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RESEARCH PAPER

Modulation of the late sodium current by ATX-II and ranolazine affects the reverse use-dependence and proarrhythmic liability of I_{Kr} blockade

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Keywords
late sodium current; reverse use-dependence; QT; $T_{p\text{-}e}$; $T_{p\text{-}e}$/QT ratio and TdP

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BACKGROUND AND PURPOSE

Drug-induced torsades de pointes (TdP) often occurs during bradycardia due to reverse use-dependence. We tested the hypothesis that inhibition or enhancement of late sodium current ($I_{Na,L}$) could modulate the drug-induced reverse use-dependence in QT and $T_{p\text{-}e}$ (an index of dispersion of repolarization), and therefore the liability for TdP.

EXPERIMENTAL APPROACH

Arterially perfused rabbit left ventricular wedge preparations were used. Action potentials from the endocardium were recorded simultaneously with a transmural ECG. The effects of *Anemonia sulcata* toxin (ATX-II) (an $I_{Na,L}$ enhancer), d,l-sotalol, clarithromycin and ranolazine (an $I_{Na,L}$ blocker) on rate-dependent changes in QT, $T_{p\text{-}e}$ and proarrhythmic events were tested, either alone or in combination. Rate-dependent QT and $T_{p\text{-}e}$ slopes and TdP score (a combined index of TdP liability) were calculated at control and during drug infusion.

KEY RESULTS

ATX-II (30 nM) and sotalol (300 μM) caused a marked increase in QT and $T_{p\text{-}e}$ intervals, steeper QT-basic cycle length (BCL) and $T_{p\text{-}e}$-BCL slopes (i.e. reverse use-dependence), and TdP. Addition of ranolazine (15 μM) to ATX-II or sotalol significantly attenuated QT-BCL, $T_{p\text{-}e}$-BCL slopes and the increased TdP scores. In contrast, clarithromycin (100 μM) moderately prolonged QT and $T_{p\text{-}e}$ without causing R-on-T extrasystole or TdP, but addition of ATX-II (1 nM) to clarithromycin markedly amplified the QT-BCL and $T_{p\text{-}e}$-BCL slopes and further increased TdP score.

CONCLUSION AND IMPLICATIONS

Modulation of $I_{Na,L}$ altered drug-induced reverse use-dependence related to QT as well as $T_{p\text{-}e}$, indicating that inhibition of $I_{Na,L}$ can markedly reduce the TdP liability of agents that prolong QT intervals.

Abbreviations

APD, action potential duration; ATX-II, *Anemonia sulcata* toxin; BCL, basic cycle length; EAD, early afterdepolarization; Endo, endocardium; I_{Kr}, rapidly activating delayed rectified K^+ current; I_{Ks}, slowly activating delayed rectifier K^+ current; $I_{Na,F}$, fast sodium current; $I_{Na,L}$, late sodium current; TdP, torsades de pointes; TDR, transmural dispersion of repolarization

Introduction

Torsades de pointes (TdP) is rare but lethal polymorphic ventricular tachycardia seen in the setting of QT interval prolongation. A number of prescription drugs, not limited to antiarrhythmic agents, have been implicated as causing drug-induced QT prolongation and TdP (Antzelevitch, 2004; Joshi *et al.*, 2004; Roden, 2004). Drug-induced TdP has emerged as one of the most significant concerns in drug safety and a major obstacle to new drug development. The last decade was marked by significant progress with respect to elucidating the pathophysiological mechanisms of drug-induced TdP (Yan *et al.*, 2001b; Fenichel *et al.*, 2004; Joshi *et al.*, 2004; Liu *et al.*, 2006). Blockade of the KCNH2-encoded, rapidly activating, delayed rectifier potassium current (I_{Kr}) is the commonest cause of the drug-induced delayed ventricular repolarization (ion channel nomenclatures follows Alexander *et al.*, 2009). I_{Kr} blockers exhibit reverse use-dependence, that is, that the drug-induced repolarization prolongation is more prominent during bradycardia and less during tachycardia, which contributes importantly to arrhythmogenesis (Joshi *et al.*, 2004; Gussak *et al.*, 2007).

Although it is believed that the slowly activating delayed rectifier potassium current (I_{Ks}) is an important modulator of rate-dependent ventricular repolarization and plays an important role in reverse use-dependence of certain QT prolonging agents (Jurkiewicz and Sanguinetti, 1993; Yang and Roden, 1996; Salata *et al.*, 1998), accumulating indirect evidence suggests that late sodium current ($I_{Na,L}$), an important inward current during ventricular repolarization, may potentially contribute to rate adaptation of ventricular repolarization and to the reverse use-dependence. It is known that ventricular M cells with a relatively larger $I_{Na,L}$ but a smaller I_{Ks} exhibit a much steeper rate-dependence of action potential duration (APD) than epicardial and endocardial cells (Antzelevitch *et al.*, 1999; Zygmunt *et al.*, 2001). In transgenic mice with long QT type 3 syndrome in which $I_{Na,L}$ is amplified, rate-dependent changes in APD become more prominent (Nuyens *et al.*, 2001). However, the role of $I_{Na,L}$ in the reverse use-dependence and its relation to the proarrhythmic liabilities of I_{Kr} blockers are not well studied.

