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Reverse rate dependency is an intrinsic property of canine cardiac preparations

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Aims Class III antiarrhythmic agents exhibit reverse rate-dependent lengthening of the action potential duration (APD). In spite of the several theories developed so far to explain this reverse rate dependency (RRD), its mechanism has not yet been clarified. The aim of the present work was to further elucidate the mechanisms responsible for reverse rate-dependent drug effects.
Methods and results Action potentials were recorded from multicellular canine ventricular preparations and isolated cardiomyocytes, at cycle lengths (CLs) varying from 0.3 to 5 s, using conventional sharp microelectrodes. APD was either modified by applying inward and outward current pulses, or by superfusion of agents known to lengthen and shorten APD. Net membrane current (I_m) was calculated from action potential waveforms. The hypothesis that RRD may be implicit in the relationship between I_m and APD was tested by numerical modelling. Both drug-induced lengthening (by veratrine, BAY-K 8644, dofetilide, and $BaCl_2$) and shortening (by lidocaine and nicorandil) of action potentials displayed RRD, i.e. changes in APD were greater at longer than at shorter CL. A similar dependency of effect on CL was found when repolarization was modified by injection of inward or outward current pulses. I_m measured at various points during repolarization was inversely proportional to APD and to CL. Model simulations showed that RRD is expected as a consequence of the non-linearity of the relationship between I_m and APD.
Conclusion RRD of APD modulation is shared, although with differences in magnitude, by interventions of very different nature. RRD can be interpreted as a consequence of the relationship between I_m and APD and, as such, is expected in all species having positive APD–CL relationship. This implies that the development of agents prolonging APD with direct rate dependency, or even completely devoid of RRD, may be difficult to achieve.

1. Introduction

Class III antiarrhythmic agents are known to display a reverse rate-dependent lengthening of action potential duration (APD).[1,2] This reverse rate-dependent nature of the APD lengthening is undesirable because it minimizes drug effects on repolarization during tachyarrhythmias and enhances their proarrhythmic potential at normal rates.[1,3,4] Several hypotheses have been developed so far to explain the mechanism of the reverse rate dependence (RRD). Based on results obtained in guinea pig ventricular myocytes, it was first suggested that the significant accumulation of I_{Ks} may occur due to the incomplete deactivation of the current at fast heart rates, which would greatly attenuate the APD lengthening effect of I_{Kr} blockade.[5] According to another hypothesis, the effect of I_{Kr} blockers is reduced, and consequently the drug-induced prolongation of APD becomes diminished, at fast heart rates due to the potassium accumulation in the sarcolemmal clefts. This theory was based on observations showing that I_{Kr} blockade by quinidine and dofetilide was attenuated when extracellular potassium concentration was elevated.[6] A third hypothesis was based on rate dependency of drug-channel interaction. Drug access to the channel and its binding and unbinding rates depend on the state of the channel,[7,8] which dynamically changes during the cardiac cycle. If channel block developed preferentially during the diastole and dissipated during the action potential, reverse rate-dependent

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prolongation of APD would be expected. Finally, we reported that, because of their kinetic properties, I_{Kr} and I_{K1} interact with rate-dependent changes in repolarization in a way potentially contributing to RRD.[9,10]

In spite of the plethora of theories developed so far to reveal the possible mechanisms underlying RRD, the exact mechanism still remains to be elucidated. We have recently proposed that RRD may simply reflect a property of the relationship linking membrane current to APD.[11] Here we address such a hypothesis by analysing the rate dependency of APD modulation by drugs that either enhance or block a variety of cardiac ion currents and by transmembrane current injection. Moreover, we test by numerical simulations to which extent the relationship between net membrane current and APD can account for the experimental observations. Canine cardiac preparations were chosen because their electrophysiological properties are believed to most resemble those of the human ones.[12–15]

2. Methods

2.1 Preparations

Adult mongrel dogs of either sex were anesthetized with intravenous injections of 5 mg/kg ketamine hydrochloride (Calypsol, Richter Gedeon Rt., Budapest, Hungary) +0.04 mg/kg xylazine hydrochloride (CP-Xilazin, CP-Pharma, Burgdorf, Germany) according to a protocol approved by the local Animal Care Committee (CAR I-74-66/2005) in compliance with the Guide for the Care and Use of Laboratory Animals (USA NIH publication NO 86-23, revised 1985). After opening the chest, the hearts were quickly removed and placed in Tyrode solution containing (in mM): NaCl 140, KCl 5.4, $CaCl_2$ 2.5, $MgCl_2$ 1.2, HEPES 5, glucose 10, at pH 7.4. A wedge-shaped section of the left ventricular wall, supplied by the left anterior descending coronary artery, was dissected and cannulated for isolation of single myocytes. Right ventricular papillary muscles and free-running Purkinje fibres were excised for standard microelectrode measurements. Chemicals used in the experiments were obtained from Sigma-Aldrich Co. (St Louis, MO, USA).

