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Class I/B antiarrhythmic property of ranolazine, a novel antianginal agent, in dog and human cardiac preparations

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ABSTRACT

The aim of this study was to investigate the cellular electrophysiological effects of ranolazine on action potential characteristics. The experiments were carried out in dog and human cardiac preparations using the conventional microelectrode technique. In dog Purkinje fibres ranolazine produced a concentration- and frequency-dependent depression of the maximum rate of depolarization (V_{max}) while action potential duration (APD) was shortened. In dog and human right ventricular papillary muscle ranolazine exerted no significant effect on APD, while it produced, like mexiletine, use-dependent depression of V_{max} with relatively fast onset and offset kinetics. In dog midmyocardial preparations the drug did not exert statistically significant effect on repolarization at 10 μM, although a tendency toward prolongation was observed at 20 μM. A moderate lengthening of APD_{90} by ranolazine was noticed in canine atrial preparations obtained from dogs in sinus rhythm and in tachypacing induced remodelled preparations. Use-dependent depression of V_{max} was more pronounced in atria from dogs in sinus rhythm than those in remodelled atria or in the ventricle. These findings indicate that ranolazine, in addition to its known late sodium current blocking effect, also depresses peak I_{Na} with class I/B antiarrhythmic characteristics. Although peak I_{Na} inhibition by ranolazine is stronger in the atria, it is also substantial (at fast stimulation frequencies) in ventricular preparations. Ranolazine also decreased the dispersion of ventricular repolarization (the difference in APD_{90} values between Purkinje fibres and papillary muscles), which can contribute to the antiarrhythmic property of the drug.

1. Introduction

Ranolazine (Ranexa®) is a novel antianginal agent shown to exert anti-ischemic effects without causing significant bradycardia or hypotension (Chaitman et al., 2004a; Louis et al., 2002; Pepine and Wolff, 1999). It was found that the drug decreased the late I_{Na} (Antzelevitch et al., 2004b) which could contribute to its therapeutic effect. In addition, ranolazine has been proposed to possess atrial-predominant antiarrhythmic properties (Antzelevitch et al., 2004a; Antzelevitch et al., 2004b; Antzelevitch and Burashnikov, 2009; Burashnikov et al., 2007; Kumar et al., 2009; Sicouri et al., 2008; Wu et al., 2004) which were principally related to the inhibition of sodium current (I_{Na}), rapid delayed rectifier potassium current (I_{Kr}) and calcium current (I_{Ca}) (Allen and Chapman, 1996; Antzelevitch et al., 2004a; Rajamani et al., 2008; Rajamani et al., 2009; Schram et al., 2004; Song et al., 2004). The drug was reported to produce atrial-predominant use dependent block of sodium channels and postrepolarization refractoriness which was postulated in the mechanism of suppressing atrial fibrillation (Antzelevitch and Burashnikov, 2009; Burashnikov et al., 2007; Kumar et al., 2009). Due to its I_{Kr} blocking effect ranolazine has been shown to cause a slight prolongation of the QT interval on the ECG (Chaitman et al., 2004a; Chaitman et al., 2004b; Schram et al., 2004). The drug proved to be effective in suppressing arrhythmogenesis in models of Long QT syndromes in guinea pig, rabbit and dog (Antoons et al., 2010; Fredj et al., 2006; Song et al., 2004; Moss et al., 2008; Sicouri et al., 2007; Wu et al., 2004; Wu et al., 2006; Wu et al., 2008). The aim of our present work was to further characterise the cellular electrophysiological effects of ranolazine in dog and human heart preparations. The effects of ranolazine were mainly investigated in dog, a species resembling human in heart size, spontaneous frequency and repolarization.

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2. Materials and methods

2.1. Dog cardiac tissues

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 1985). The protocols were approved by the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development, Hungary (15.1/01031/006/2008).

Adult mongrel dogs (8–16 kg) of either sex were used. Following anaesthesia (sodium pentobarbital, 30 mg kg^{-1} administered intravenously), the heart of each animal was rapidly removed through right lateral thoracotomy. The hearts were immediately rinsed in oxygenated Locke's solution containing (in mM): NaCl, 120; KCl, 4; $CaCl_2$, 2; $MgCl_2$, 1; $NaHCO_3$, 22; and glucose, 11. The pH of this solution was 7.35 to 7.45 when gassed with 95% O_2 and 5% CO_2 at 37 °C. Papillary muscles were obtained from the right ventricle, and free-running Purkinje fibres (false tendons) were isolated from both ventricles of the hearts. The preparations were placed in a tissue bath and allowed to equilibrate for at least 1 h while superfused with oxygenated Locke's solution (flow rate 4–5 ml min^{-1}) warmed to 37 °C. To prepare midmyocardial ventricular preparations a piece from the basal part of the left ventricle was glued with tissue adhesive directly to top of the cutting stage of a vibratome (Vibratome 3000 PELCO 100 Vibratome Sectioning System, generous donation from Mr. Tamás Leisztinger). Tangential slices were cut in cold (4 °C) Locke's solution with a steel blade. The slices were placed in a preincubation chamber filled with oxygenated Locke's solution at room temperature for at least 3 h.

Table 1
The electrophysiological effects of ranolazine in dog Purkinje fibre (*top*), papillary muscle (*middle*) and midmyocardial preparation (*bottom*).

