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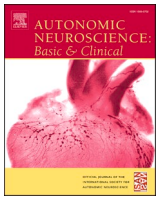

Role of beta-adrenergic modulation of action potential duration in arrhythmogenesis in Long QT Syndrome Type 1 & 2

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ABSTRACT

Introduction: Congenital Long QT Syndromes type 1 and 2 (LQT1 and LQT2) are life-threatening conditions that arise from functional impairment of delayed rectifier potassium channels and predispose individuals to ventricular arrhythmias (VAs) such as polymorphic ventricular tachycardia and ventricular fibrillation (VF). Sympathetic surges have been associated with VA onset in LQTS, but mechanisms of initiation are not fully understood. It is therefore necessary to investigate the effects of β-adrenergic receptor (β-AR) stimulation on ventricular electrophysiology in LQT1 and LQT2.

Methods: HMR-1556 (0.1 μM, 0.5 μM, 1.0 μM) and E4031 (0.02 μM, 0.05 μM, 0.10 μM) were applied to selectively inhibit the slowly and rapidly activating delayed rectifier potassium currents (IKs and IKr), thereby pharmacologically modelling LQT1 and LQT2, respectively. The effects of β-AR stimulation using isoproterenol (ISO) were investigated on monophasic action potential duration ($MAPD_{90}$), effective refractory period (ERP), $MAPD_{90}$ restitution (RT $Slope_{max}$) and VF threshold (VFT).

Results: Both HMR and E4031 displayed concentration dependent bradycardic effects and increased $MAPD_{90}$, ERP and VFT. β-AR stimulation on both LQTS models induced tachycardia, and reduced $MAPD_{90}$, ERP and VFT. In LQT1, the presence of ISO caused a greater decrease in VFT and flattening of RT $Slope_{max}$, but in LQT2 RT $Slope_{max}$ was steeper.

Conclusion: The preliminary data suggest that both LQT1 and LQT2 are associated with an increase in VF susceptibility when sympathetic activity is enhanced. However, the dichotomy in the effect on RT $slope_{max}$ suggest that each LQT subtype may have different arrhythmogenic mechanisms.

1. Introduction

Congenital long QT syndrome (LQTS) is a group of inherited cardiac channelopathies characterised by delayed ventricular repolarisation, QT interval prolongation, and increased susceptibility to malignant ventricular arrhythmias, including torsades de pointes and ventricular fibrillation (Crotti et al., 2008; Roden, 1998). Beyond the underlying ion channel defect, autonomic influences are now recognised as important modifiers of arrhythmic risk in LQTS.

In particular, sympathetic activation is a well-established trigger for arrhythmic events, with clear gene-specific patterns of susceptibility; events in LQT1 are typically associated with exercise or emotional stress, whereas events in LQT2 are often precipitated by sudden arousal or auditory stimuli (Herring et al., 2019). More broadly, recent work has emphasised the central role of neuro-cardiac interactions in arrhythmogenesis, highlighting the importance of sympathetic modulation of cardiac electrophysiology in both inherited and acquired arrhythmia syndromes (Habecker et al., 2025). In addition to acute adrenergic triggers, there is increasing evidence that inherited arrhythmia syndromes may also involve intrinsic abnormalities in the cardiac

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sympathetic nervous system, further contributing to arrhythmia susceptibility (Winbo et al., 2021; Li et al., 2026).

These observations provide a strong rationale for investigating the electrophysiological effects of β-adrenergic stimulation in experimental models of LQT1 and LQT2.

LQTS types 1 (LQT1) and 2 (LQT2) account for 90% of congenital LQTS cases (Crotti et al., 2008), with both subtypes causing cardiac events such as sudden cardiac death and syncopal episodes, during surges in sympathetic activity. This is due to reduced activity of two different delayed rectifier potassium currents (Roden, 1998; Moss et al., 1991). LQT1 is caused by mutations in the KCNQ1 gene which encodes Kv7.1, the α-subunit of the channel that conducts I_{Ks} (the slowly activated delayed rectifier potassium current) (Schwartz et al., 2001), while LQT2 is caused by mutations in the KCNH2 gene (also known as the human ether-a-go-go-related gene, hERG1) which encodes the Kv11.1 channel subunit of the channel that conducts the rapidly activated delayed rectifier potassium current, I_{Kr} (Wang et al., 1996; Sanguinetti et al., 1995; Trudeau et al., 1995). Reduced activity of these potassium currents leads to a decrease in the repolarisation capacity of ventricular cells, which becomes more apparent in the presence of adrenergic stimulation, and predisposes the heart to arrhythmogenesis.