Single myocytes were obtained by enzymatic dispersion using the segment perfusion technique.[16] Briefly, the left anterior descending coronary artery was perfused using a Langendorff apparatus. Ca^{2+}-free JMM solution (Minimum Essential Medium Eagle, Joklik modification), supplemented with 2.5 g/L taurine, 175 mg/L pyruvic acid, 750 mg/L ribose, 13.5 mg/L allopurinol, and 200 mg/L NaH_2PO_4, was used during the initial 5 min of perfusion to remove Ca^{2+} and blood from the tissue. After addition of 1.3 g/L $NaHCO_3$, the pH of this perfusate was adjusted to 6.9 by equilibrating the solution with a mixture of 95% O_2 and 5% CO_2. Cell dispersion was performed from the endocardial layer of the ventricular wall for 30 min in the same solution containing also 660 mg/L collagenase, 2 g/L bovine albumin, and 50 μM $CaCl_2$. During the isolation procedure, the solutions were gassed with carbogen and the temperature was maintained at 37°C. The cells were stored in JMM solution until use. They were rod shaped and showed clear striation when the external calcium was restored.

2.2 Electrophysiology

The cells were sedimented in a plexiglass chamber allowing continuous superfusion with oxygenized Tyrode solution at 37°C. Transmembrane potential (V_m) was recorded using 3 M KCl filled sharp glass microelectrodes having tip resistance between 20 and 40 MΩ. These electrodes were connected to the input of an Axopatch-200B amplifier (Axon Instruments, Foster City, CA, USA) under current clamp conditions, allowing for injection of inward or outward current pulses. These current pulses, having durations longer than the expected APD, were injected from the upstroke of every 20th action potential. Thus, action potentials with current injections were separated from one another by 19 regular action potential waveforms. The cells were paced through the recording electrode using 1 ms wide rectangular current pulses with twice threshold amplitude. The cycle length (CL) was initially set to 5 s, and following stabilization of action potential configuration the CL was continuously varied to the shorter values. Action potentials were digitized at 100 kHz using Digidata 1200 A/D card (Axon Instruments) and stored for later analysis. Since the cytosol was not dialyzed, time-dependent changes in APD were negligible for at least 60 min under these experimental conditions.

Pharmacological studies were performed in right ventricular papillary muscles and Purkinje fibres. Multicellular preparations were selected to prevent the limitations inherent to single myocyte studies (absence of intercellular clefts, potential damage to channel proteins, etc.) in the generalization to the *in vivo* condition. I_{CaL} and I_{Na} blockers (nicorandil and lidocaine) were tested in Purkinje fibres, in which the blocked currents are strongly represented during repolarization. Preparations were paced through a pair of platinum electrodes; the pacing CL was initially set to 1 s for at least 60 min allowing the preparations to equilibrate before starting the measurement, then it was gradually reduced from 5 to 3, 2, 1.5, 1, 0.7, 0.5, 0.4, and 0.3 s. In case of Purkinje strands, the longest CL was 3 s. After taking control records, the preparations were superfused for 40 min with the drug tested and the measurement was repeated at each pacing CL. Glass microelectrodes, filled with 3 M KCl and having tip resistances of 5–20 MΩ, were used to record V_m. Efforts were made to maintain the same impalement throughout each experiment. If, however, an impalement became dislodged, adjustment was attempted, and if the action potential characteristics (both APD_{90} and maximum rate of depolarization) of the re-established impalement deviated by less than 5% from the previous measurement, the experiment continued.