	MDP (mV)	APA (mV)	APD_{50} (ms)	APD_{90} (ms)	V_{max} (Vs^{-1})
Purkinje fibre					
Control	−86.9 ± 0.8	127.2 ± 1.2	174.8 ± 9.2	238.7 ± 10.4	669.7 ± 27.2
Ranolazine 10 μM	−85.5 ± 0.3	125.6 ± 1.4	138.6 ± 4.8[b]	215.4 ± 7.1[b]	634.7 ± 22.7
Ranolazine 20 μM	−85.0 ± 0.5	123.3 ± 1.3[a]	111.7 ± 5.6[b]	203.9 ± 5.8[b]	584.6 ± 20.2[b]
Ranolazine 50 μM	−85.9 ± 0.5	122.2 ± 1.6[b]	79.7 ± 7.0[b]	192.6 ± 4.2[b]	548.4 ± 14.8[b]
Papillary muscle					
Control	−84.2 ± 0.6	105.0 ± 2.2	178.0 ± 9.2	219.1 ± 10.1	258.0 ± 9.2
Ranolazine 10 μM	−84.3 ± 0.5	103.7 ± 2.4	175.2 ± 7.2	218.2 ± 8.0	233.9 ± 9.4
Ranolazine 20 μM	−84.0 ± 0.4	103.6 ± 2.1	173.9 ± 7.2	220.7 ± 7.5	219.7 ± 14.9[a]
M-cells					
Control	−84.5 ± 0.6	107.8 ± 1.9	206.2 ± 11.6	255.6 ± 13.8	328.6 ± 20.2
Ranolazine 10 μM	−85.1 ± 1.9	105.4 ± 2.8	205.4 ± 11.4	257.0 ± 13.5	301.3 ± 26.1
Ranolazine 20 μM	−84.3 ± 1.6	106.3 ± 2.3	213.7 ± 20.3	271.8 ± 24.2	283.5 ± 24.8[a]

MDP, maximum diastolic potential; APA, action potential amplitude; APD_{50} and APD_{90}, action potential durations at 50% and 90% of repolarization; V_{max}, maximum rate of depolarization. Results are mean ± S.E.M. Purkinje fibre: n = 11 for 10 and 20 μM, n = 9 for 50 μM, BCL = 500 ms; dog papillary muscle: n = 9, BCL = 1000 ms; dog midmyocardial preparation: n = 7, BCL = 1000 ms.
[a] P < 0.05 vs control.
[b] P < 0.01 vs control.

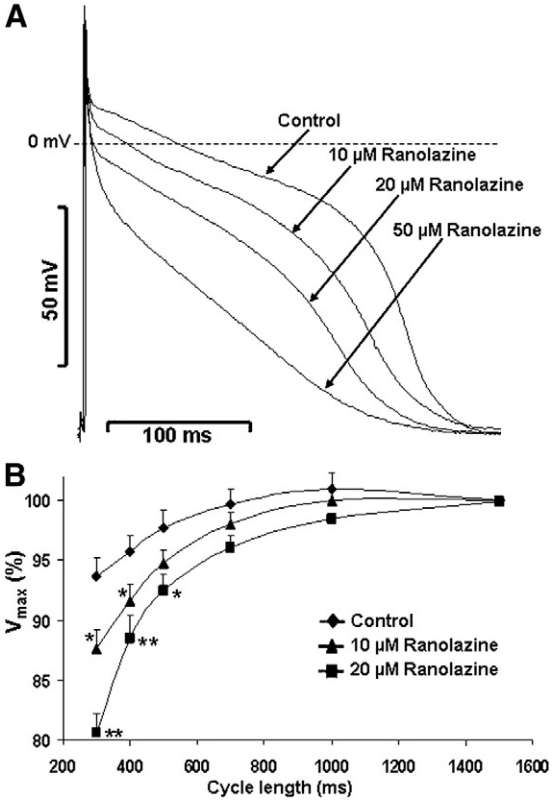

Fig. 1. Effect of ranolazine (A) on the action potential waveform and (B) on the maximum rate of depolarization (V_{max}) in dog Purkinje fibre. The dotted line represents the zero voltage level. Voltage and time calibrations for the action potential recordings are indicated on the left. Values were expressed as percentage of V_{max} measured at cycle length of 1500 ms. Results are mean ± S.E.M. * P < 0.05 and ** P < 0.01 vs control, n = 11.

Papillary muscles and midmyocardial preparations were initially stimulated (HSE [Hugo Sachs Elektronik] stimulator type 215/II, March-Hugstetten, Germany) at a basic cycle length of 1000 ms, using 2 ms rectangular constant voltage pulses (twice diastolic threshold in intensity) isolated from ground and delivered across bipolar platinum electrodes in contact with the preparation. Purkinje fibres and atrial preparations (trabecules from the free wall of the right atria) were stimulated at the basic cycle length of 500 ms. Each preparation was allowed to equilibrate for at least 1 h while they were continuously superfused with Locke's solution. Temperature of the superfusate was kept constant at 37 °C.

To obtain tachypacing induced remodelled dog atrial preparations, beagle dogs (12–16 kg) were subjected to implantation of two pacemakers (Biotronik, Berlin, Germany) with pacemaker electrodes placed into the right atrium and into the right ventricle. Radiofrequency catheter ablation of the AV node was performed to avoid high atrial pacing rates propagating into the ventricles. The ventricular pacemaker was set to between 80 and 90 beat min^{-1}, mimicking the baseline heart rate of the dog before the operation. Following recovery from surgery (3 days), high frequency right atrial pacing was started at 400 beat min^{-1} and was maintained for at least 4 weeks before the experiments started to allow full electrical remodelling of the atria. Electrical remodelling (Nattel et al., 2008) was confirmed by measuring atrial effective refractory period (i.e. AERP < 80 ms) and atrial fibrillation inducibility (by 800/min right atrial pacing bursts) *in vivo* in conscious dogs.

2.2. Human cardiac tissue

Non-diseased human hearts that were technically unusable for transplantation (based on logistical, not patient-related, considerations) were obtained from general organ donors. Before cardiac explanation, organ donor patients did not receive medication except dobutamine, furosemide and plasma expanders. The investigations

conform to the principles outlined in the *Declaration of Helsinki* of the World Medical Association. All experimental protocols were approved by the Scientific and Research Ethical Committee of the Medical Scientific Board at the Hungarian Ministry of Health (ETT-TUKEB), under ethical approval No 4991-0/2010-1018EKU (339/PI/010). Human cardiac tissue was stored in cardioplegic solution at 4 °C for 4–8 h. Papillary muscles were obtained from the right ventricle. Preparations were initially stimulated at a basic cycle length of 1000 ms and allowed at least 1 h to equilibrate while they were continuously superfused with Locke's solution. Temperature of the superfusate was kept constant at 37 °C.

2.3. Conventional microelectrode technique

Transmembrane potentials were recorded using conventional microelectrode technique as described earlier in detail (Varro et al., 2000). Microelectrodes filled with 3 M KCl and having tip resistances of 5–15 MOhm were connected to the input of a high impedance electrometer (Experimetria microelectrode amplifier type 309, Budapest, Hungary), which was connected to the ground. The voltage outputs from all amplifiers were displayed on a dual beam memory oscilloscope (Hitachi oscilloscope V-555, Japan).