A current theory is that TdP may be initiated via a mechanism known as action potential duration (APD) restitution. APD restitution is a natural property of myocytes and is defined as the relationship of APD to its preceding diastolic interval (DI), i.e. how the APD adapts to changes in heart rate. If the relationship between APD and DI is steep, i.e. greater than 1, then APD is changing greatly over a small range of DIs, which leads to dynamic instability and can cause the break-up of spiral waves of electrical activity into oscillations, then facilitating the initiation of ventricular fibrillation (VF). This has been supported in both mathematical and biological models (Cao et al., 1999; Nolasco and Dahlen, 1968; Karma, 1994; Gilmour Jr. and Chialvo, 1999; Ng et al., 2007). Importantly, experimental work in intact heart preparations, including that by Nash et al. (Nash et al., 2006), has highlighted the dynamic nature of restitution properties and their modulation by physiological conditions, reinforcing their relevance to arrhythmogenesis in the whole heart.

Ng et al. previously showed in rabbit hearts that sympathetic stimulation increased the restitution curve slope, which was directly related to the generation of VF (Li et al., 2026). However, the relevance of this in LQTS is yet to be investigated. β-adrenergic stimulation is commonly modelled experimentally using the non-selective β-adrenoceptor agonist isoprenaline (isoproterenol). Isoprenaline activates both β1 and β2 adrenoceptors and provides a well-established pharmacological approach for investigating β-adrenergic modulation of cardiac electrophysiology.

The purpose of this study was to use IKs and IKr channel blockers to investigate the effects of LQT1 and LQT2 on cardiovascular electrophysiology and the development of ventricular arrhythmias. Studying these pharmacological models of LQT1 and LQT2 in the presence of β-adrenergic activation will enable a better understanding of the significance of the restitution hypothesis and sympathetic stimulation in arrhythmogenesis in LQTS.

2. Materials and methods

2.1. Isolated whole guinea pig hearts

Whole hearts were isolated from adult male Dunkin Hartley guinea pigs (n = 25,390-680 g) using procedures that fulfilled the criteria for the Animal Scientific Procedure Act (ASPA, 1986) and the Guide for the Care and Use of Laboratory Animals from the US National Institute of Health (NIH Publication No. 85–23, revised 1985) and EU legislation on the protection of animals used for scientific purposes (Directive 2010/63/EU, 2010). Guinea pigs were culled by cervical dislocation and the heart was immersed in cold Tyrode solution with 1000 IU of heparin.

2.2. Langendorff preparation

The hearts were perfused using a modified Langendorff perfusion technique. This was performed by cannulating the aorta, and perfusing the heart in a retrograde manner with Tyrode solution heated to 37 °C and oxygenated with 95% O_2 / 5% CO_2 to maintain pH 7.4. The modified Tyrode solution contained the following (in mM): Na^+ 138.0, K^+ 4.0, Ca^{2+} 1.8, Mg^{2+} 1.0, HCO_3^- 24, $H_2PO_4^-$ 0.4, Cl^- 124, Glucose 11. The heart was perfused using a Gilson Minipulse 3 peristaltic pump (Anachem, Luton, UK) at a constant flow rate of 20 mL/min, with a catheter placed in the left ventricular apex to drain thebesian venous effluent. A fluid-filled latex balloon was inserted into the left ventricle via the left atrium to measure left ventricular pressure (LVP), which was secured to the left atrial appendage. The balloon was inflated to an end diastolic pressure of 0–5 mmHg and connected to a solid-state pressure transducer (MLT0380/D ADInstruments Ltd., Charlgrove, UK), which was also used to record heart rate. Perfusion pressure (PP) was also monitored using a pressure transducer connected in series with the perfusion line.

2.3. Cardiac electrical recording and pacing

Action potentials were recorded at apical and basal sites on the epicardial surface of the left ventricle, using two contact monophasic action potential (MAP) electrodes (Harvard Apparatus Ltd., Holliston, Massachusetts, US. Model number 73–0150), connected to a custom-made DC-coupled high input impedance differential amplifier (Biomedical Joint Workshop, University of Leicester, UK).

Two hook electrodes were connected to the right atrium and an earthing source to record an atrial electrogram, allowing comparison of atrial electrical activity with that of the ventricles.

A bipolar catheter (ADInstruments Ltd., Charlgrove, UK) was inserted into the right ventricle and placed in contact with the apical endocardium ventricle to deliver a stimulus to the heart at double the diastolic pacing threshold using a constant current stimulator for cardiac pacing during the protocols.