2.3 Numerical simulations

The experimental results of this study suggested that RRD is the consequence of a general mechanism, i.e. one independent of the specific intervention through which APD modulation is achieved. The purpose of the numerical simulations was to test the working hypothesis that such a general mechanism could be provided by the features of the relation linking net transmembrane current to APD. 'Classical' action potential models were not suitable for this purpose because they include a description of specific channel gating properties potentially contributing to RRD. Exclusion of such a contribution was strictly required for unequivocal testing of the working hypothesis, which is by definition independent of specific channel gating mechanisms. The approach used was a calculation of the point-by-point change in the time-course of repolarization caused by addition of a constant current. The procedure was applied to real action potential waveforms, previously acquired from canine myocytes at two CLs. The expectation was that, because of their different duration, the action potentials recorded at different CLs would be differently prolonged (or shortened) by addition of an identical amount of current. It should be stressed that such an approach, tailored to address the working hypothesis suggested by the experimental results of this study, would be unsuitable to predict action potential modulation by changes in individual conductances.

The simulation is based on the following principles. In a single cell (i.e. under space-clamp conditions), total membrane current during the action potential is null,[17,18] therefore the net transmembrane ionic current (I_m) equals the capacitive current (I_c) with an opposite sign.[17] The capacitive current can be calculated by multiplying membrane potential velocity ($V_m' = dV_m/dt$) by membrane electrical

capacitance (C_m) ($I_c = V_m' * C_m$); thus:

$$I_m = -C_m * V_m' \quad \text{or} \quad V_m' = -\frac{I_m}{C_m} \tag{1}$$

This implies that, in a single myocyte (with constant C_m), membrane potential velocity (V_m') is representative of net transmembrane current (I_m); therefore, the latter can be estimated by differentiation of the action potential waveform.

During repolarization membrane capacitance is charged by I_m; since this process is continuous in time (i.e. the interval between subsequent V_m values is $1/\infty$), I_m magnitude sets the time required for achievement of a given V_m difference [as implied by Eq. (1)]. A digitized action potential waveform is a $V_m(t)$ matrix in which t is finite and determined by the sampling interval (1 ms).

The simulation was implemented as follows (the term 'array' is used for a matrix of two dimensions (e.g. V_m and t). The term 'vector' refers to a mono-dimensional matrix (e.g. (t)); positions within a vector are defined by the index 'i'.): the repolarization phase of endocardial action potentials recorded at CLs of 3 and 5 s from the same impalement (sampling rate 1 kHz) were used as control waveform arrays ($V_m(t_{cont})$); the respective control V_m' arrays were obtained by numerical differentiation and filtered for noise minimization (FFT algorithm, Microcal Origin). The effect of adding a constant inward I_m (-0.066 pA/pF) was calculated as a delay in the achievement of each V_m value during repolarization; the calculation was repeated for all the values of V_m to obtain a time vector ($t_{test(i)}$) defining the repolarization courses during injection of the test I_m:

$$t_{test}(i) = t_{cont}(i-1) + \frac{dV_m}{V_{m'(i)}} \tag{2}$$

where t_{cont} is the control time vector and dV_m is the V_m change between two consecutive time points. The repolarization course during current injection is thus described by $V_m(t_{test})$ arrays, one for each CL, compared with the respective $V_m(t_{cont})$ arrays in *Figure 5A*.

The cumulative point-by-point change in repolarization time caused by current injection was calculated by digital subtraction of the respective time vectors ($t_{test(i)} - t_{cont(i)}$) (*Figure 5B*). Differentiation of the subtraction vector (instantaneous difference, *Figure 5C*) provides an estimate of how the impact of a change in I_m on APD may vary as a function of the portion of repolarization during which it occurs.

3. Results

3.1 Experimental studies

Rate-dependent effects of several compounds were tested on APD in multicellular canine cardiac preparations including ventricular papillary muscles and Purkinje fibres. These drugs were chosen to represent a variety of modes of action. Some of them, like the I_{Kr} blocker dofetilide, the I_{K1} blocker $BaCl_2$, the I_{Na} activator veratrine, or the I_{Ca} activator Bay K 8644 lengthened action potentials. Other drugs, including the $I_{K(ATP)}$ activator nicorandil and the dominantly I_{Na} blocker lidocaine, caused shortening of APD. However, all these agents shared a common feature: their shortening and lengthening effects on APD showed reverse rate-dependent properties. Specifically, both the absolute magnitude of APD changes and also their percentage form were greater at longer than at shorter pacing CLs independently of the mode of action of the drug, or the type of the preparation studied (*Figure 1*).