The maximum diastolic potential, action potential amplitude, maximum rate of depolarization (V_{max}, equivalent to the dV/dt_{max}) and APD measured at 50% and 90% repolarization (APD_{50-90}) were obtained, using a software developed in our Department (APES) on an IBM personal computer connected to the digital output of the oscilloscope. Impulse conduction time (CT) was measured as the time difference between the end of the stimulus artefact and the midtime of the upstroke of the action potential.

The following types of stimulation 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 (or to 2000 ms in the case of Purkinje fibres to prevent spontaneous diastolic depolarization). To determine the recovery kinetics of V_{max}, extra test action potentials were elicited by using single test pulses (S_2) in a preparation driven at a basic cycle length of 400 ms. The S_1–S_2 interval was increased progressively from the end of the refractory period. The effective refractory period was defined as the longest S_1–S_2 interval at which S_2 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. To determine the onset kinetics the preparations were continuously stimulated at cycle length of 1000 ms, the stimulation was interrupted for 1 min, and then a train of 40-beat stimuli was applied with a cycle length of 400 ms. Control recordings were obtained after equilibrium period. The effects of ranolazine were determined at the given concentrations, with recordings started 30 min after the addition of each concentration of the drug.

2.4. Drugs

Ranolazine (Sequoia Research Products Ltd., Pangbourne, United Kingdom) was dissolved in distilled water at stock solution concentration of 10 mM L^{-1}. The stock solution was further diluted in the

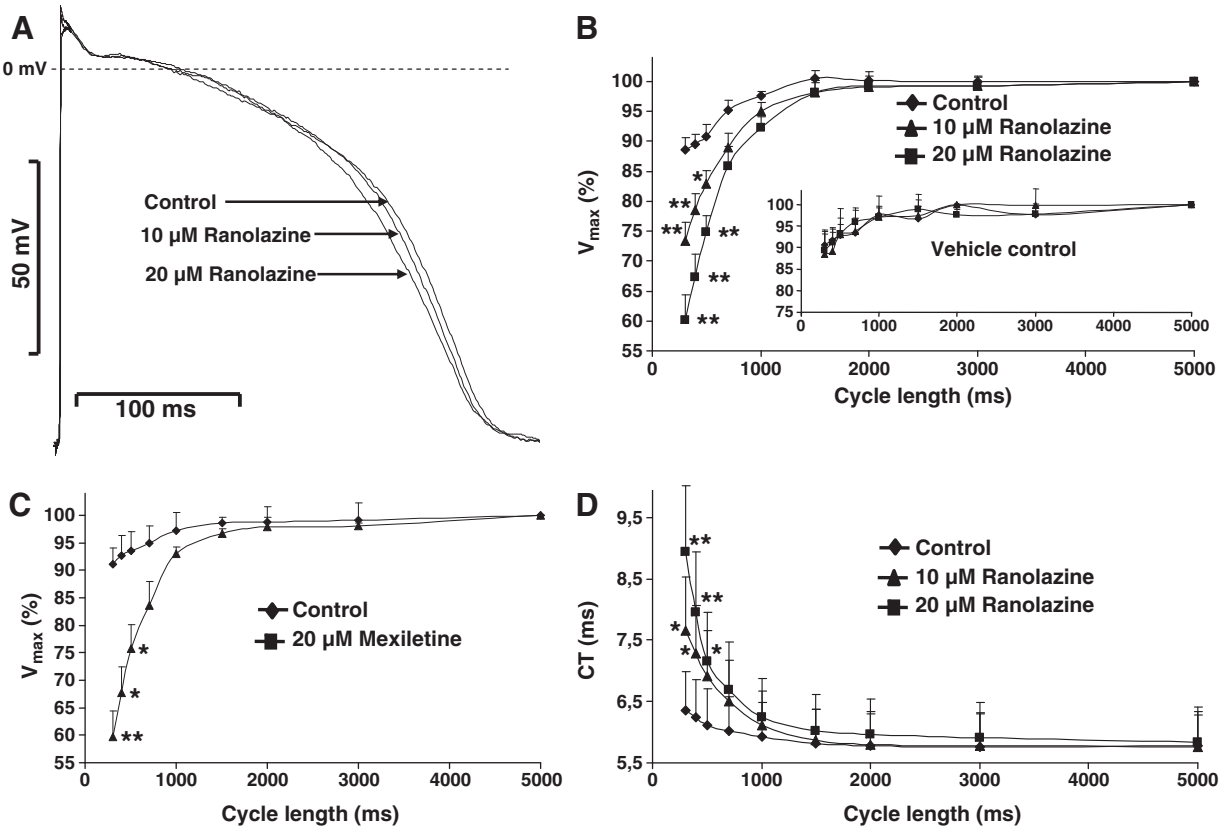

Fig. 2. Effect of ranolazine on the action potential waveform, maximum rate of depolarization and conduction time in dog papillary muscle. Comparison with mexiletine. In panel A: effect of ranolazine on the action potential waveform. The dotted line represents the zero voltage level. Voltage and time calibrations for the action potential recordings are indicated on the left. In panel B: frequency dependent effect of ranolazine on maximum rate of depolarization (V_{max}) in dog papillary muscle (inset shows vehicle control, n = 4). In panel C: frequency dependent effect of 20 μM mexiletine on V_{max} in dog papillary muscle. In panel D: frequency dependent effect of ranolazine on conduction time (CT) in dog papillary muscle. V_{max} values were expressed as percentage of V_{max} measured at long (5000 ms) cycle length. Results are mean ± S.E.M. * $P<0.05$ and ** $P<0.01$ vs control. n = 9 for B, n = 5 for C and n = 8 for D.

tissue bath to obtain the desired final drug concentrations. Mexiletine (Sigma, St. Louis, USA) was diluted in the tissue bath from a 10 mM L^{-1} aqueous stock solution.

2.5. Statistical analyses

Statistical analysis was performed using one way repeated measures analysis of variance (ANOVA) followed by Tukey's test, taking $P<0.05$ as significant. Post hoc tests were done only in case of significant F value. Data are expressed as mean ± standard error of the mean (S.E.M.).

