. On the contrary, in coupled preparations, amiodarone increased APD in such a manner to result in a significant decrease of dispersion of repolarization, measured as ΔAPD_{90}.

Effects of dofetilide

In coupled preparations, acutely administered dofetilide ($n = 8$, 50 nM) induced a marked increase in APD90, APD75 and APD50 ($p < 0.001$) values in PF compared to control measurements (Table 2, Fig. 3B). In VM, APD90 and APD75 values were also prolonged ($p < 0.01$ and $p < 0.05$ respectively), and APD50 was markedly increased. The more pronounced prolongation of AP duration in PFs led to an increase in ΔAPD90 to 75.2 ± 12.6 ms from 47.0 ± 11.1 ms

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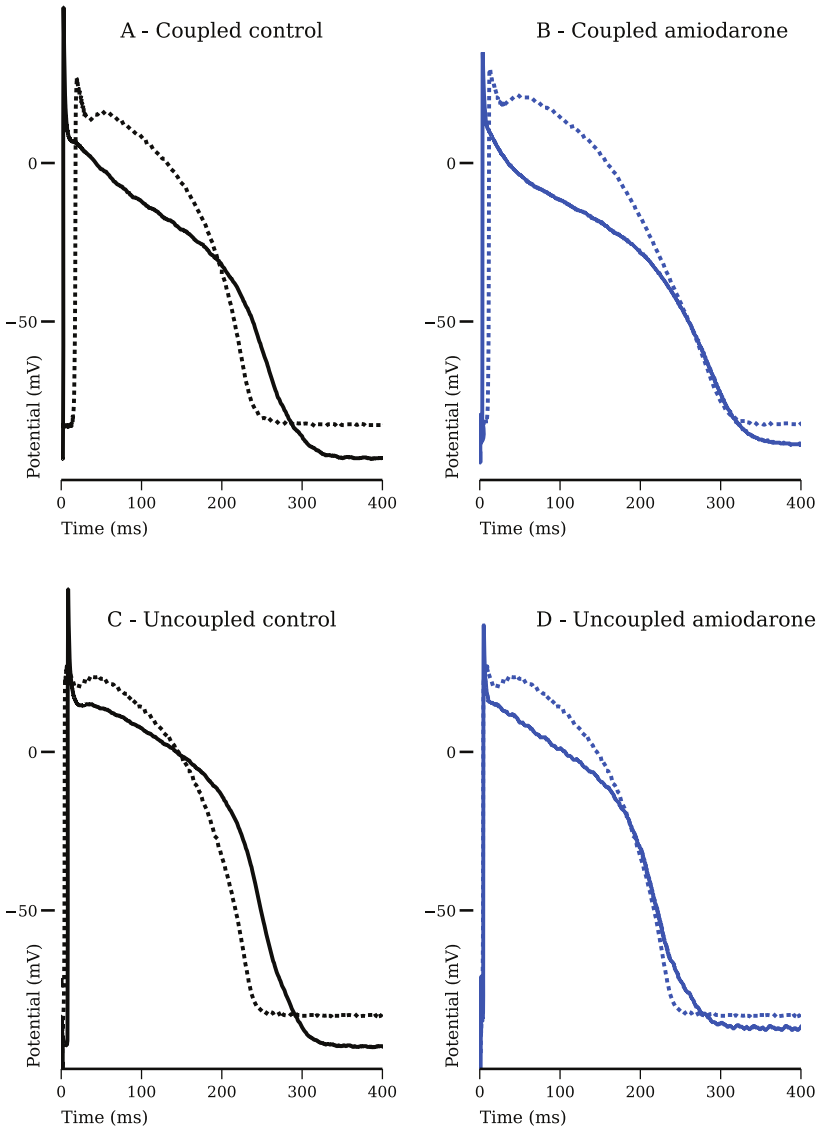
Fig. 2. The effect of chronic amiodarone (50 mg·kg^{-1}·day^{-1}) in (A and B) coupled and (C and D) uncoupled action potentials. Solid lines represent Purkinje fiber potentials and dotted lines represent ventricular action potentials; stimulation frequency was 1 Hz. [Colour online.]