Based on the results above, the possibility arises that simply the longer action potentials can be lengthened or

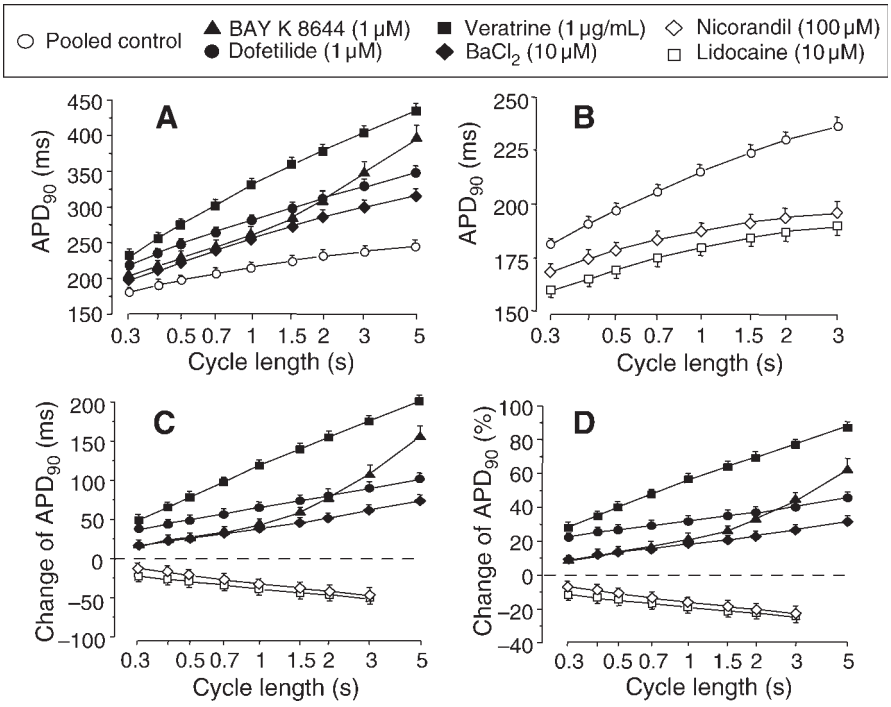

Figure 1 Changes in action potential duration (APD_{90}) induced by various ion channel blockers and activators in multicellular canine cardiac preparations. APD was lengthened by 1 μM dofetilide ($n = 13$), 10 μM $BaCl_2$ ($n = 11$), 1 μM BAY K 8644 ($n = 6$), and 1 μg/mL veratrine ($n = 7$) in right ventricular papillary muscles, while it was shortened by 100 μM nicorandil ($n = 7$) and 10 μM lidocaine ($n = 7$) in Purkinje fibres. The pacing CL was gradually decreased (from 5 to 0.3 s in papillary muscles and from 3 to 0.3 s in Purkinje fibres) before and 40 min after the administration of drugs. (*A* and *B*) Average APD_{90} values obtained under various experimental conditions as a function of CL in papillary muscles and Purkinje fibres, respectively. (*C*) Drug-induced changes of APD_{90} given in ms, (*D*) changes normalized to the control values and expressed as percentage. Symbols and bars denote mean ± SEM values.

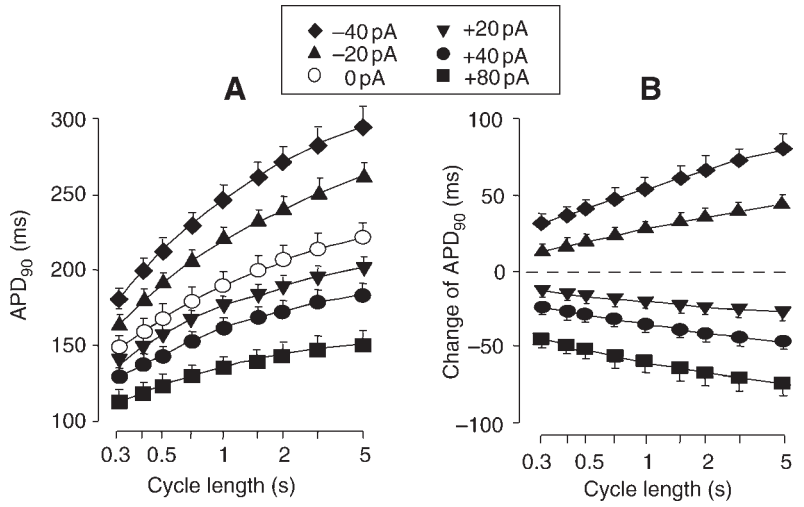

Figure 2 Rate-dependent changes in action potential duration induced by depolarizing (inward) and hyperpolarizing (outward) current pulses, injected throughout the entire duration of the action potential, in 26 single canine ventricular myocytes as a function of the pacing CL. (*A*) Measured APD_{90} values, (*B*) changes of APD induced by current pulses. Symbols and bars denote mean ± SEM values.