Therefore, we planned experiments investigating the role of $I_{Na,L}$ in the genesis of TdP induced by I_{Kr} blockers, and specifically tested the hypothesis that modulation of $I_{Na,L}$ by *Anemonia sulcata* toxin (ATX-II) or ranolazine can significantly modify the reverse use-dependence of I_{Kr} blockers and therefore their proarrhythmic potentials. Specifically, enhancing $I_{Na,L}$ by ATX-II could unmask arrhythmogenic effect of non-antiarrhythmic QT prolonging drugs, whereas inhibition of $I_{Na,L}$ by ranolazine could attenuate the proarrhythmic potential of Class III antiarrhythmic drugs.

Methods

Arterially perfused rabbit left ventricular wedge preparations

The isolated arterially perfused rabbit left ventricular wedge model was used to test the above hypothesis. This model has been previously validated in a blinded fashion for preclinical assessment of drug-induced proarrhythmias (Liu *et al.*, 2006; Wang *et al.*, 2008).

All animal care and experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC). Surgical preparation of the rabbit left ventricular wedge has been described in detail in previous publication (Yan *et al.*, 2001a). Briefly, rabbits (New Zealand White) weighing 2.3–2.8 kg in either sexes were anticoagulated with heparin and anesthetized by intramuscular injection of xylazine (5 $mg \cdot kg^{-1}$) and intravenous administration of ketamine HCl (30–35 $mg \cdot kg^{-1}$). The chest was opened via a left thoracotomy, and the heart was excised and placed in a cardioplegic solution consisting of cold (4°C) normal Tyrode's solution. The left circumflex branch of the coronary artery was cannulated and perfused with the cardioplegic solution. Unperfused areas of the left ventricle, which were easily identified by their reddish appearance due to the existence of unflushed erythrocytes, were removed. The preparation was then placed in a small tissue bath and arterially perfused with Tyrode's solution containing 4 mM K^+ buffered with 95% O_2 and 5% CO_2 (temperature: 35.7 ± 0.1°C, mean perfusion pressure: 35–45 mmHg). The preparation was paced from endocardium (Endo) at basic cycle lengths (BCL) of 2000 ms, 1000 ms and 500 ms. The ventricular wedge was allowed to equilibrate in the tissue bath for 1 h prior to electrical recordings.

Electrophysiological recordings from rabbit ventricular wedge preparations

Transmembrane action potentials were recorded from Endo using a floating glass microelectrode. A transmural ECG signal was recorded using extracellular silver/silver chloride electrodes placed in the Tyrode's solution bathing the preparation 1.0 to 1.5 cm from the epicardial and endocardial surfaces. The QT interval was defined as the time from the onset of the QRS to the point at which the final downslope of the T wave crossed the isoelectric line. The $T_{p\text{-}e}$ interval, which closely approximates transmural dispersion of repolarization (TDR) (Yan and Antzelevitch, 1998; Liu *et al.*, 2006; Wang *et al.*, 2008), was defined as the time from the peak to the end of the T wave. QT-BCL and $T_{p\text{-}e}$-BCL slopes were defined as the changes in QT interval and $T_{p\text{-}e}$ interval as the function of BCL respectively.

The QT and $T_{p\text{-}e}$ intervals were measured manually in three consecutive beats within the last minute of the recording and the values were then averaged.

Late sodium current recording

Late sodium current recording in the single rabbit myocytes has been described in detail in our previous publications. (Guo *et al.*, 2010) Briefly, both fast sodium current ($I_{Na,F}$) and $I_{Na,L}$ were recorded at room temperature (22–24°C) using a whole-cell patch-clamp technique. $I_{Na,F}$ was recorded during an 80 ms depolarizing voltage step from a holding potential of –100 mV to a test potential of –30 mV. $I_{Na,L}$ currents were recorded using a 2000 ms depolarizing pulse from –140 mV to –20 mV at the stimulating rate of 0.1 Hz (holding potential is –140 mV). The amplitude of $I_{Na,L}$ was measured at 200 ms after membrane depolarization.