($p < 0.01$). APA and V_{max} did not change. Conduction times (CTs) slightly increased after dofetilide treatment.

In uncoupled PF preparations (n = 6–6), APD_{90}, APD_{75}, and APD_{50} were also prolonged after dofetilide treatment ($p < 0.001$), but prolongation was more pronounced compared to the change in coupled preparations (Table 4; Fig. 3D). In uncoupled VM preparations, APD_{90} to APD_{25} were all prolonged after dofetilide treatment ($p < 0.01$). Even though the change in uncoupled VM APD_{90} is comparable to that of the coupled preparations, the AP of PFs showed a more pronounced prolongation, unlike with that of amiodarone treatment, indicated by the greatly increased difference in average APD_{90} values.

Discussion

Under control conditions, PF APDs were shorter in coupled preparations compared to uncoupled preparations, while VM APDs were longer when coupled with PF, as indicated in the control values of Tables 1 and 2, when compared to those of Tables 3 and 4. APs recorded from canine transitional cells in the PMJs have been previously described to have longer APD than VM and slower maximum rate of depolarization than PF but limited to the immediate surroundings of the PMJ (Martinez-Palomo et al. 1970), suggesting that the slight prolongation we observed in VM and abbreviation in PFs when measured in electrotonically coupled preparations may be attributable to the electrotonic interaction between VM and PFs. This is not the case in dissected ventricular and Purkinje preparations (i.e., individual PFs, papillary muscle, or trabecule). Therefore, these latter preparations are not affected by electrotonic coupling, which also leads to an exaggerated difference in APD; thus, when calculating dispersion from individual, uncoupled preparations, dispersion is likely to be overestimated.

Prolongation of APs in individual VM preparations has been previously reported after chronic amiodarone treatment, while PF has been not changed or shortened. Thus, the differences in APD have been decreased between PF and VM in uncoupled

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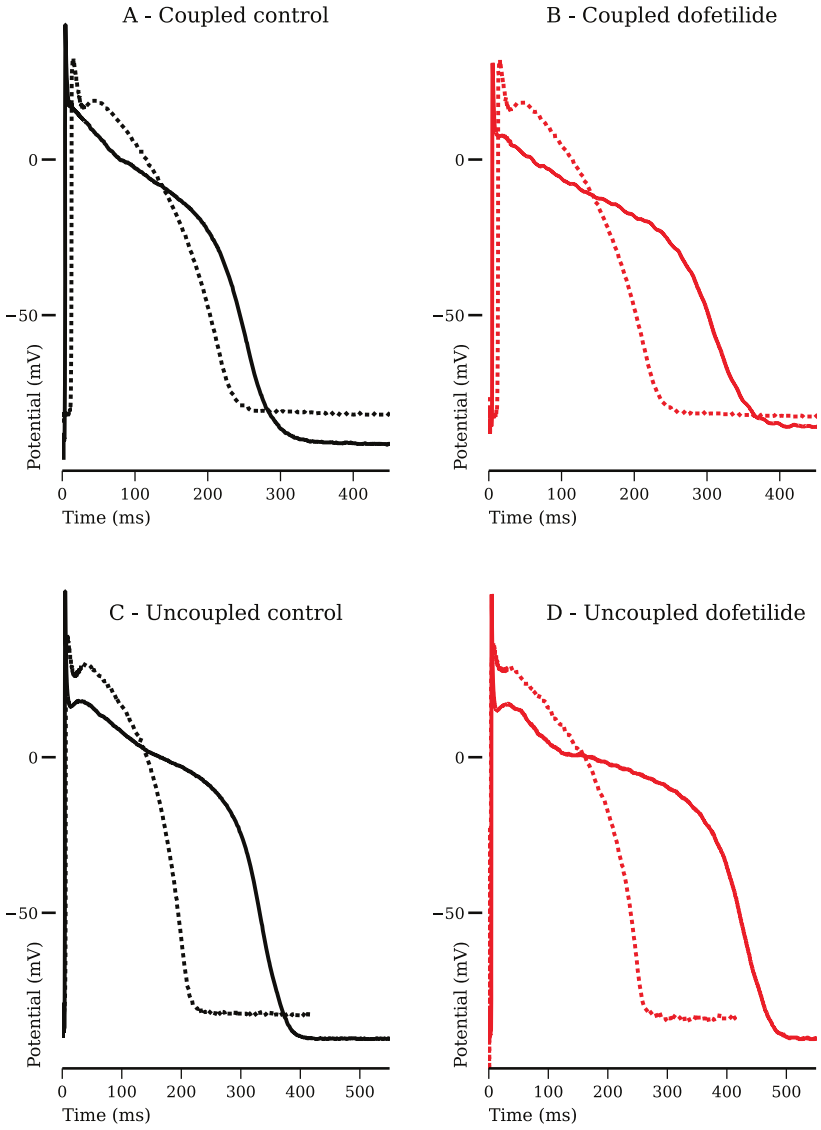
Fig. 3. The effect of dofetilide (50 nmol/L) in (A and B) coupled and (C and D) uncoupled action potentials. Solid lines represent Purkinje fiber potentials and dotted lines represent ventricular action potentials; stimulation frequency was 1 Hz. [Colour online.]