3. Results

3.1. Dog Purkinje fibre

Ranolazine (dose- and rate-dependently) decreased the maximum rate of rise of the action potential upstroke (V_{max}) in isolated dog cardiac Purkinje fibres (Fig. 1B). Action potential duration measured at 50% and 90% of repolarization (APD_{50}, APD_{90}) was shortened in a concentration-dependent manner at pacing with a constant cycle length of 500 ms (Fig. 1A and Table 1). The reduction of APD_{50} was more pronounced than that of APD_{90} which resulted in an action potential with a triangular shape (Fig. 1A). The depression of V_{max} evoked by ranolazine was strongly dependent upon stimulation frequency ("use-dependency"); i.e., as pacing cycle length was decreased, the depression of V_{max} was increased. The block was statistically significant only at stimulation rates faster or equal of 2 Hz (Fig. 1B).

3.2. Dog papillary muscle

In dog right ventricular papillary muscle at a stimulation cycle length of 1000 ms ranolazine moderately abbreviated the action potential repolarization in 5 and lengthened it in 4 (out of 9) experiments. Therefore, on an average the drug exerted no statistically significant effect on action potential duration (Fig. 2A and Table 1). Ranolazine decreased the V_{max} and increased the impulse conduction time (CT) dose- and rate-dependently (Fig. 2B and D). The degree and frequency dependent behaviours of ranolazine induced V_{max} block were similar to those of 20 μM mexiletine (Fig. 2C). This effect was significant also at the concentration of 5 μM at basic cycle length of 300–500 ms. The decrease in V_{max} was more pronounced in case of elevating potassium concentration from 4 to 6 mM (Fig. 3A and B) and produced a shift in the normalised V_{max}-membrane potential curve to more negative potentials (Fig. 3C).

3.3. Human papillary muscle

In human right ventricular papillary muscle ranolazine moderately shortened the APD in 2 out of 4 experiments at a stimulation cycle length of 1000 ms. On average ranolazine exerted no statistically significant effect on APD (Fig. 4A and Table 2). Ranolazine dose- and rate-dependently decreased the V_{max} (Fig. 4B). This effect, like in dog papillary muscle, was similar to that of mexiletine (Fig. 4D). In human preparations pronounced biphasic effect was evident during the incubation, i.e. first shortening (faster development of late sodium channel block) and later prolongation of APD (formation of I_{Kr} block) was detected (Fig. 4C) suggesting multiple ion channel block with different drug binding kinetics.

3.4. Onset and offset kinetics of V_{max} block

In dog papillary muscles, after 1 min of rest, train of stimuli driven at the cycle length of 400 ms, the onset kinetics of 20 μM ranolazine induced V_{max} block was fitted by a single exponential, resulting in the onset rate kinetic constant of $\tau = 3.5 \pm 0.8$ $beat^{-1}$ (Fig. 5A). At the same

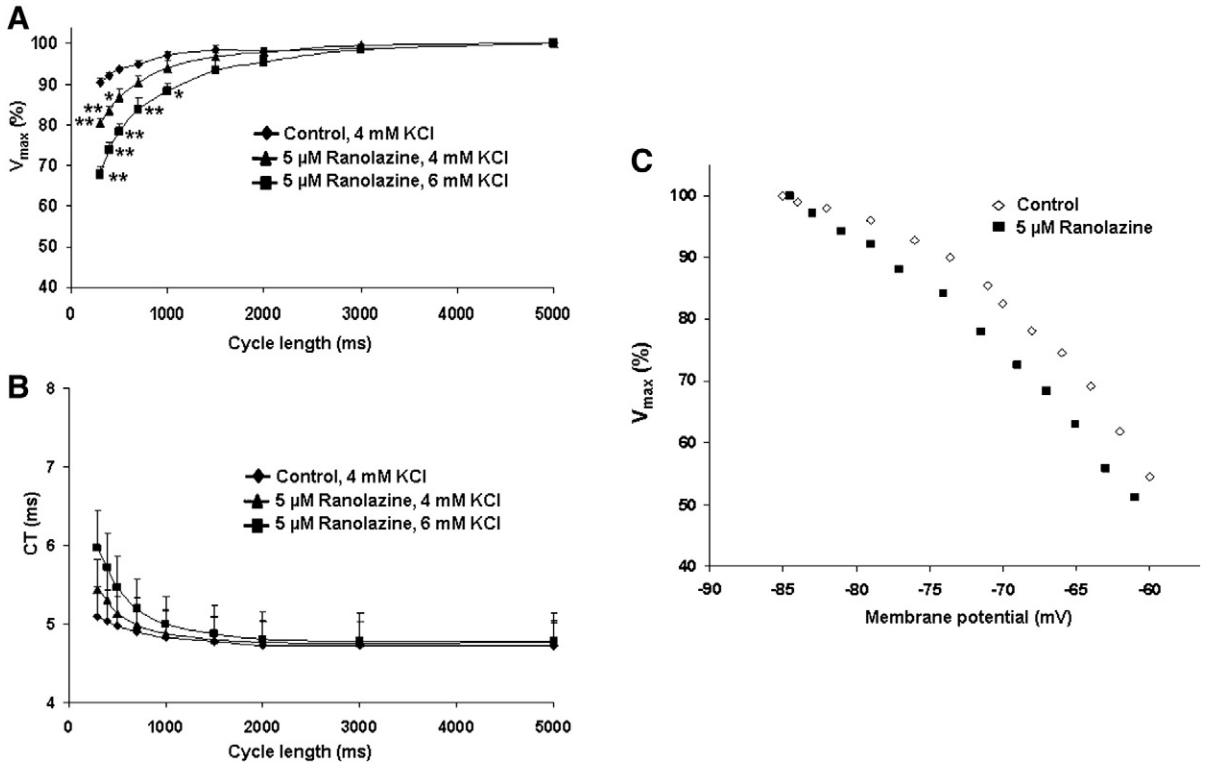

Fig. 3. Effect of 5 μM ranolazine in dog papillary muscle. In panel A and B: frequency dependent effect of 5 μM ranolazine in case of normal (4 mM) and elevated (6 mM) potassium concentration on maximum rate of depolarization (V_{max}) and on conduction time (CT), respectively. V_{max} values were expressed as percentage of V_{max} measured at long (5000 ms) cycle length. Results are mean ± S.E.M. In panel C: typical example of the effects of 5 μM ranolazine on the relationship between V_{max} and membrane potential at basic cycle length of 1000 ms. V_{max} is plotted for control conditions and 5 μM of ranolazine in the same experiment. * $P<0.05$ and ** $P<0.01$ vs control. $n=7$ for A and B.