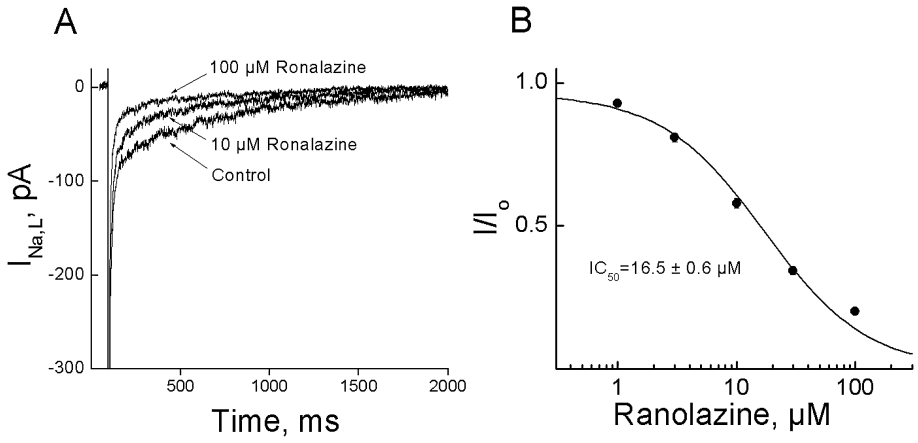

Figure 1

Effect of ranolazine on late sodium currents ($I_{Na,L}$) in rabbit myocytes. A. The superimposed currents show responses to step depolarizations ranging from –140 to –20 mV from a holding potential of –140 mV, obtained under control conditions and after 3 min of superfusion with various concentrations of ranolazine. B. Concentration–response relationship of ranolazine on $I_{Na,L}$. The amplitude of $I_{Na,L}$ was measured at 200 ms after membrane depolarization. Data were fitted with an equation $I/I_o = 1/(1 + [C]/[IC_{50}])$. Data were expressed as Mean ± SEM, n = 8, two cells per rabbit.

Experimental protocols and endpoint evaluations

The following three groups of experiments were carried out. The sample size of each group was five preparations:

- control perfusion for 1 h and then perfusion with ATX-II at 30 nM for 30 min, followed by ATX-II (30 nM) plus ranolazine (15 μM)
- control perfusion for 1 h and then perfusion with d,l-sotalol at 300 μM for 30 min, followed by d,l-sotalol (300 μM) plus ranolazine (15 μM)
- control perfusion for 1 h and then perfusion with clarithromycin at 100 μM for 30 min, followed by clarithromycin (100 μM) plus ATX-II (1 nM)

The relative TdP risk of each compound was estimated according to the criteria reported previously (Liu *et al.*, 2006; Wang *et al.*, 2008). The following three core parameters were used in scaling the relative TdP risk of each compound: delayed ventricular repolarization (QT prolongation), dispersion of repolarization (the $T_{p\text{-}e}$/QT ratio) and the incidence of early afterdepolarization (EAD), with and without closely coupled extrasystoles, and the development of TdP. Among the three parameters, the development of EAD-induced extrasystoles received the greatest weight. TdP score was defined and calculated as described before (Liu *et al.*, 2006; Wang *et al.*, 2008). The maximum TdP score was 14 and the minimum TdP score was –2.

Statistics

Statistical analysis of the data was performed using Student's *t*-test or ANOVA (one-way, with *post hoc* Newman–Keuls multiple comparison test or two-way with Bonferroni test; see Figure legends) as appropriate. The χ^2-test was used for the comparison between two groups for event incidences. Data are presented as mean ± SEM.

Materials

We used ATX-II (Alomone Laboratories, Jerusalem, Israel) to increase the magnitude of $I_{Na,L}$, and a novel anti-anginal drug ranolazine (Sigma, St Louis, USA) was used to reduce $I_{Na,L}$ (Antzelevitch *et al.*, 2007). The commonly prescribed antibiotic clarithromycin (Sigma) was chosen as a non-antiarrhythmic I_{Kr} blocker(Redfern *et al.*, 2003) as compared with d,l-sotalol (Sigma), a Class III antiarrhythmic drug that blocks I_{Kr}.

Results

Effects of ranolazine on $I_{Na,L}$

This series of experiments were performed to determine the IC_{50} of ranolazine on $I_{Na,L}$ in the rabbit ventricular myocytes (Figure 1) and, as this was 16.5 ± 0.6 μM (n = 8), we used ranolazine at 15 μM to suppress $I_{Na,L}$ in the subsequent experiments.

Effects of ATX-II, d,l-sotalol and clarithromycin on QT, $T_{p\text{-}e}$ Interval and incidence of proarrhythmic events

As shown in Figure 2, ATX-II at 30 nM, d,l-sotalol at 300 μM and clarithromycin at 100 μM produced a significant increase in QT and $T_{p\text{-}e}$ intervals, in the preparations paced at a BCL of 2000 ms (Figure 2). Marked QT and $T_{p\text{-}e}$ prolongation by ATX-II and d,l-sotalol during bradycardia, that is, at a BCL of 2000 ms, was associated with the development of R-on-T extrasystoles and TdP (Figure 3, Table 1). Although clarithromycin at 100 μM produced EAD in three of five preparations, no R-on-T extrasystoles and TdP were observed.