preparations (Papp et al. 1996). In our experiments, amiodarone was found to prolong APD of PF significantly in electrotonically coupled, but not in uncoupled, preparations, as seen in Fig. 2, and in uncoupled preparations resulted in no difference between the APD_{90} of PFs and VM. However, after chronic amiodarone treatment in electrotonically coupled preparations, we observed slight to moderate prolongation of PF repolarization accompanied by a much more pronounced prolongation of VM, leading to a decrease of dispersion of repolarization, reflected as lower ΔAPD_{90} (Fig. 4).

The effect of dofetilide in uncoupled cardiac PF and VM preparations is well documented (Gwilt et al. 1991; Knilans et al. 1991; Bányász et al. 2009), but such measurements have not been reported in ex vivo coupled preparations. Therefore, in this study, direct comparison between coupled and uncoupled preparations was possible after dofetilide administration. In coupled preparations, dofetilide increased ΔAPD_{90} by causing a much greater prolongation in PFs than in VM (Table 2). This difference in mean APD_{90} values was further increased in uncoupled preparations, as seen in Table 4.

The different changes in dispersion may be partially explained by the different effects of each drug on ion channels: dofetilide is considered as a selective inhibitor of the delayed rectifier outward potassium current (I_{Kr}) (Carmeliet 1992; Kiehn et al. 1994; Mounsey and DiMarco 2000), while amiodarone also inhibits inward currents, such as I_{NaL} and I_{CaL} (Follmer et al. 1987; Kodama et al. 1996; Nishimura et al. 1989), and outward currents, such as I_{Ks}, apart from I_{Kr} (Balser et al. 1991; Bertran et al. 1998; Kodama et al. 1996; Sato et al. 1994; Varró et al. 1996). Since I_{NaL} is considered more prominent in PFs than in VM (Baláti et al. 1998; Haufe et al. 2005), blocking I_{NaL} by amiodarone would limit APD lengthening in PFs more than in VM, resulting in less dispersion of repolarization.

In this work, we did not study the possible role of calcium signaling in either tissue type. Nevertheless, it is worth mentioning that in previous studies performed in uncoupled PF and VM

Table 3. The electrophysiological effects of 50 mg·kg^{-1}·day^{-1} amiodarone in uncoupled ("S") Purkinje fiber (PF) and ventricular muscle (VM) preparations at a basic cycle length of 1000 ms.