2.4. Protocols

2.4.1. Effective refractory period (ERP) and standard restitution protocol

A standard single extrastimulus protocol was used to obtain APD restitution curves. The heart was paced for 25 S1 drive train beats at a cycle length (CL) of 200 ms, followed by a single extrastimulus (S2) with an initial cycle length of 200 ms. This was repeated with progressively shorter S1-S2 intervals, shortened by 3 ms on each repeat until ERP was reached, which was defined as the longest S1-S2 coupling interval that failed to electrically capture the ventricles. APD was measured from the time of activation (T_{act}) to 90% repolarisation ($MAPD_{90}$) using a custom-made program, NewMap (Francis Burton, Glasgow University, UK). These values were used to construct restitution curves by plotting the $MAPD_{90}$ of S2 vs. DI (DI = interval between the S1-and S2- MAP signals minus S1-MAPD90). Electrical restitution curves were constructed using Microcal Origin Software (v6.0, Origin, San Diego, CA, US) by plotting MAPD90 as a function of the preceding diastolic interval (DI). Data were fitted using a monoexponential function: MAPD90 = MAPD90max × (1 − e^(−DI/τ)), where MAPD90max represents the asymptotic maximal action potential duration and τ is the time constant describing the rate of restitution. The maximal slope of the restitution curve (RTSlopemax) was determined as the maximal slope of the fitted restitution curve within the range of experimentally observed DI. This represents the steepest slope within the fitted DI range rather than the theoretical maximum of the exponential function (MAPD90max/τ) at DI = 0.

This approach allows estimation of the maximal gradient of the restitution relationship based on the fitted curve rather than relying on slopes calculated directly from individual DI measurements.

2.4.2. Ventricular fibrillation threshold (VFT) protocol

A standard VFT protocol was used to investigate inducibility of VF, adapted from previously published protocol in the rabbit (Ng et al., 2007). The heart was paced for 25 beats at 200 ms cycle length, followed by rapid burst pacing of 30 beats at 30 ms cycle length. There was then a 10 s rest period before the protocol was repeated at a higher current, increased in increments of 0.5 mA. This was repeated until sustained VF was achieved. VFT was defined as the minimum current required to induce sustained VF (>10 s). Cardioversion was performed using a bolus of KCl (40 mM) and the heart was given a recovery period of 10–15 min to allow values to return to baseline.

2.5. Using HMR-1556 and E4031 to mimic LQT1 and 2

2.5.1. HMR-1556 and E4031

Concentration responses were conducted for HMR-1556 and E4031. HMR-1556 is a well-established and selective inhibitor of the slowly activating delayed rectifier potassium current (IKs), while E4031 is a selective blocker of the rapidly activating delayed rectifier potassium current (IKr), and both have been widely used to pharmacologically model LQT1 and LQT2 phenotypes in experimental preparations (Gögelein et al., 2000; Sanguinetti and Jurkiewicz, 1990). The concentrations of HMR-1556 (Sanofi Aventis, Guildford, UK) applied were 0.1 μM (low), 0.5 μM (medium) and 1.0 μM (high) and for E4031 (Tocris Bioscience, Bristol, UK) were 0.02 μM (low), 0.05 μM (medium) and high 0.1 μM (high). Drugs were applied cumulatively in increasing concentrations to establish concentration–response relationships.

To investigate pharmacological models of LQT1 and LQT2, separate groups of hearts were exposed to the IKs blocker HMR-1556 or the IKr blocker E4031. Separate preparations were used for each drug because these agents can exhibit slow or incomplete washout in isolated heart preparations, making sequential application unreliable.

Where washout periods were performed, hearts were perfused with drug-free Tyrode's solution for approximately 30–60 min, or until electrophysiological parameters stabilised and approached baseline levels.

2.5.2. Applying Isoprenaline (ISO) to mimic adrenergic activation

10 nM of the non-specific β-adrenergic receptor agonist Isoprenaline (ISO;, Sigma Aldrich, Dorset, UK) were used to cause an increase in β-adrenergic activity. ISO was dissolved in ethanol (10 mmol/L) and diluted to a final concentration of 10 nM in Tyrode solution. LVP, PP, heart rate and APD at apical and basal sites were measured during constant pacing at 200 ms cycle length in all recording conditions; control, β-adrenergic stimulation (ISO), simulated LQT1/2 plus β-adrenergic stimulation.

2.6. Data recording and statistical analysis

All signals were recorded using Powerlab 16/30 (ADInstruments Ltd., Charlgrove, UK) and processed at 2 kHz with LabChart software (ADInstruments Ltd). For statistical analysis, dose-response data was compared using a one-way ANOVA (Bonferroni post-hoc) or a Student's paired *t*-test; ISO data were analysed using a two-way ANOVA (Tukey post-hoc); using GraphPad Prism (v.5, California, USA). Percentage changes (%Δ) were calculated from paired measurements obtained from the same heart under control and experimental conditions. Statistical comparisons were performed on the calculated percentage change values. Data is presented as the mean ± SEM, with a p-value <0.05 considered statistically significant.