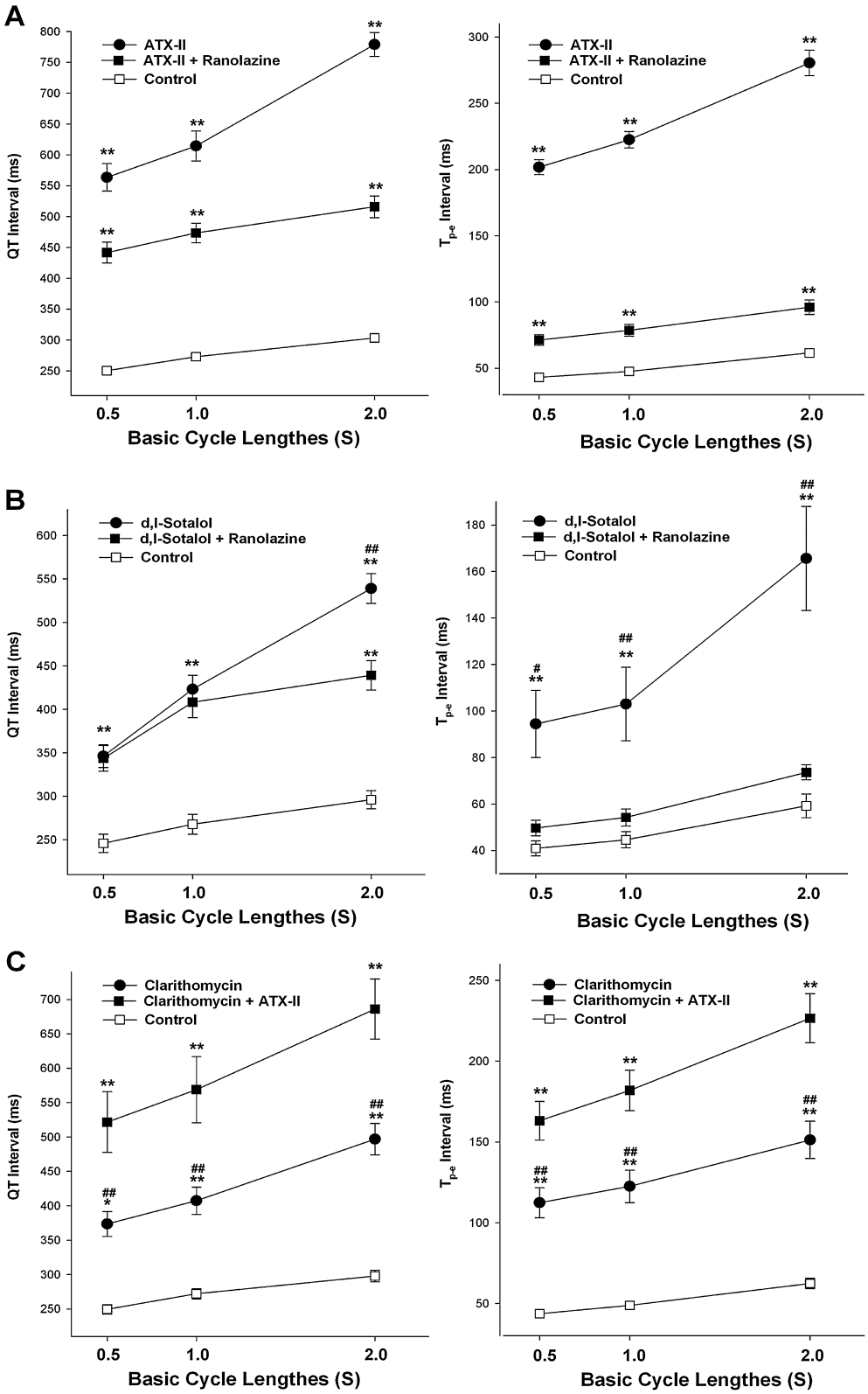

Figure 2

Rate-dependent changes in QT and T_{p-e} after infusion of ATX-II (30 nM) and ATX-II (30 nM) plus 15 μM ranolazine (A), d,l-sotalol (300 μM) and d,l-sotalol (300 μM) plus 15 μM ranolazine (B), and clarithromycin (100 μM) and clarithromycin (100 μM) plus 1 nM ATX-II (C). *P < 0.05 and **P < 0.01 compared with controls; #P < 0.05 and ##P < 0.01 compared with the groups plus additional modulation of $I_{Na,L}$ with ATX-II or ranolazine; two-way ANOVA analysis.