	Sample	CT (ms)	RP (mV)	APA (mV)	V_{max} (V/s)	APD_{90} (ms)	APD_{50} (ms)	APD_{90} difference (ms)
Control	PF-S (6)	5.6±0.6	−87.6±1.9	128.6±4.2	535.7±42.4	272.3±16.2	181.9±9	65.3
Amiodarone (50 mg·kg^{-1}·day^{-1})	PF-S (7)	4.1±0.7	−85.7±3.7	120.8±6.1	510.3±50.9	302.3±14.6	161.4±21.2	72.2
Control	VM-S (10)	6.1±0.4	−85.8±0.8	108.2±3.2	238.9±30.9	207±4.4	169.9±4	
Amiodarone (50 mg·kg^{-1}·day^{-1})	VM-S (11)	4.6±0.1$^{\#\#}$	−84.5±1.6	101.9±2.6	173.9±13.7	230.1±3.6$^{\#\#\#}$	180.3±3.7	

Note: CT, conduction time; RP, resting potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{90} and APD_{50}, action potential durations at 90% and 50% of repolarization. Results are means ± SEM. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$, Student's *t* test for unpaired data.

Table 4. The electrophysiological effects of 50 nM dofetilide in uncoupled ("S") Purkinje fiber (PF) and ventricular muscle (VM) preparations at a basic cycle length of 1000 ms.

	Sample	CT (ms)	RP (mV)	APA (mV)	V_{max} (V/s)	APD_{90} (ms)	APD_{50} (ms)	APD_{90} difference (ms)
Control	PF-S (7)	5.5±0.6	−87.2±1.8	133.5±2.6	487.3±42.3	280±23.5	181.9±18.5	81.3
Dofetilide (50 nmol/L)	PF-S (7)	6.1± 0.8	−88.8±1.6	134.6±2.1	446.9±40.5	409.9±12***	252.7±9.6***	168.1
Control	VM-S (6)	6.2±0.9	−86.2±2.4	116.7±3.8	192±20.3	198.7±3.1	163.8±3	
Dofetilide (50 nmol/L)	VM-S (6)	6.3±0.76	−84.6±2.5	120.6±3	195.7±22.8	241.8±6.6**	196.6±6.3**	

Note: CT, conduction time; RP, resting potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD_{90} and APD_{50}, action potential durations at 90% and 50% of repolarization. Results are means ± SEM. $^{**}p < 0.01$, $^{***}p < 0.001$, Student's *t* test for unpaired data.