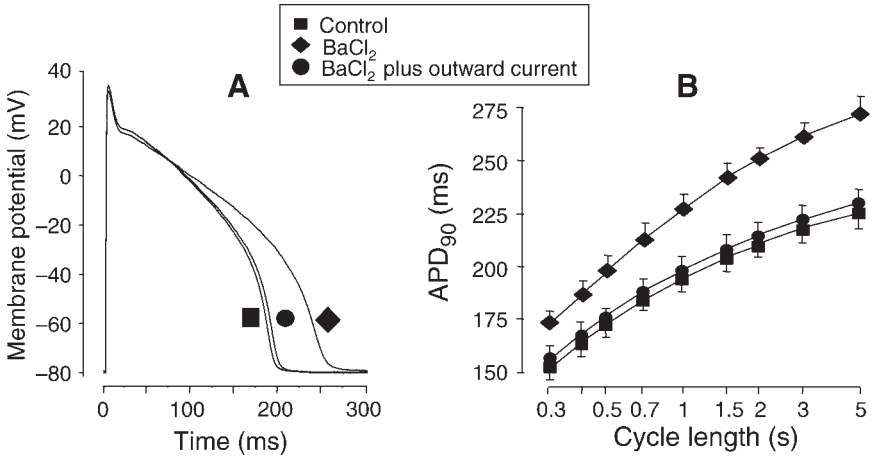

Figure 3 Combined effects of $BaCl_2$ and outward current on the rate-dependent changes of APD. (*A*) Representative superimposed action potentials recorded at 0.2 Hz in control, in the presence of 10 μM $BaCl_2$, and in case of outward current injection in the presence of $BaCl_2$. (*B*) Average values of APD_{90} obtained in seven myocytes. Symbols and bars denote mean ± SEM values.

shortened in a greater extent than the shorter ones. This hypothesis was tested in 26 isolated ventricular myocytes using inward and outward current pulses to lengthen and shorten APD, respectively, and APD was plotted against the pacing CL. *Figure 2* indicates that the lengthening or shortening effect of a given inward or outward current pulse is increasing with increasing the pacing CL, when the action potentials are *sui generis* longer. This finding suggests that the magnitude of APD prolonging effect is exclusively a function of the initial APD and it is independent of the nature of the current change (electrical impulse or drug action). This point is conclusively demonstrated in *Figure 3*, showing the effects of reversing drug-induced (10 μM $BaCl_2$) APD prolongation by concomitant injection of outward current. APD prolongation by $BaCl_2$ is the consequence of I_{K1} blockade. Neither I_{K1}, nor its inhibition by $BaCl_2$ are significantly rate-dependent; thus making this experiment best suited to unveil a mechanism of RRD independent of specific channel or drug properties. In the absence of current injection, $BaCl_2$ prolonged APD (*Figure 3A*) and made its dependency on CL steeper (*Figure 3B*), i.e. $BaCl_2$ effect showed clear cut RRD. The $BaCl_2$-induced prolongation of APD was completely offset at all CLs by concomitant injection of the same amount of current (39 ± 3 pA in seven experiments, *Figure 3A*). At the same time, current injection also reduced the steepness of its CL dependence back to control value, thus eliminating RRD of the $BaCl_2$ effect. This implies that the current change induced by $BaCl_2$ was the same at all CLs; thus, the resulting APD prolongation was only a function of control APD value, which in turn proportional to CL.