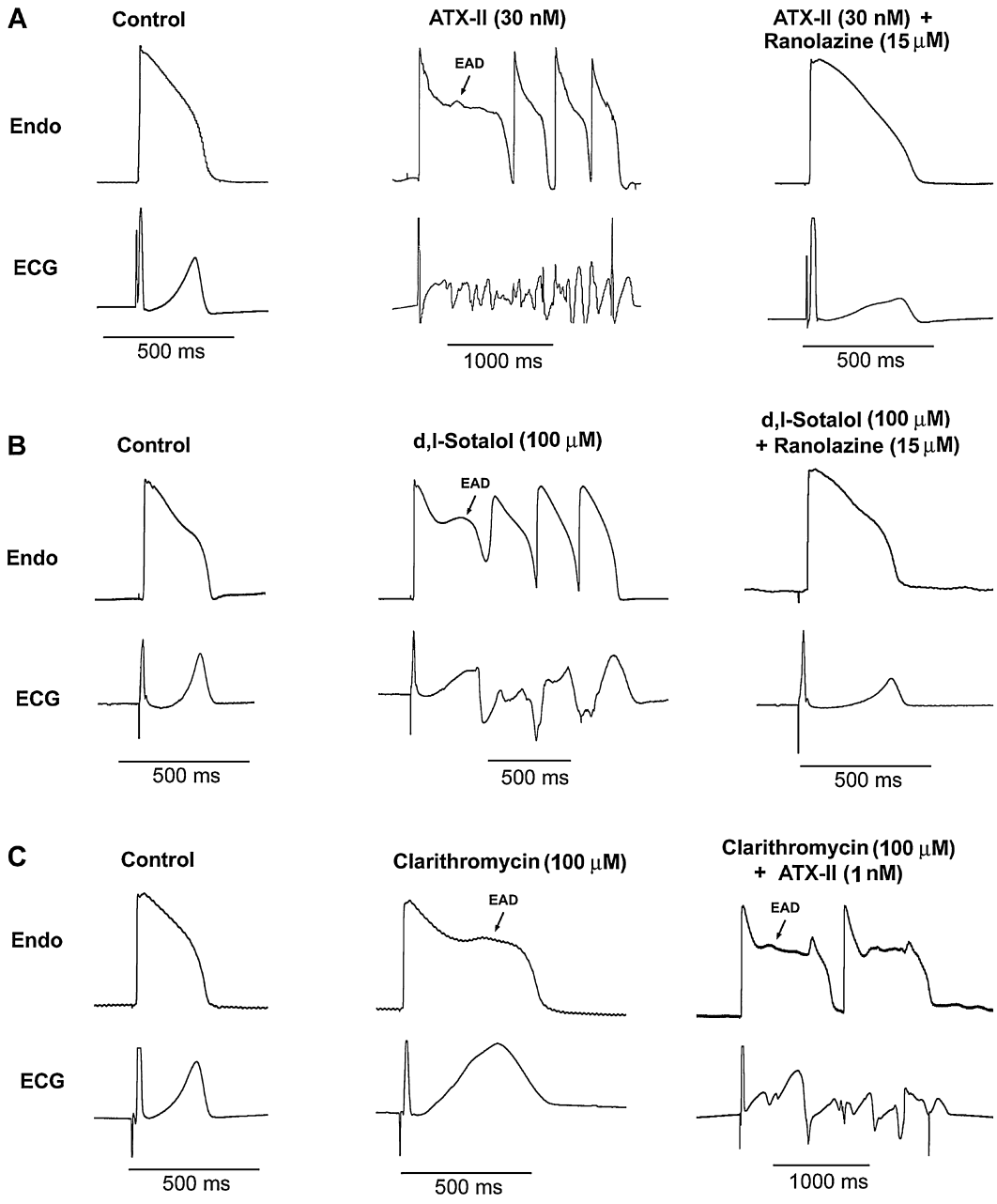

Figure 3

Original tracings of early afterdepolarization (EAD) and spontaneous torsades de pointes (TdP) induced by ATX-II (A), d,l-sotalol (B) and clarithromycin (C), either alone or in combination. Please note that there are different scales. Endo: endocardium; basic cycle length = 2000 ms.

Effect of modulation of $I_{Na,L}$ on QT, $T_{p\text{-}e}$ interval and incidence of proarrhythmic events

Interestingly, reduction of $I_{Na,L}$ by ranolazine at 15 μM significantly attenuated the effects of ATX-II and d,l-sotalol on the QT and $T_{p\text{-}e}$ intervals (Figure 2) In contrast, with infusion of 100 μM clarithromycin, addition of 1 nM ATX-II further increased the QT interval (Figure 2). Ranolazine decreased the incidence of proarrhythmic events including EAD, R-on-T extrasystoles and TdP, whereas ATX-II increased this incidence significantly (Table 1).

The reverse use-dependence of I_{Kr}: the effects of modulation of $I_{Na,L}$

As shown in Figure 2, ATX-II, d,l-sotalol and clarithromycin caused more QT prolongation during slowing pacing rates, illustrating the reverse use-dependence. Interestingly, the reverse use-dependence related to $T_{p\text{-}e}$ prolongation induced by these compounds appeared similar to that related to QT prolongation. ATX-II (30 nM), d,l-sotalol (300 μM) and clarithromycin (100 μM) increased the QT-BCL slope (Figure 4). Similarly, the $T_{p\text{-}e}$-BCL slope was steeper in the presence of these compounds (Figure 5). Enhancement of