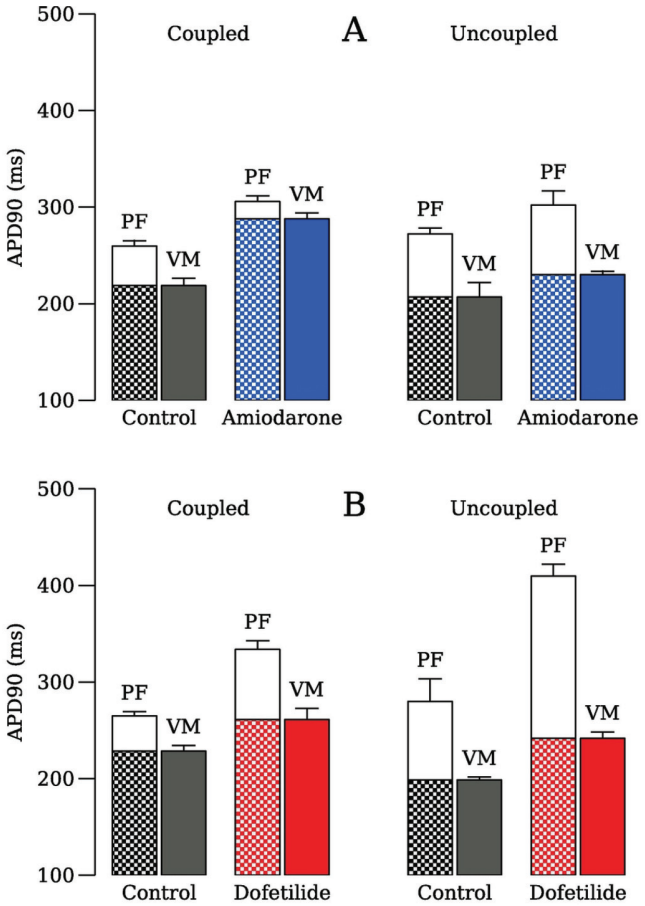


Fig. 4. The action potential differences between Purkinje fibers (PF) and ventricular muscle (VM) in electrotonically coupled and uncoupled preparations during control conditions after chronic amiodarone treatment (50 mg·kg^{-1}·day^{-1}) and in the presence of dofetilide (50 nmol/L). Bars represent means ± SEM. The white areas in the PF bars represent the difference in action potential duration between PF and VM; stimulation frequency was 1 Hz. [Colour online.]

preparations, amiodarone abolished EADs and delayed afterdepolarizations (Varró et al. 2001). On the contrary, several studies have shown that dofetilide evoked EADs in various cardiac preparations (Horváth et al. 2015; Nalos et al. 2012; Fedida et al. 2006). In some preliminary, additional experiments, we found that in coupled preparations, dofetilide evoked EADs only when administered in combination with CsCl and Bay K8644, i.e., a situation where repolarization reserve had been previously attenuated and calcium current had been activated. Therefore, understanding calcium signaling in electrotonically coupled preparations should be an aim of further studies.

The extent of AP prolongation elicited by each drug also varies between the electronically coupled and uncoupled preparations. Dofetilide elicited a more pronounced prolongation in uncoupled PFs compared to changes measured when coupled with VM. This can be witnessed when comparing Figs. 3B and 3D. PF prolongation caused by chronic amiodarone treatment was similar in coupled and uncoupled conditions, but VM prolongation was more pronounced in coupled preparations (Fig. 4). These differences may be explained by the undisturbed electrotonic coupling between the PFs and the subendocardial VM, since this interaction may lead to a slight decrease in the measured APD of PFs and slight increase in that of VM, partially evening out the distinct difference in APDs measured when the conduction system and the myocardium are dissected. Accordingly, the extent of PF prolongation caused by dofetilide may partially be modulated by the neighboring ventricular muscle, and the prolongation of VM caused by amiodarone may be, in a similar manner, potentiated by the interaction with PFs. Thus, our data from coupled and uncoupled preparations suggest that the electrotonic interaction between PFs and subendocardial myocardium affects not only the baseline electrophysiology of tissues studied using the conventional microelectrode technique but also the measured effects elicited by antiarrhythmic agents.

Therefore, amiodarone ex vivo decreased not only transmural dispersion, previously demonstrated on canine (Sicouri et al. 1997) and human (Drouin et al. 1998) preparations, but also between PFs and VM. In the clinical setting, the QT/QTc prolongation increase is associated with an increased risk of VT/VF. However, drugs that not only increase QT/QTc but also decrease the dispersion of QT/QTc or JT are accompanied by a lower proarrhythmic risk (e.g., amiodarone), while agents that prolong QT/QTc without a decrease in dispersion (e.g., quinidine) have a higher risk of arrhythmic events (Cui et al. 1994; Antzelevitch et al. 1998). It has also been reported that dofetilide increases

electrical heterogeneity in the human heart (Stabenau et al. 2020). Dofetilide is associated with a higher risk of causing torsades than amiodarone (Brendorp et al. 2002). Amiodarone has also been shown to decrease dispersion of monophasic APD_{90} (Osaka et al. 2011). The decrease in dispersion between the cardiac conductive system and myocardium may also be a beneficial action of antiarrhythmic agents, similar to the reduction of transmural dispersion, by decreasing the diversity in refractoriness between adjacent cardiac regions, consequently decreasing the risk of extra beats propagating by unidirectional block.