3. Results

3.1. Effects of HMR-1556 & E4031 on physiological parameters

3.1.1. Effects of HMR-1556 & E4031 on cardiac function

Heart preparations were perfused with low, medium and high concentrations of HMR-1556 and E4031 to measure the effect of concentration on cardiac function. LVP, PP and HR were measured during sinus rhythm before and during drug perfusion ($n = 10$). HMR-1556 produced a significant concentration-dependent reduction in LVP; from 71.4 ± 29.7 mmHg in control to 35.5 ± 13.6 mmHg and 23.0 ± 15.2 mmHg in medium and high concentrations of HMR-1556 respectively ($p < 0.01$)(Fig. 1A). HMR-1556 in medium and high concentrations also resulted in a lower HR when compared to control but this was not significant and there was minimal difference between the HR response with the medium and high concentrations. E4031 produced a significant concentration-dependent reduction in LVP and HR (Fig. 1B & 1D), particularly with the medium concentration for LVP (54.8 ± 20.9 mmHg to 34.0 ± 14.2 mmHg; $p < 0.05$) and with all concentrations for HR (Control: 185.5 ± 23.7 bpm to low: 153.1 ± 14.6 bpm, medium: 137.7 ± 13.9 bpm and high: 133.8 ± 10.4 bpm ($p < 0.001$)). The LVP and HR responses for the medium and high concentrations were similar. No significant difference in PP was found in different concentrations of HMR-1556 and E4031.

3.1.2. Effects of HMR1556 & E4031 on action potential duration ($MAPD_{90}$) during constant pacing at 200 ms cycle length

Heart preparations were perfused with low, medium and high concentrations of HMR-1556 and E4031 to measure the effect of concentration on monophasic action potential duration ($MAPD_{90}$) at the apex and base of the left ventricle. Apex $MAPD_{90}$ was significantly longer in the medium concentration of HMR-1556 compared to control; 122 ± 9 ms vs 140 ± 15 ms; $p < 0.01$ (Fig. 2A & 2C). This was also observed at the base (Fig. 2E & 2G); 120 ± 12 ms vs 141 ± 18 ms; $p < 0.05$. Interestingly, this response did not appear to be concentration dependent as the high concentration of HMR-1556 produced a shorter apical (125 ± 6 ms) and basal (123 ± 9 ms) $MAPD_{90}$ than the medium concentration. Like HMR-1556, E4031 had a significant effect on $MAPD_{90}$ prolongation at medium concentrations compared to control at both apex (Fig. 2B & 2D) and base (Fig. 2F & 2H); 120 ± 7 ms vs 144 ± 18 ms; 109 ± 17 ms vs 133 ± 21 ms; control vs medium concentration at apex and base respectively; $p < 0.05$ for both. This response appeared to be concentration dependent with similar responses seen at both medium and high concentrations. Analysis comparing differences between apex and base $MAPD_{90}$ for HMR-1556 and E4031 yielded no significance.

3.1.3. Effects of HMR-1556 & E4031 on ERP and VFT

The effect of concentration on ERP and VFT was investigated using low, medium and high concentrations of HMR-1556 & E4031. HMR-1556 increased ERP significantly at medium and high concentrations; 128 ± 15 ms vs 152 ± 14 ms and 157 ± 13 ms; control vs medium and high concentration respectively; $p < 0.05$ (Fig. 3A). There was also an increase in VFT at medium and high concentrations; 2.9 ± 1.3 mA vs 18.8 ± 12.7 mA* and 21.9 ± 13.9 mA*; control vs medium and high concentration respectively; $p < 0.05$ (Fig. 3C).

E4031 also caused a significant increase in ERP at medium concentrations (128 ± 15 ms vs 144 ± 14 ms) when compared to control. VFT was significantly different from control at medium and high concentrations; 1.8 ± 1.6 mA vs 16.1 ± 11.4 mA and 16.7 ± 14.2 mA; control vs medium and high, ERP and VFT data respectively; $p < 0.05$ for both (Fig. 3B & 3D). With both drugs, a concentration dependent response was observed and there was minimal difference between medium and high concentrations.

3.1.4. Effects of HMR-1556 & E4031 on restitution slopes (RT $slope_{max}$)

Restitution data was collected in the presence of low, medium and high concentrations of HMR-1556 & E4031 to investigate the effects of concentration on RT $slope_{max.}$ Restitution slopes for HMR-1556 were observed to be steepest with the medium concentration at both apex and base; 1.5 ± 0.8 vs 4.4 ± 2.7; 1.6 ± 0.7 vs 3.0 ± 0.5; control vs medium at apex and base respectively; $p > 0.05$ (Fig. 4). This response was not concentration dependent as the greatest response was seen with the