Table 1

Comparison of the incidence of EADs, R-on-T extrasystole, TdP and TdP score

Study groups		EADs	R-on-T	TdPs	TdP score
A	ATX-II (30 nM)	5/5	5/5	4/5	13.6 ± 0.3
	ATX-II (30 nM) + Ranolazine (15 μM)	2/5	0/5**	0/5*	4.2 ± 1.0**
B	d,l-sotalol (300 μM)	5/5	5/5	2/5	12.8 ± 0.5
	d,l-sotalol (300 μM) + Ranolazine (15 μM)	3/5	0/5**	0/5	3.8 ± 1.2**
C	Clarithromycin (100 μM)	3/5	0/5	0/5	8.2 ± 1.2
	Clarithromycin (100 μM) + ATX-II (1 nM)	5/5	5/5**	3/5	13.2 ± 0.5*

*$P < 0.05$; **$P < 0.01$ compared between the corresponding drug's effects with and without modulation of $I_{Na,L}$ within each group.
All arrhythmic events occurred at a basic cycle length of 2000 ms.
ATX-II, *Anemonia sulcata* toxin; EAD, early afterdepolarization; TdP, torsades de pointes.

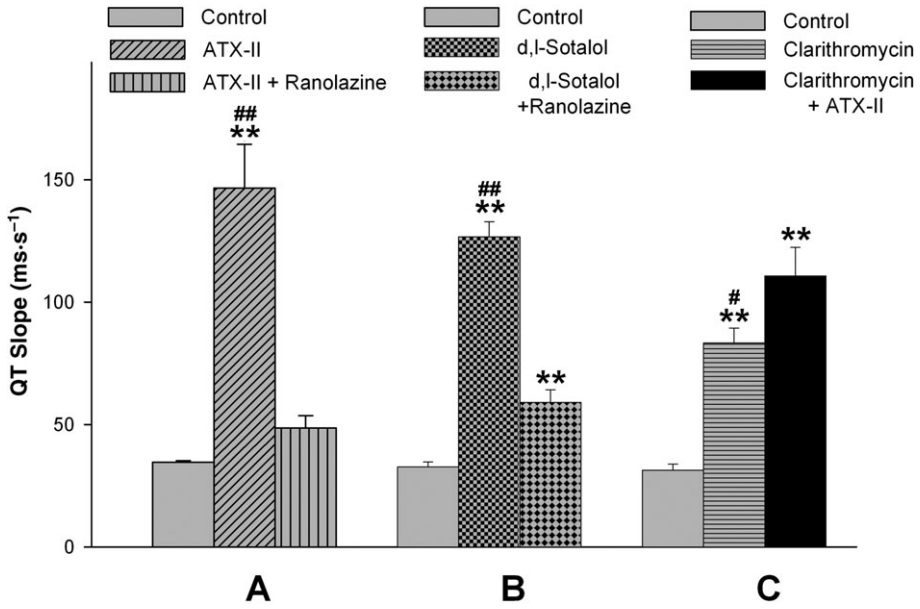

Figure 4

QT-BCL slopes (changes in QT interval as the function of basic cycle lengths), after infusion of ATX-II (30 nM) and ATX-II (30 nM) plus 15 μM ranolazine (A), d,l-sotalol (300 μM) and d,l-sotalol (300 μM) plus 15 μM ranolazine (B), and clarithromycin (100 μM) and clarithromycin (100 μM) plus 1 nM ATX-II (C). **$P < 0.01$ compared with controls; #$P < 0.05$ and ##$P < 0.01$ compared with the groups plus additional modulation of $I_{Na,L}$ with ATX-II or ranolazine; one-way ANOVA analysis.

$I_{Na,L}$ by ATX-II increased both the QT-BCL and the $T_{p\text{-}e}$-BCL slopes, whereas inhibition of $I_{Na,L}$ by ranolazine attenuated both of them (Figures 4 and 5).

The $T_{p\text{-}e}$/QT ratio: the effects of modulation of $I_{Na,L}$

Previous studies have shown that the $T_{p\text{-}e}$ interval represents dispersion of ventricular repolarization (Yan and Antzelevitch, 1998; 1999; Yan *et al.*, 2001b; Patel *et al.*, 2009). Its ratio to the QT interval ($T_{p\text{-}e}$/QT) has been shown to be an important arrhythmic index particularly related to the TdP risk of the QT prolonging agents (Liu *et al.*, 2006; Gupta *et al.*, 2008; Wang *et al.*, 2008). Therefore, the changes in the $T_{p\text{-}e}$/QT ratio by modulation of $I_{Na,L}$ were also calculated. As shown in Figure 6, ATX-II (30 nM), d,l-sotalol (300 μM) and clarithromycin (100 μM) all produced a marked increase in the $T_{p\text{-}e}$/QT ratio that could be attenuated by ranolazine or further enhanced by ATX-II.

As a result of these effects, reduction of $I_{Na,L}$ by ranolazine significantly reduced the estimated TdP scores of ATX-II and d,l-sotalol. In contrast, ATX-II at a low dose (1 nM) significantly increased the TdP score of clarithromycin (Table 1). The proarrhythmic events related to TdP, before and after modulation of $I_{Na,L}$, is also shown in Table 1.