Conclusion

This study demonstrated that amiodarone, like dofetilide, lengthened cardiac repolarization but unlike dofetilide, it decreased dispersion of repolarization in a preparation that preserves electrotonic coupling between PFs and subendocardial VM. Also, cardiac electrophysiological drug effects can be better established in preparations with preserved electrotonic coupling than in uncoupled tissues. The observed marked differences between the effects of amiodarone and dofetilide on dispersion of repolarization in both well-coupled and uncoupled PFs and VM fibers provide a further explanation why amiodarone has a significantly less proarrhythmic risk than dofetilide, in spite of both drugs exerting a similar degree of QT lengthening in patients. This effect of amiodarone, unlike that of dofetilide, suggests an antiarrhythmic effect without a significant proarrhythmic risk. In addition, this study highlights the importance of studying dispersion of repolarization between PFs and VM in well-coupled preparations, since drug effects can be over- and underestimated in uncoupled preparations.

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ORAL PRESENTATION

Varied Effects of Selective Ion Channel Inhibitors in Human, Rat, Dog, Rabbit and Guinea Pig Cardiac Ventricular Preparations: a Comparison of Models in Cardiac Electrophysiology

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Abstract

Background: Rodents are commonly used as model organisms in health sciences, including cardiac research, due to their high accessibility and the availability of the toolset for their genetic manipulation. However, distinct differences exist between large animal models and rodents in terms of their ion channel expression profiles and action potential shapes,[1] possibly limiting the translational value of findings obtained in rodents. In this study, we aimed to directly compare the possible impact of selective inhibition of ion channels on the cardiac repolarization in preparations obtained from human hearts and from commonly utilized model species.
Methods: We applied the standard microelectrode technique at 37 °C on cardiac ventricular preparations (papillary muscles and trabecules) from human (n=63), dog (n=47), guinea pig (n=53), rat (n=43), and rabbit (n=16) hearts, paced at 1 Hz. To selectively block the I_{Ca} current, 1 µM nisoldipine; I_{Kur} current, 1 µM XEND101; I_{K1} current, 10 µM barium chloride; I_{Kr} current, 50 nM dofetilide; I_{Ks} current, 500 nM HMR-1556; and I_{to} current, 100 µM chromanol293B were applied directly to the tissue bath.
Results: The inhibition of I_{Ca} shortened action potential duration (APD) (6 %–13 %) in a similar manner between species. The block of I_{Kur} and I_{K1} elicited significantly more prominent prolongation of APD in rats (35.6 % and 67.9 %, respectively) when compared to the other species, including preparations obtained from human hearts (1.0 % and 2.6 % respectively). On the other hand, I_{Kr} block did not affect APD in rat preparations (1.6 %), whereas it elicited marked prolongation in other species (from 16.9% in dog to 47.7% in rabbit) especially being pronounced in human preparations (60.3 %). I_{Ks} inhibition elicited similar but minor APD prolongation (from 1.1 % in human to 11.4 % in rat). Inhibition of I_{to} moderately lengthened APD in dog (22.3 %) and rabbit (17.5 %), but elicited no change of APD in human preparations. In contrast, block of I_{to} caused marked APD prolongation in rat preparations (33.2 %).
Conclusion: Our findings suggest that the specific inhibition of various ion channels elicits fundamentally different effects in rodent ventricular action potential when compared to that of other species, including human. This signifies the crucial species differences in repolarization reserve and, consequently, in drug-induced proarrhythmic effects. Therefore, from a translational standpoint, rodent models in cardiac electrophysiological and arrhythmia research should be utilized with great caution.

Key words: Action potential; Animal models; Heart; Ion channels.

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