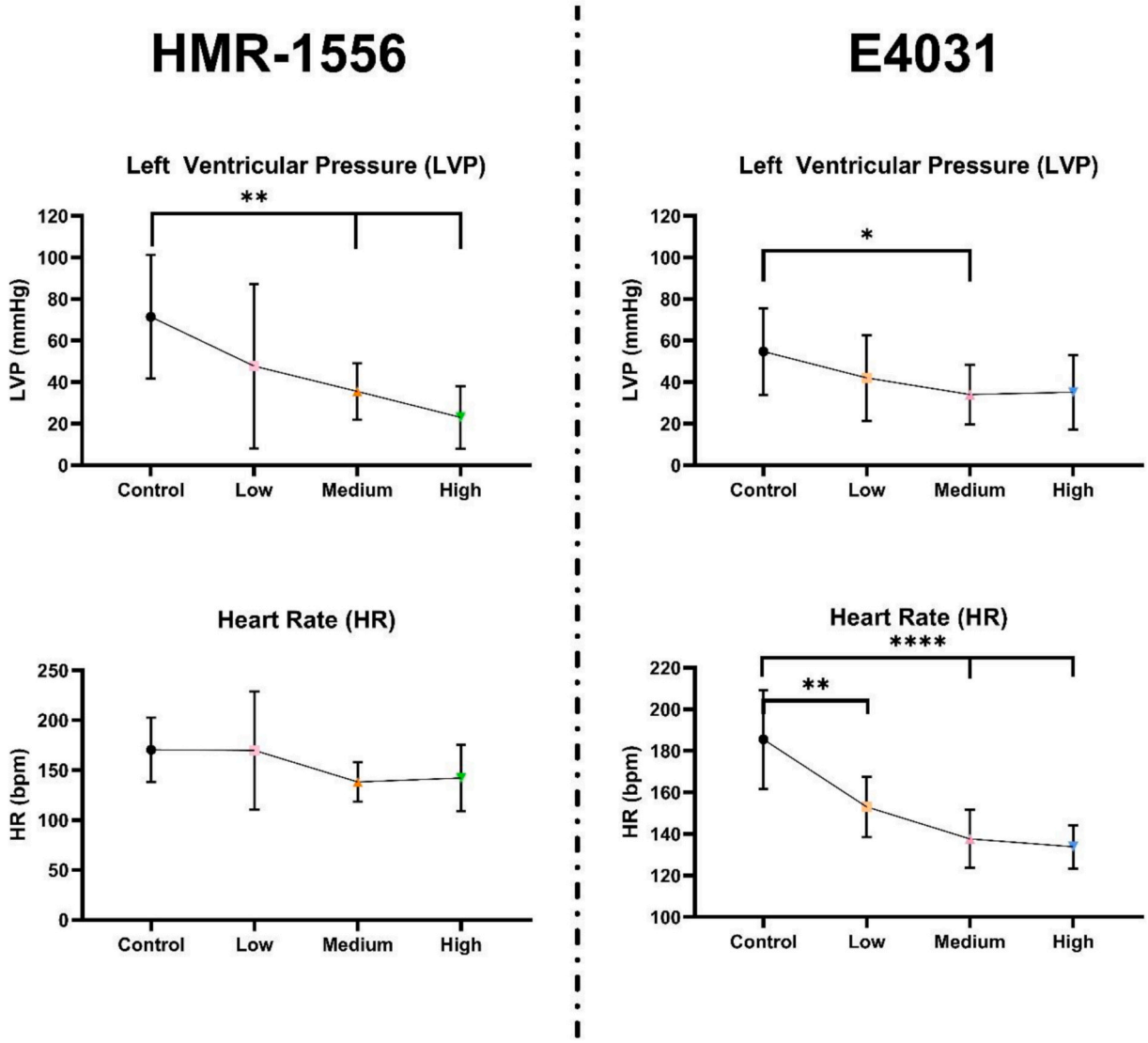

Fig. 1. Effects of HMR-1556 and E4031 on Left Ventricular Pressure and Heart Rate. The effects of HMR-1556 (low: 0.1 μM, medium: 0.5 μM, high: 1.0 μM), on left ventricular pressure (LVP) (A) and heart rate (C). The effects of E4031 (low: 0.02 μM, medium: 0.05 μM, high: 0.10 μM) on LVP (B) and heart rate (D). Data are mean ± SEM, $n = 10$ animals. *$p < 0.05$, **$p < 0.01$, $p < 0.0001$, one way ANOVA.

medium concentration.

Apex and base RT $Slope_{max}$ for E4031 were also determined to be steepest at medium concentration; 1.6 ± 0.1 vs 2.6 ± 1.5; 1.0 ± 0.1 vs 1.9 ± 0.1; control vs medium at apex and base respectively; $p < 0.05$ (Fig. 4) however the RT $slope_{max}$ values with the high concentration were similar. The steepest RT $slope_{max}$ values were observed with HMR-1556.