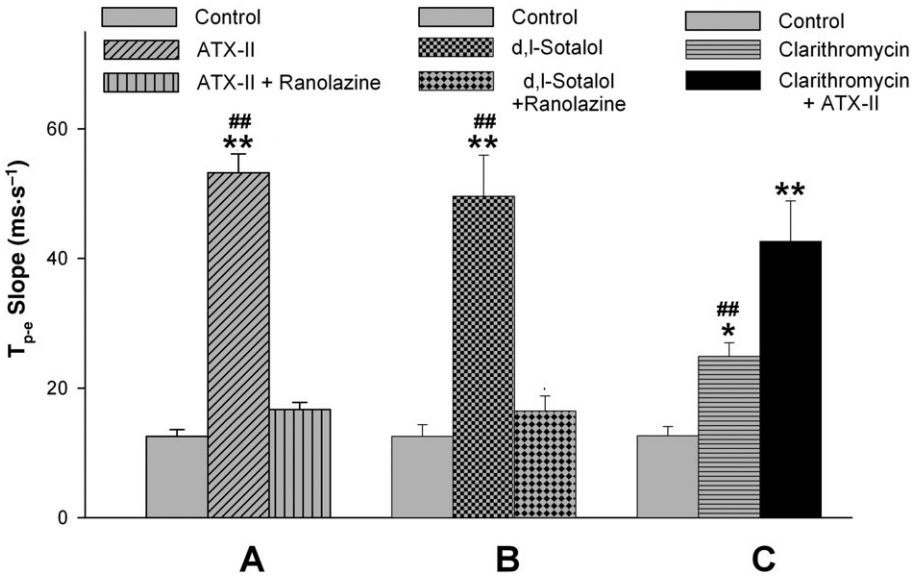

Figure 5

T_{p-e}-BCL slopes (changes in T_{p-e} interval as the function of basic cycle lengths), after infusion of ATX-II (30 nM) and ATX-II (30 nM) plus 15 μM ranolazine (A), d,l-sotalol (300 μM) and d,l-sotalol (300 μM) plus 15 μM ranolazine (B), and clarithromycin (100 μM) and clarithromycin (100 μM) plus 1 nM ATX-II (C). *P < 0.05 and **P < 0.01 compared with controls; ##P < 0.01 compared with the groups plus additional modulation of $I_{Na,L}$ with ATX-II or ranolazine; one-way ANOVA analysis.

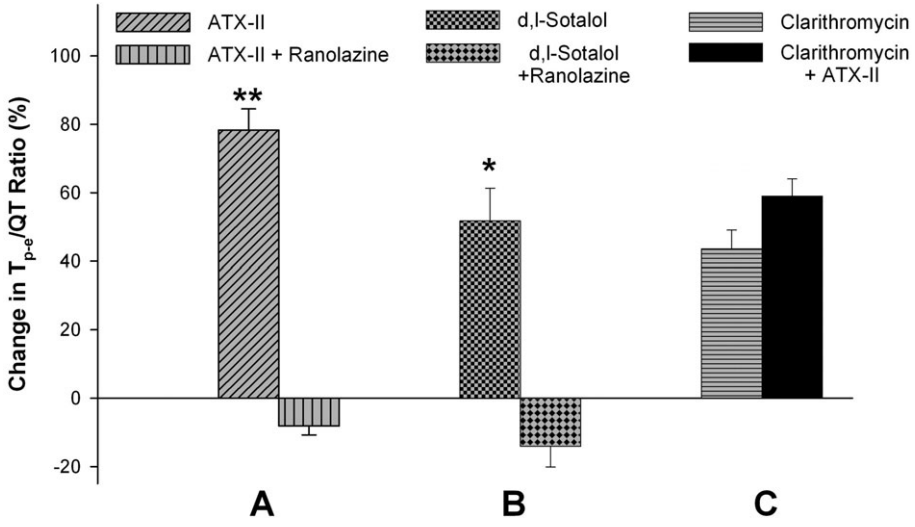

Figure 6

Changes in T_{p-e}/QT ratios after infusion of ATX-II (30 nM) and ATX-II (30 nM) plus 15 μM ranolazine (A), d,l-sotalol (300 μM) and d,l-sotalol (300 μM) plus 15 μM ranolazine (B), and clarithromycin (100 μM) and clarithromycin (100 μM) plus 1 nM ATX-II (C). *P < 0.05 and **P < 0.01 when compared between two interventions within the same groups (Student's t-test).

Discussion

Our results have provided novel insights into the role of $I_{Na,L}$ in the pathogenesis of drug-induced TdP. Our principal findings are: (i) blockade of I_{Kr} or augmentation of $I_{Na,L}$ by ATX-II exhibited significant reverse use-dependence, not only associated with the QT interval but also with the T_{p-e} interval, an index of TDR, in rabbit ventricular wedge preparations; (ii) enhancement of $I_{Na,L}$ by ATX-II significantly amplified the reverse use-dependence of QT and T_{p-e} intervals and increased the arrhythmogenic potential of I_{Kr} blockers; and (iii) inhibition of $I_{Na,L}$ by ranolazine reduced the reverse use-dependence of QT and T_{p-e} intervals and conferred significant antiarrhythmic effects.