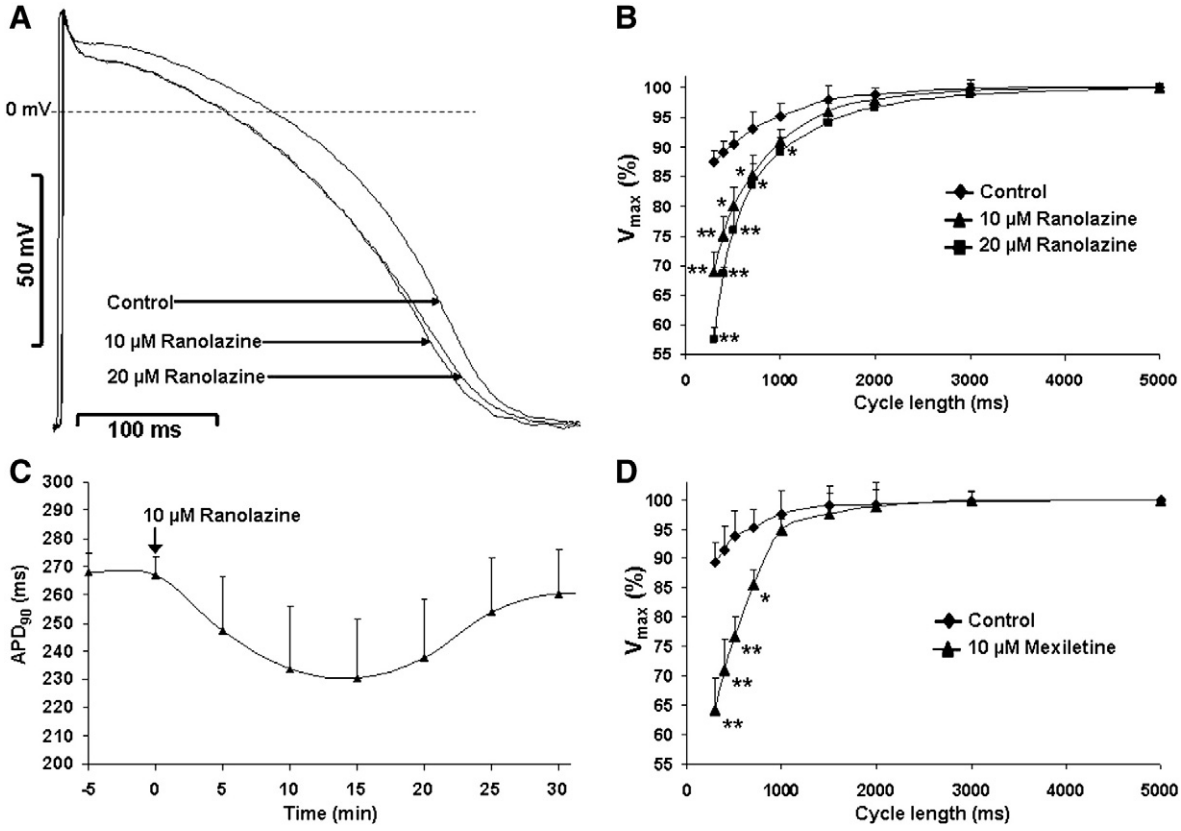

Fig. 4. Effect of ranolazine on the action potential waveform in human papillary muscle (A). The dotted line represents the zero voltage level. Voltage and time calibrations for the action potential recordings are indicated on the left. In panel B: frequency dependent effect of ranolazine on the maximum rate of depolarization (V_{max}) in human papillary muscle. Values were expressed as percentage of V_{max} measured at long (5000 ms) cycle length. In panel C: biphasic effect of ranolazine during the incubation on action potential duration at basic cycle length of 1000 ms. In panel D: frequency dependent effect of 10 μM mexiletine on V_{max} in human papillary muscle. Results are mean ± S.E.M. * $P<0.05$ and ** $P<0.01$ vs control. n = 4 for B, n = 3 for C and n = 5 for D.

stimulation cycle length of 400 ms, the recovery of V_{max} (offset kinetics) during control was best fitted to a single exponential relation. The time constant for recovery of V_{max} during control was fast ($\tau_{fast} = 35.7 \pm 5.4$ ms) and before final repolarization of the basic action potential, it was almost complete. In the presence of 20 μM ranolazine the recovery kinetics of V_{max} was best fitted with a two exponential relation. In addition to a fast component ($\tau_{fast} = 29.1 \pm 2.9$ ms) which reflects recovery of the drug-free sodium channels (Hondeghem and Katzung, 1984), a slow component ($\tau_{slow} = 1.58 \pm 0.25$ s) of recovery of V_{max} was revealed following exposure to ranolazine. This second slow component for recovery of V_{max} may reflect effects on drug-affected sodium channels (Hondeghem and Katzung, 1984) (Fig. 5B).

Table 2
The electrophysiological effects of ranolazine in human papillary muscle at basic cycle length of 1000 ms.

Human papillary muscle	MDP (mV)	APA (mV)	APD_{50} (ms)	APD_{90} (ms)	V_{max} (Vs^{-1})
Control	−85.3 ± 0.4	112.4 ± 1.1	230.0 ± 22.9	289.8 ± 21.9	318.0 ± 8.8
Ranolazine 10 μM	−85.8 ± 1.6	112.1 ± 1.3	210.5 ± 29.6	284.5 ± 30.6	309.1 ± 27.5
Ranolazine 20 μM	−85.9 ± 0.9	111.5 ± 3.2	203.9 ± 26.6	290.5 ± 30.8	291.8 ± 21.6

MDP, maximum diastolic potential; APA, action potential amplitude; APD_{50} and APD_{90}, action potential durations at 50% and 90% of repolarization; V_{max}, maximum rate of depolarization. Results are mean ± S.E.M. n = 4.