3.1.5. Summary of using HMR-1556 and E4031 to mimic LQT1 & 2

Medium dose of HMR-1556 and E4031 (0.5 μM and 0.05 μM respectively) were determined to be an appropriate dose to simulate LQT1 & 2 (sLQT1 & sLQT2), based on the data investigating concentration-response relationship. This is based particularly on the significant changes in functional parameters of the heart such as a reduction in HR and LVP, homogeneous prolongation of APD at apex and base, and increase in electrophysiological parameters such as ERP, VFT and RT $slope_{max.}$

3.2. Investigating β-adrenergic activation in LQT1&2

3.2.1. Effects of ISO on cardiac function in control and sLQTS

ISO was used to investigate the effects of β-adrenergic stimulation on cardiac function in the presence of HMR-1556 to simulate LQT1 (sLQT1) and with E4031 to simulate LQT2 (sLQT2). In sLQT1 experiments, LVP was increased on perfusion of ISO, although not significantly. When ISO was applied to sLQT1, a significant increase in LVP was observed; 34.7 ± 6.5 mmHg vs 72.2 ± 27.6 mmHg; sLQT1 vs ISO + sLQT1; $p < 0.05$ (Fig. 5A). HR was also increased significantly with ISO when added in control conditions (151.2 ± 12.5 bpm vs 263.5 ± 26.6 bpm; $p < 0.0001$) and with sLQT1(136.8 ± 23.19 bpm vs 218.7 ± 3.1 bpm; $p < 0.0001$) (Fig. 5C).

In sLQT2 experiments, LVP was not significantly increased on perfusion of ISO; 62.1 ± 23.2 mmHg vs 71.6 ± 21.8 mmHg; Control vs ISO. A significant increase was observed with ISO perfusion with sLQT2; 35.4 ± 14.1 mmHg vs 62.1 ± 24.1 mmHg; sLQT2 vs ISO + sLQT2; $p < 0.001$ (Fig. 5B). HR was also increased significantly in control ISO conditions and in sLQT2 in presence of ISO; 188.7 ± 28.2 bpm vs 265.6 ± 34.9 bpm and 137.8 ± 16.9 bpm vs 239.1 ± 23.3 bpm; Control vs ISO and sLQT2 vs ISO + sLQT2 respectively; $p < 0.0001$ for both (Fig. 5D).