Equations (1) and (3) predict that APD depends on net membrane current (I_m) according to a non-linear function. This prediction was tested by plotting I_m at 50% of APD [I_{m50}, calculated from V_m by Eq. (1)] as a function of APD at various CLs and in the presence or absence of inward and outward current pulses (*Figure 4A*). I_{m50} was inversely related to APD according to a non-linear relation, and I_{m50} values fell on the same curve irrespective of whether APD changes were induced by pacing or by current injection (either outward or inward). The shape of the I_m–APD relationship was preserved when I_m was measured at other

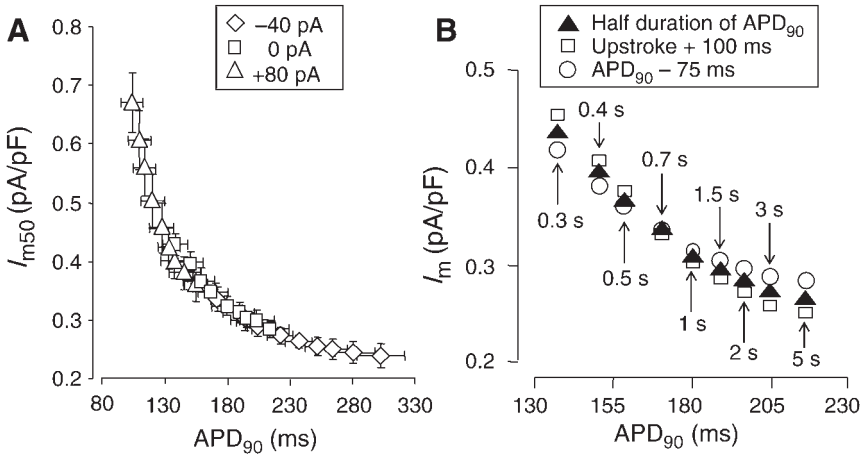

Figure 4 (*A*) Net membrane current flowing at half duration of the action potential (I_{m50}) plotted against the respective APD_{90} values. I_m was determined from the slope of the membrane potential changes (dV/dt). Data are obtained from the experiments shown in *Figure 2*, displaying results of 0 pA (control), −40 pA, and +80 pA current injections at the nine studied CLs. Symbols and bars denote mean ± SEM values obtained from 26 myocytes. (*B*) Relationship between I_m and APD_{90}, determined at half duration of the action potential (I_{m50}), at 100 ms after the upstroke, and at 75 ms before terminal repolarization in myocytes paced at various constant CLs under control conditions (without current injections).

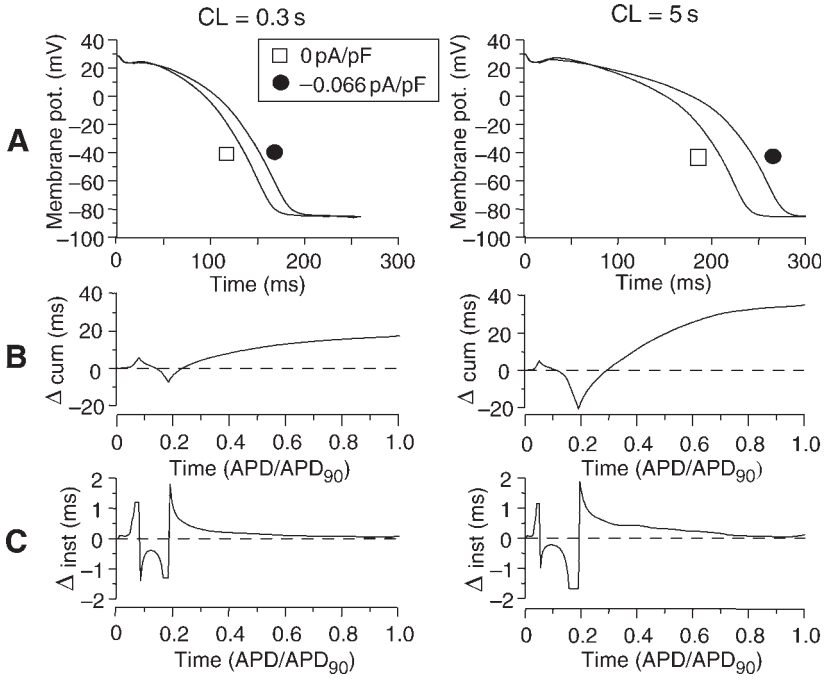

Figure 5 (*A*) Numerical simulation of changes in repolarization course, induced by injection of constant inward current (−0.066 pA/pF), at two pacing CLs. (*B*) Cumulative differences in repolarization time, Δcum (see methods for computation), induced by current injection, plotted as a function of time. (*C*) Time plot of the instantaneous (point-by-point) difference in repolarization time, Δinst, induced by current injection (see methods for computation). The instantaneous difference provides an estimate of how the effect of I_m on APD may vary according to the time of its occurrence during repolarization (e.g. at a CL of 5 s, the same 'point' I_m would prolong APD by 0.5 or 0.2 ms, if injected at 30 or 60% of APD_{90}, respectively). In (B) and (C), time is normalized to APD_{90} (e.g. 0.2 means 20% of the time required for 90% repolarization).