3.5. Dog midmyocardial preparations

In dog midmyocardial tissue (M-cells) 10 μM ranolazine exerted no significant effect on APD at basic cycle length of 1000 ms, however, at higher concentration (20 μM) it produced a slight prolongation of APD (Fig. 6 and Table 1). In these preparations ranolazine decreased the maximum rate of rise of the action potential upstroke (V_{max}) which was significant only in higher concentration (20 μM) at basic cycle length of 1000 ms (Table 1).

3.6. Dispersion of repolarization

In dog heart ranolazine decreased the dispersion of repolarization i.e. the difference in APD_{90} values between Purkinje fibres and papillary muscles (Fig. 7). The drug produced abbreviation in APD of Purkinje fibre and at the same time exerted no significant effect on the subendocardial layers (papillary muscle).

3.7. Dog atria

In atria from dog in sinus rhythm (SR) a statistically significant prolongation of the APD was observed in the presence of 10 μM ranolazine and this lengthening further increased at 20 μM (Figs. 8A and 9). In addition, the drug exerted a marked and significant use-dependent depression of V_{max} at basic cycle length of 300–700 ms (Fig. 8C). This block was significantly more expressed in atria than in ventricle at fast stimulation frequencies (BCL = 300–400 ms). In tachypacing induced remodelled (AF) dog atrial preparations ranolazine also produced statistically significant prolongation of APD_{90} with a

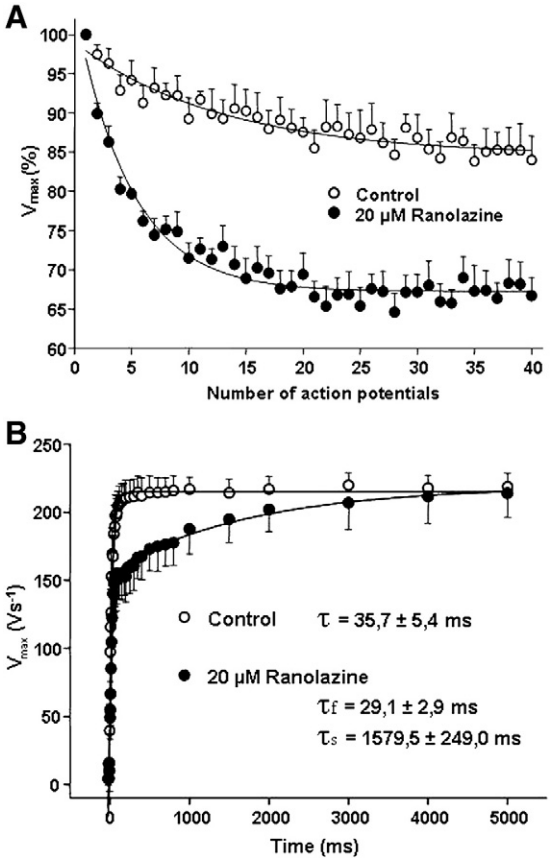

Fig. 5. Onset (A) and offset (B) kinetics of V_{max} block by ranolazine in dog right ventricular papillary muscle (BCL = 400 ms). τ_f: time constant of the fast component τ_s: time constant of the slow component. Results are mean ± S.E.M. n = 9 for A, n = 8 for B.

concomittant shortening of APD_{50} (Figs. 8B and 9). The repolarization (APD_{90}) lengthening and V_{max} blocking effects were more pronounced in SR, than in AF.

4. Discussion

The main new finding of the present study is that ranolazine at relatively high concentrations (5–20 μM) in dog and human cardiac preparations – in addition to its well-known late I_{Na} and I_{Kr} blocking effects – produces a concentration- and frequency-dependent depression of V_{max} with rather fast onset and offset kinetics, i.e. exerts mexiletine-like Class IB antiarrhythmic action not only in normal and

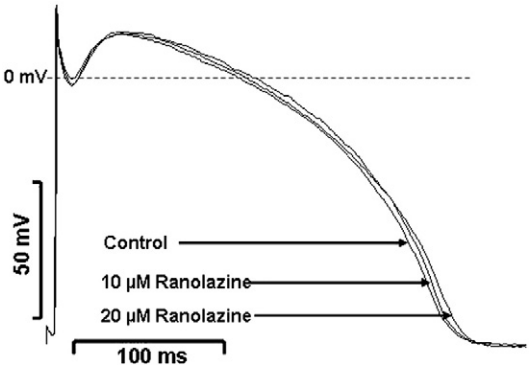

Fig. 6. Effect of ranolazine on the action potential waveform in dog midmyocardial heart slice (BCL = 1000 ms). The dotted line represents the zero voltage level. Voltage and time calibrations for the action potential recordings are indicated on the left.

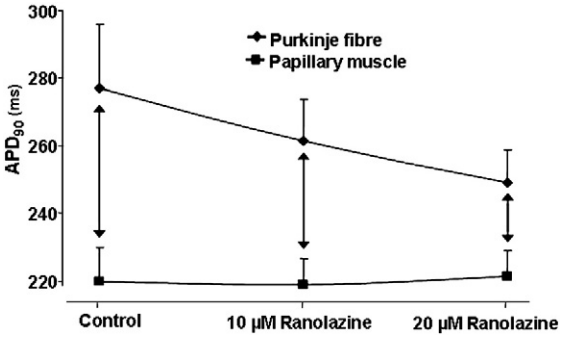

Fig. 7. Effect of ranolazine on dispersion of repolarization between dog Purkinje fibre and papillary muscle at basic cycle length of 1000 ms. Results are mean ± S.E.M. n = 11 for Purkinje fibre and n = 9 for papillary muscle.

remodelled atria, but also in the ventricle. The other important finding is that due to its multiple ion channels blocking property, ranolazine alters the repolarization in a complex manner in remodelled atria.