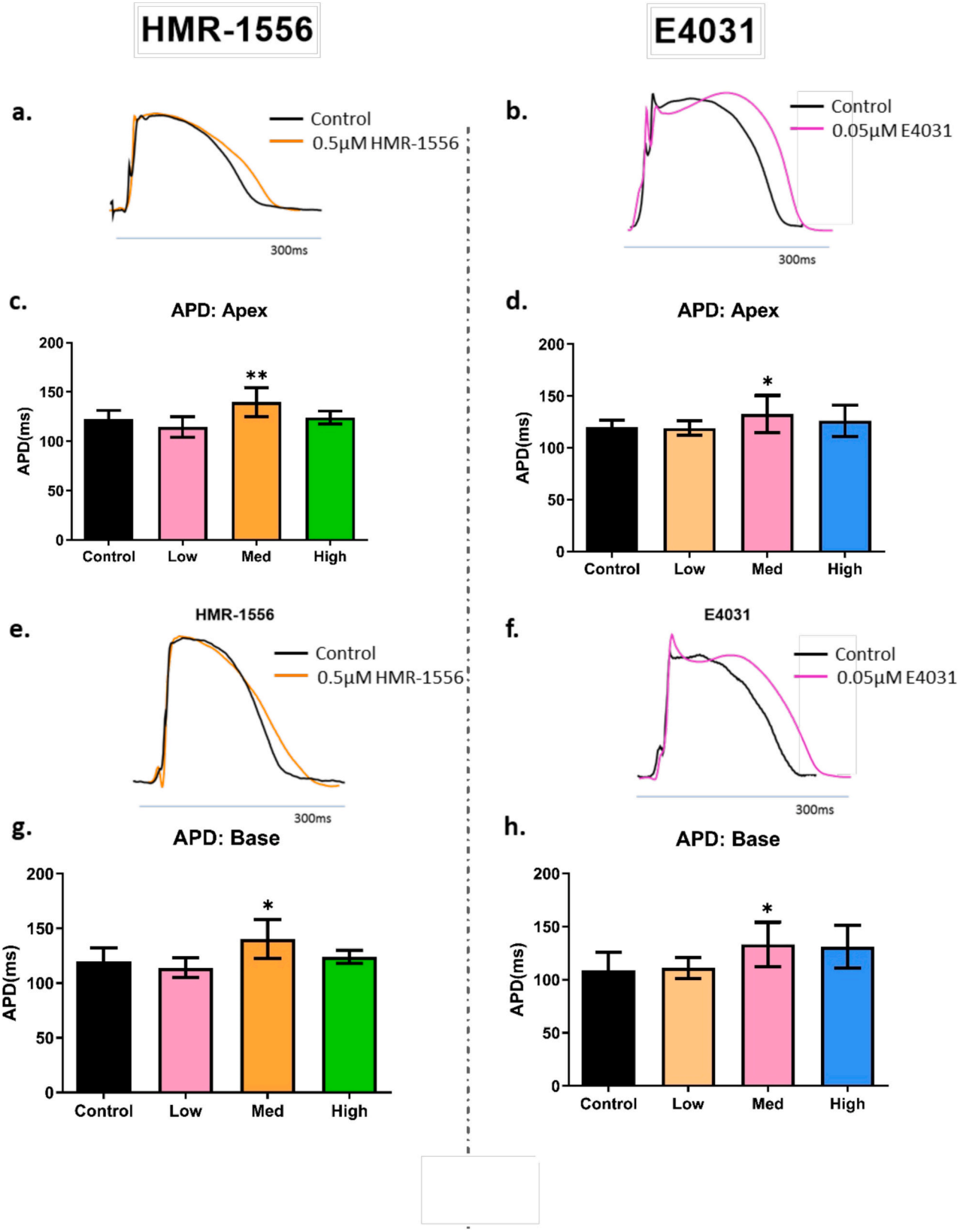

Fig. 2. Effects of HMR-1556 and E4031 on Monophasic Action Potential Duration ($MAPD_{90}$) during constant pacing. A&E shows raw MAP traces when pacing at 200 ms in control conditions and with 0.5 μM HMR-1556 at apex and base respectively. B&F shows raw MAP traces in control conditions and with 0.05 μM E4031 apex and base respectively. C&G show the effects of HMR-1556 (low: 0.1 μM, medium: 0.5 μM, high: 1.0 μM), and D&H show the effects of E4031 (low: 0.02 μM, medium: 0.05 μM, high: 0.10 μM) on $MAPD_{90}$ at apex and base. Data are mean ± SEM, n = 10 animals. *$p < 0.05$, **$p < 0.01$, one way ANOVA.

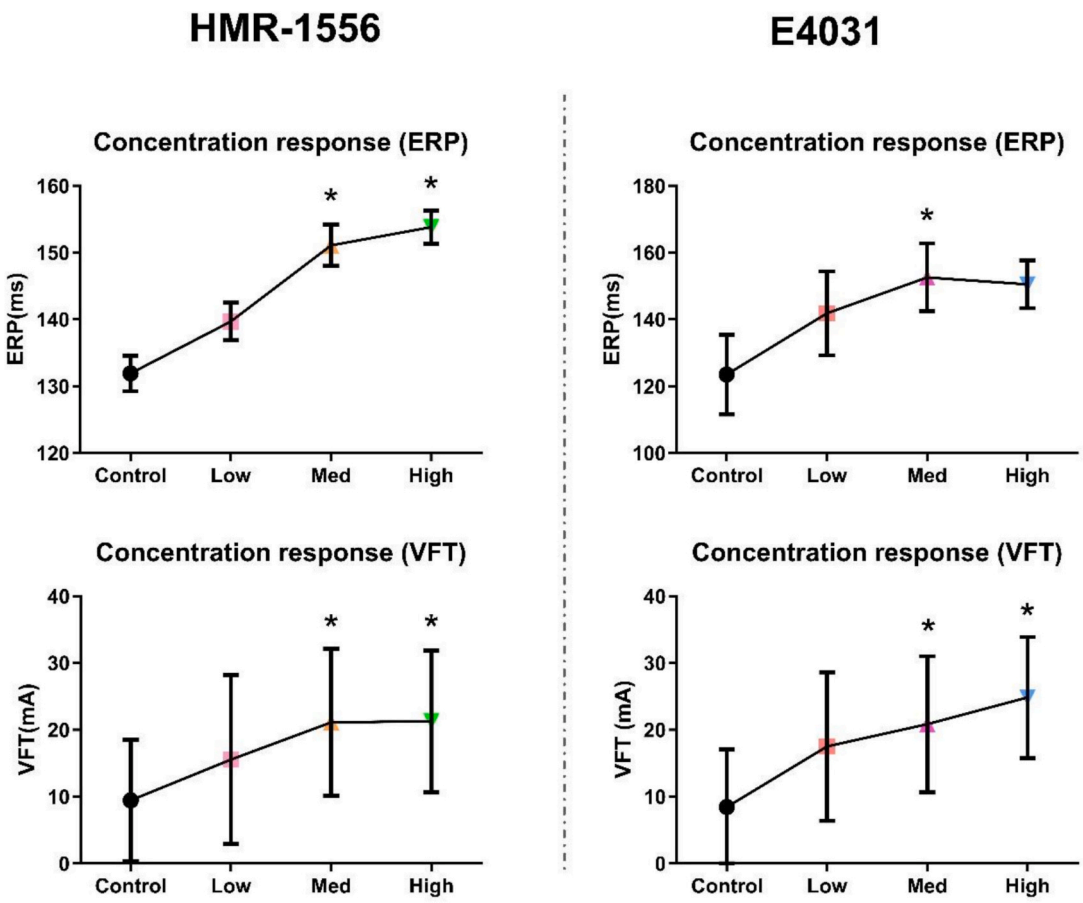

Fig. 3. Effects of HMR-1556 and E4031 on Effective Refractory Period (ERP) and Ventricular Fibrillation Threshold (VFT). A&B show the mean ERP data and C—D show the mean VFT data during control conditions and with HMR-1556 (low: 0.1 μM, medium: 0.5 μM, high: 1.0 μM), and E4031 (low: 0.02 μM, medium: 0.05 μM, high: 0.10 μM). Data are mean ± SEM, n = 10 animals. *p < 0.05, one way ANOVA.