time points during repolarization (100 ms after the upstroke, or 75 ms before terminal repolarization); however, its steepness slightly decreased at later time points (*Figure 4B*). This can be explained by considering that CL-dependent changes mainly concern early repolarization, when the ratio between I_m and APD changes may consequently look larger.

3.2 Numerical simulations

The relationship between V_m' (or I_m) and APD is non-linear and this can be readily appreciated by assuming V_m' constant during repolarization:

$$APD = \frac{\Delta V_m}{V_{m'}} \quad \text{or} \quad APD = -\Delta V_m * \frac{C_m}{I_m} \tag{3}$$

where ΔV_m is the total V_m excursion during repolarization. The purpose of numerical simulations was to assess whether, when applied to real action potential waveforms, non-linearity of APD dependency on I_m may result in RRD of APD response to an I_m change.

As shown in *Figure 5A*, when the same inward current (−0.066 pA/pF) was added to I_m, APD_{90} was prolonged more at a CL of 5 s (35.4 ms) than at a CL of 0.3 s (17.4 ms); the difference in APD_{90} prolongation between the two CLs, reflecting RRD, amounted to 18 ms (*Figure 5A* and *B*). In experiments, RRD of APD changes was smaller for outward current injection (to shorten APD) when compared with inward current injection (e.g. compare −20 and +20 pA in *Figure 2B*). When injection of +0.066 pA/pF was simulated, the difference in APD_{90} prolongation between CLs of 5 s and 0.3 s was only 12.2 ms (not shown). Thus, the model also predicts asymmetry of RRD between inward and outward current injection, observed in experiments (*Figure 2*).

Most ion channel currents are time-dependent; thus, they have a specific time profile during repolarization. The mechanism of RRD predicts that such profile may be important in determining the APD response to current modulation. This is illustrated by *Figure 5C*, describing the delay between consecutive V_m values, induced by current injection, according to the instant of repolarization during which the added current flows. This analysis predicts that APD is most sensitive to I_m changes affecting phases with slower repolarization (e.g. compare values at 20 and 80% of APD_{90}). Moreover, it shows that because of its non-monotonic course, the spike-and-dome phase of repolarization is very sensitive to I_m changes. If restricted to this phase, an inward change in I_m (e.g. −0.066 pA) may paradoxically shorten APD, as shown by the negative portions of the traces in *Figure 5B* and *C*. Such negative portion was larger at the longer CL, because of the deeper notch between the spike and the dome; however, the time profile of the spike-and-dome phase may also change independently of CL; therefore, generalization of this feature as a property of intrinsic RRD may be unwarranted.

4. Discussion

The major finding of this study was to show that a drug-induced lengthening or shortening of APD is more pronounced at longer than at shorter CL in multicellular canine cardiac preparations, independently of the type of the ion channel blocked or stimulated. Similar results were obtained in single ventricular cells using inward or outward current pulses.

Previous interpretations of RRD were based on the features of the blocked channel or of drug-channel interaction.[5,6,19] A general difficulty with such interpretations was that drug-channel interaction is, in most of the cases, directly rate-dependent and therefore unsuitable to account for RRD. For instance, a major disparity in the RRD of APD prolongation by I_{Kr} and I_{Ks} blockers is a feature of guinea-pig myocytes[5,20] and was initially attributed to the different rate dependency of the two currents.[5] Whereas these experiments used a conventional voltage-clamp approach, later ones performed by action-potential clamp showed that guinea-pig I_{Kr} and I_{Ks} conductances are similarly increased at faster rates and that the proportion of total current provided by each of them is rate-independent.[9] According to these observations, rate dependency of I_{Kr} and I_{Ks} may be unsuitable to explain RRD of APD modulation. In canine ventricular myocytes, I_{Kr} and I_{K1} provide most of repolarizing current and are rate-independent;[21] nevertheless, their blockade affects APD with clear-cut RRD. Intrinsic rate dependency of persistent Na^+ current is relatively small,[22,23] while lidocaine[24,25] and veratrine[26–28] preferentially bind to Na^+ channels at higher heart rates; nevertheless, the effects of lidocaine and veratridine on APD show remarkable RRD (*Figure 1*). To summarize, although RRD of APD modulation has been reported for many agents with different mechanisms of action, a unifying interpretation of the phenomenon is missing.