The mammalian ventricular repolarization at steady state, represented by the QT interval on the surface ECG, is

inversely proportional to heart rate (Carmeliet, 1977). In other words, tachycardia usually shortens ventricular repolarization and bradycardia prolongs it. The QT prolonging agents particularly with a pure I_{Kr} blockade property often amplify the physiological rate adaptation of ventricular repolarization, leading to reverse use-dependence. It is popularly believed that I_{Ks}, which can accumulate during tachycardia, is a major modulator for the reverse use-dependence of the I_{Kr} blockers (Jurkiewicz and Sanguinetti, 1993; Yang and Roden, 1996). However, evidence suggests that I_{Ks} may not be as important as we thought in modulation of the rate-dependence of cardiac APD. In the canine left ventricle, M cells exhibit a much steeper rate-APD slope than in the epicardial and endocardial cells, although the M cells have a much weaker I_{Ks} compared with the other two cell types (Liu and Antzelevitch, 1995). More directly, block of I_{Ks} by HMR 1556 was also associated with reverse use-dependent APD prolongation, that is, amplification of the intrinsic rate-dependent changes in APD (Stengl *et al.*, 2003).

On the other hand, contribution of $I_{Na,L}$, an important inward current participating in maintaining the plateau of the action potential and contributing to a great extent to intrinsic heterogeneity of myocardium (Antzelevitch *et al.*, 2004), has so far received relatively little attention in reverse use-dependence of QT/APD prolongation. Amongst three electrophysiologically distinct myocardial cell types spanning the ventricular wall, M cells have intrinsically larger $I_{Na,L}$ and weaker I_{Ks}. Due to this, M cells have the longest duration of action potential and more prominent rate-dependent changes in QT/APD, contributing most importantly to TDR that manifests as $T_{p\text{-}e}$ on the ECG (Antzelevitch *et al.*, 1999; Yan *et al.*, 2001b). Prominent TDR and reverse use-dependence in drug-induced TdP is well recognized now (Yan *et al.*, 2001a; Hondeghem, 2005; Liu *et al.*, 2006; Antzelevitch *et al.*, 2007). In the rabbit left ventricle, cells with M cell characteristics occupy the entire Endo (Xu *et al.*, 2001; Yan *et al.*, 2001a,b). Based on the findings of the present study, larger TDR during bradycardia may be the consequence of heterogeneous rate-dependent changes in APD at least partially due to heterogeneous distribution of $I_{Na,L}$ across the ventricular wall. This is supported by the previous findings that blockade of the sodium current reduced TDR (Liu *et al.*, 2006).

It is also interesting to note that potent blockade of $I_{Na,L}$ by ranolazine outweighed the combined I_{Kr} blockade by sotalol and ranolazine itself in the reverse use-dependence of QT and $T_{p\text{-}e}$ prolongation. Our finding that combinations of mild augmentation of $I_{Na,L}$ and inhibition of I_{Kr} could have synergistic effect and could amplify the risk of drug-induced TdP, has clinical relevance and implications for drug development. Inhibition of $I_{Na,L}$ could have potential therapeutic benefit especially in conditions with reduced repolarizing forces like left ventricular hypertrophy and long QT 3 (enhanced $I_{Na,L}$) and other pathological conditions (Nuyens *et al.*, 2001; Maltsev and Undrovinas, 2008; Moss *et al.*, 2008) or other forms of acquired long QT syndromes (reduced potassium currents) (Wu *et al.*, 2006; 2008; Hale *et al.*, 2008). In agreement with these basic research observations, Scirica *et al.* (2007) have recently demonstrated the antiarrhythmic efficacy of ranolazine in a clinical trial involving more than 6000 patients admitted for non-ST-elevation myocardial infarction.

The ionic basis of our findings that $I_{Na,L}$ contributes importantly to the reverse use-dependence of drug-induced QT and $T_{p\text{-}e}$ prolongation is unknown. $I_{Na,L}$, unlike fast I_{Na} that inactivates in a few milliseconds and recovers fast, has very slow inactivation and recovery kinetics ranging from hundreds of milliseconds to minutes (Carmeliet, 1987; Undrovinas *et al.*, 2002). Therefore, it is expected that $I_{Na,L}$ is sensitive to changes in rates. The results of our work may raise interest in investigating the kinetics of $I_{Na,L}$ and its changes in amplitude to different rates because inhibition of $I_{Na,L}$ is useful in long QT 3 (Moss *et al.*, 2008) and other pathological conditions with enhanced $I_{Na,L}$.

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Conflict of interest

There is no conflict of interest for any of the authors.

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