4.1. Dog cardiac tissue

The repolarization lengthening effect of ranolazine could be best explained by the drug evoked I_{Kr} block, while dose- and frequency dependent V_{max} block could be attributed to the inhibition of the fast/peak Na^+ current. V_{max} measurements are indicative for I_{Na} function, but they cannot be used for quantitative estimation of sodium channel availability, since it could be underestimated (Sheets et al., 1988). It was demonstrated that V_{max} could be regarded as a nonlinear indicator of the fast inward sodium current (Cohen et al., 1984). It is well established that block of sodium current is more pronounced at depolarized membrane potential (Pu et al., 1998). The decrease in V_{max} is greater in the atria (which has less negative resting potential) than in the ventricles and Purkinje fibres. In addition, the greater density of sodium channels and the more negative half-inactivation voltage in atrial myocytes could make the sodium channel block more vigorous (Antzelevitch and Burashnikov, 2009; Burashnikov et al., 2007). Similarly to that described by others earlier (Burashnikov et al., 2007; Antzelevitch and Burashnikov, 2009) the V_{max} block was more pronounced in the atria than in the ventricle. However, in our experiments the V_{max} block and consequently the CT were also substantial (at fast stimulation frequencies) in ventricular preparations. This effect of ranolazine was similar in magnitude and frequency-dependent characteristic than that of mexiletine, a well established class I/B antiarrhythmic drug. The therapeutically relevant concentration of ranolazine should be interpreted with great caution. In previous studies the authors interpreted 2–10 μM ranolazine as therapeutically relevant concentration, but they did not provide a cross reference to a factual effective plasma level (Burashnikov et al., 2007; Burashnikov and Antzelevitch, 2008; Rajamani et al., 2009; Sicouri et al., 2008; Wu et al., 2009). Authors report steady state through levels at a therapeutic dose in man to be 464 ng/ml (Abdallah and Jerling, 2005). This equates to a plasma concentration of 1 μM. Thus the doses used in our study may 5- to 20-fold higher than therapeutic concentration according to this article. Since ranolazine produced significant depression of V_{max} at fast stimulation frequencies, it can therefore be expected that the drug may suppress the impulse conduction at fast heart rate i.e. during tachycardia or that extrasystoles with short coupling interval during cardiac arrhythmias without affecting impulse conduction in normal situation. However the possible contribution of this effect to the clinical benefit of the drug is still unclear. This effect could be more pronounced in case of elevated potassium concentration, e.g. in the presence of an ischaemic cardiac substrate (Gettes, 1991). In previous studies the investigators did not apply properly wide range of stimulation

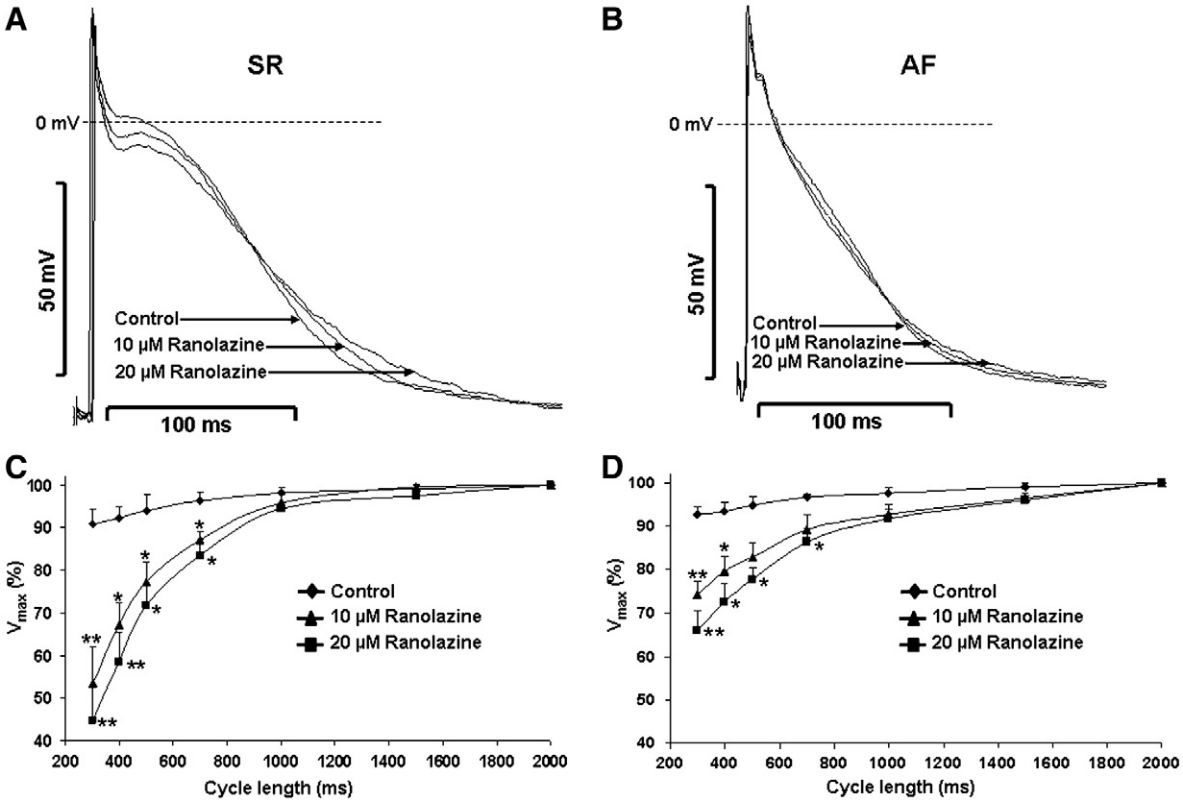

Fig. 8. Effect of ranolazine on the action potential waveform at stimulation frequency of 2 Hz in atria from dog in sinus rhythm (SR, A) and atrial fibrillation/tachypaced (AF, B). In panes C and D: frequency dependent effect of ranolazine on maximum rate of depolarization (V_{max}) in atria from dog in sinus rhythm and atrial fibrillation, respectively. V_{max} values were expressed as percentage of V_{max} measured at long (2000 ms) cycle length. The dotted line represents the zero current level. Voltage and time calibrations for the action potential recordings are indicated on the left of the figure. Results are mean ± S.E.M. * $P<0.05$ and ** $P<0.01$ vs control. n = 5 for C and n = 4 for D.