The present experimental results, in which APD was modulated by a variety of agents and current injection, show that RRD occurs independently of the underlying mechanism, thus suggesting that it may be a general property of APD modulation. Furthermore, the hypothesis that such general property was inherent to the relation between I_m and APD was confirmed by numerical simulations. We will refer to this mechanism as 'intrinsic', to differentiate it from others, potentially concurring to rate-dependent APD modulation. In simple terms, this interpretation predicts that the impact of a given change in I_m on APD is proportional to the initial APD value which, in turn, decreases with increasing heart rate. Such a two-step link results in RRD, i.e. the inverse proportionality between APD modulation and heart rate. This conclusion is based on simple physical principles of general value; therefore, RRD of APD modulation is expected as an obligatory feature of all species in which APD is prolonged at lower heart rates, such as guinea-pig, dog, and man. In line with this argumentation, drug effects are expected to become directly rate-dependent if APD response to pacing rate is reversed. Indeed, in rabbit papillary muscles, levkromakalim was shown to shorten APD with direct rate dependency in a range of CLs (0.7 and 3.0 s) in which APD responded with prolongation to an increase in rate.[29]

The data in *Figure 1* show that, albeit RRD characterized APD response to all drugs, its extent and shape changed between different drugs. This indicates that drug-specific mechanisms also contribute to determine the rate dependency of APD modulation. This is expected based on the time-dependency of the targeted conductance and of the drug-channel interaction, both of which are specific for each drug. If present, such drug-specific rate dependency (either direct or reverse) coexists with intrinsic RRD and modulates its expression. For example, the specific mechanism of action of Bay-K might account for the supra-linear RRD observed in the present experiments (*Figure 1*);[30] also, the strong rate-dependent augmentation of I_{Ks} might explain why APD modulation by I_{Ks} blockade shows minimal RRD in guinea-pigs.[20]

To summarize, the present evidence does not argue against the existence of drug-specific mechanisms of rate-dependent APD modulation, but shows that they are necessarily superimposed on a background of intrinsic RRD. This may explain why RRD is so common and is often difficult to explain if only drug-specific mechanisms are considered.

Although based on very simple principles the mechanism of intrinsic RRD has some relevant consequences that may be difficult to appreciate at first sight and are disclosed by numerical simulations. Among them is the complexity in APD response to current injection resulting from the presence of a spike-and-dome phase. If net inward current is increased throughout repolarization (as in the simulation of *Figure 5*), APD prolongation is predicted to be larger if

the spike-and-dome is absent, as in endocardial myocytes. Indeed, the overall APD change reflects the sum of contrasting effects occurring during the spike-and-dome phase and the monotonic portion of repolarization. Nevertheless, it is important to stress that, in keeping with its purpose, the present model only takes into account the relation between I_m and APD. Therefore, although it may provide clues in interpreting the mechanisms of APD modulation, it is unsuitable to predict the overall effect of an intervention. Classical action potential models, which beside the I_m–APD relation also consider the properties of specific conductances, are required for this purpose.

Intrinsic RRD is based on a simple mechanism but, by affecting repolarization rate, may have practical consequences. For instance, suitably slow repolarization may favour recovery of I_{CaL} channels and be severely distorted by their subsequent reactivation during repolarization.[31,32] Repolarization rate also affects the rate of onset of I_{Kr} and I_{K1} during the action potential, with consequences on the repolarization reserve.[10,33]

The present results suggest that the development of channel blockers with suitable features (or targeting suitable channels) may, at best, reduce RRD of APD modulation, because intrinsic RRD needs to be offset in the first place. Thus, prolongation of APD with direct rate dependency, although clinically desirable as an antiarrhythmic intervention, may be difficult to attain. A more promising approach may be to combine APD prolongation with interventions suitable to minimize its arrhythmogenic effect at slow heart rates. This can probably be achieved by blockade of plateau inward currents, such as I_{CaL} and the persistent Na^+ current.[34,35] This view is supported by the results obtained by either combining two distinct molecules,[36–38] or by applying single drugs having intrinsically combined modes of action.[39]

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