frequencies (BCL = 300–5000 ms) in the presence of ranolazine at therapeutically meaningful concentration using the conventional microelectrode technique. Therefore the effect of ranolazine on peak I_{Na} and conduction in the ventricle might have been underestimated and neglected. In a previous study the investigators found that ranolazine blocked peak I_{Na} with high IC_{50} values (at 1, 2 and 5 Hz were 260, 157 and 154 μM, respectively) in HEK293 cells using whole-cell patch-clamp technique at room temperature (Rajamani et al., 2009) which is about one order of magnitude higher than our results based on V_{max} measurements and should be extrapolated with caution to intact heart including humans. Fredj et al. found preferential ranolazine block of sustained vs peak Na^+ channel current for LQT-3 mutant channels (IC_{50} = 15 vs 135 μM) also in HEK293 cells using patch-clamp technique at room temperature which ratio in our experiments – in 'healthy' cardiac preparations at 37 °C – is almost 1:1. The effects of ranolazine on onset and offset kinetics of V_{max} indicate that ranolazine kinetically resembles fast/intermediate (Class IB) antiarrhythmic agents. Similar results were described by others earlier at high concentration (Antzelevitch and Burashnikov, 2009; Burashnikov et al., 2007) using different protocol to determine unbinding kinetics.

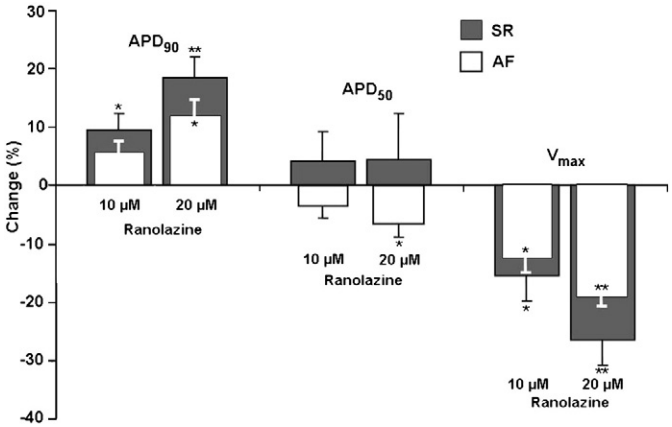

Fig. 9. The electrophysiological effects of ranolazine in atria from dog in sinus rhythm (SR) and atrial fibrillation/tachypaced (AF) at basic cycle length of 500 ms. Results are mean ± S.E.M. * $P<0.05$ and ** $P<0.01$ vs control. n = 5 for both groups.

In Purkinje fibres, where the late I_{Na} is robust, ranolazine produced shortening of the action potential duration in accordance with a previous study (Antzelevitch et al., 2004b). This ability can be considered as an advantageous property by decreasing the dispersion of repolarization in the subendocardial layers of the ventricle (Balati et al., 1998). Excessive shortening of the Purkinje fibre APD may increase the risk of reentry (Shryock and Belardinelli, 2008), but in the case of ranolazine the subsidiary I_{Kr} blocking effect and the postrepolarization refractoriness attenuate this potential risk factor. In other ventricular preparations, the effect of ranolazine on APD depends on the different density of various ion channels (late I_{Na}, late I_{Ca}, I_{Kr}, and I_{Ks}) and the extent of blocking effects of the compound on these channels. In dog midmyocardial preparations, at the concentration of 20 μM ranolazine, produced prolongation of repolarization in accordance with the observation that the drug can cause a slight prolongation of the QT interval on the ECG (Chaitman et al., 2004a; Chaitman et al., 2004b; Schram et al., 2004).

4.2. Atrial preparations

The investigation of the effects of ranolazine in atria from tachypacing induced remodelled dogs (used as an established model for atrial fibrillation) also yielded novel finding suggesting that the repolarization lengthening effect of the drug is not to be expected to vanish in remodelled preparations or during chronic atrial fibrillation. In AF the strong use-dependent Na^+-channel block by ranolazine most likely enhances postrepolarization refractoriness in addition to the repolarization lengthening in the remodelled atria. Despite the prolongation in the APD_{90}, ranolazine decreased the APD_{50} value in remodelled atria, which may reflect inhibitory effect on late I_{Na}. The latter current was recently reported to be upregulated in isolated human atrial myocytes obtained from AF patients in experiments with patch-clamp technique (Sossalla et al., 2010). The frequency dependent V_{max} block was less pronounced in AF than in SR. This is in accordance with a previous study in which reduced peak I_{Na} density was detected in AF (Sossalla et al., 2010). The less pronounced effect on V_{max} by ranolazine in remodelled atrial tissue can be explained by shorter repolarization and, as a consequence, longer diastolic intervals allowing more recovery at each cycle.

4.3. Human cardiac tissue

To our knowledge the present study is the first in which the effects of ranolazine were investigated in human ventricular muscle preparations with conventional microelectrode technique. We observed a rate dependent depression of the V_{max} to similar extent as in dog papillary muscles. Also our results suggest that late I_{Na} has less contribution to repolarization in dog than in human ventricular preparations. APD_{50} values were shortened in human but not in dog ventricular muscle (Table 1 and 2). In this context it should be noted that biphasic time dependent changes were observed (especially in human preparations) with ranolazine, i.e. the APD shortening effect always preceded the drug evoked tendency to repolarization lengthening. The initial shortening of APD was much greater in human than in dog, which may also indicate, that late I_{Na} has less contribution to repolarization in dog.

4.4. Clinical implications

We concluded that relatively high concentrations of ranolazine in addition to blocking the late I_{Na} and I_{Kr}, also inhibit the fast/peak sodium channels with rather fast onset and offset kinetics. Also ranolazine decreases the dispersion of repolarization between Purkinje fibres and the subendocardial muscle layers. These properties of ranolazine resemble that of chronically administered amiodarone and may represent antiarrhythmic property. Clinical findings support the suggestion that ranolazine might be efficacious not only in supraventricular but also in ventricular arrhythmias (Scirica et al., 2007). Although proarrhythmic side effects do not seem to be a major concern during ranolazine application, they cannot be completely ruled out in some pathological conditions such as certain types of long QT syndrome or any kind of attenuated repolarization reserve. Therefore, further studies with ranolazine are needed to determine its safety and efficacy in future clinical use.

Conflicts of interest

None.

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