There were no effects on PP that achieved statistical significance.

3.2.2. Effects of ISO on monophasic action potential duration ($MAPD_{90}$) in control and sLQTS during constant pacing at 200 ms cycle length

ISO was used to investigate the effects of β-adrenergic stimulation on monophasic action potential duration ($MAPD_{90}$) in sLQT1 and sLQT2 conditions. ISO shortened $MAPD_{90}$ at both apex and base when compared to control conditions (Fig. 6). In sLQT1 experiments, ISO was still able to shorten the $MAPD_{90}$ at the apex (140 ± 16 ms vs 104 ± 29 ms; $p < 0.001$) and base (141 ± 19 ms vs 97 ± 27 ms; $p < 0.0001$). Similarly, ISO shortened $MAPD_{90}$ at both apex (144 ± 19 ms vs 116 ± 30 ms; $p < 0.05$) and base (133 ± 23 ms vs 107 ± 39 ms; $p > 0.05$) during sLQT2 experiments. There were no significant differences between apex and base $MAPD_{90}$ or between sLQT1 and sLQT1 $MAPD_{90}$.

3.2.3. Effects of ISO on ERP and VFT in control and sLQTS

ERP and VFT were measured in the presence of ISO in both sLQT1 and sLQT2 conditions. ISO caused a significant reduction in ERP from control ($p < 0.0001$). In sLQT1 experiments, ERP was reduced from 160 ± 6 ms to 143 ± 16 ms with ISO ($p < 0.01$) (Fig. 7A). A similar result was also observed in sLQT2 experiments where ISO had the effect of decreasing ERP from 152 ± 12 ms to 118 ± 10 ms ($p < 0.0001$)(Fig. 7B) however a greater decrease in ERP was seen in these conditions.

VFT was also reduced with ISO in comparison to control conditions. In sLQT1 experiments, VFT decreased from 26.2 ± 8.0 mA to 8.45 ± 8.8 mA with ISO ($p < 0.0001$)(Fig. 7C). A reduction in VFT was also observed in sLQT2 experiments from 23.4 ± 9.7 mA to 8.4 ± 11.2 mA with ISO ($p < 0.0001$) (Fig. 7D).

Percentage changes in ERP (%ΔERP) and VFT (%ΔVFT) were calculated (Fig. 7E & 7F). In sLQT1 experiments, there was a significantly smaller change in ERP (−16.2 ± 16.7%) when compared to control conditions (−31.5 ± 4.7%) ($p < 0.05$). In contrast, there was no significant difference in ERP between control (−31.0 ± 12.3%) and sLQT2 (−34.9 ± 14.0%) ($p > 0.05$). There was also a difference in %Δ VFT between the two models in presence of ISO, where there was a greater increase in VFT from control to sLQT1 (−6.1 ± 3.7% vs −17.8 ± 10.2%, $p < 0.01$) than control to sLQT2 (−5.1 ± 5.2% vs −15.0 ± 10.5%, $p < 0.05$).

3.2.4. Effects of ISO on action potential duration restitution kinetics in control and sLQTS

ISO was used to investigate the effects of β-adrenergic stimulation on action potential duration restitution in sLQT1 and sLQT2 conditions. ISO was observed to increase the $MAPD_{90}$ restitution slope gradient (RT $Slope_{max}$) at the apex and base (Fig. 8A-D), although this did not reach significance. However, RT $Slope_{max}$ decreases in sLQT1, in presence of β-adrenergic stimulation; 3.1 ± 3.1 vs 0.9 ± 0.7 and 3.0 ± 2.2 vs 0.9 ± 0.8*; sLQT1 vs ISO + sLQT1 at apex and base respectively; $p = 0.10$ and $p = 0.05$ respectively.

In sLQT2 experiments, baseline β-adrenergic stimulation showed an increase in RT $Slope_{max}$ at the apex and base (Fig. 8B & D); 1.2 ± 0.4 vs 1.7 ± 0.4 and 0.9 ± 0.3 vs 1.4 ± 0.5*, Control vs ISO at apex and base respectively; $p = 0.09$ for apex and $p < 0.05$ for base. In contrast to the data obtained from sLQT1, RT $Slope_{max}$ at the apex and base were determined to be steeper during β-adrenergic stimulation of sLQT2; 1.7 ± 0.6 vs 3.8 ± 2.7 and 1.5 ± 0.6 vs 2.0 ± 0.9; sLQT2 vs ISO + sLQT2 at apex and base respectively; $p = 0.09$ for apex and $p = 0.07$ for base.

Percentage change in RT $Slope_{max}$ (%ΔRT $Slope_{max}$) was also

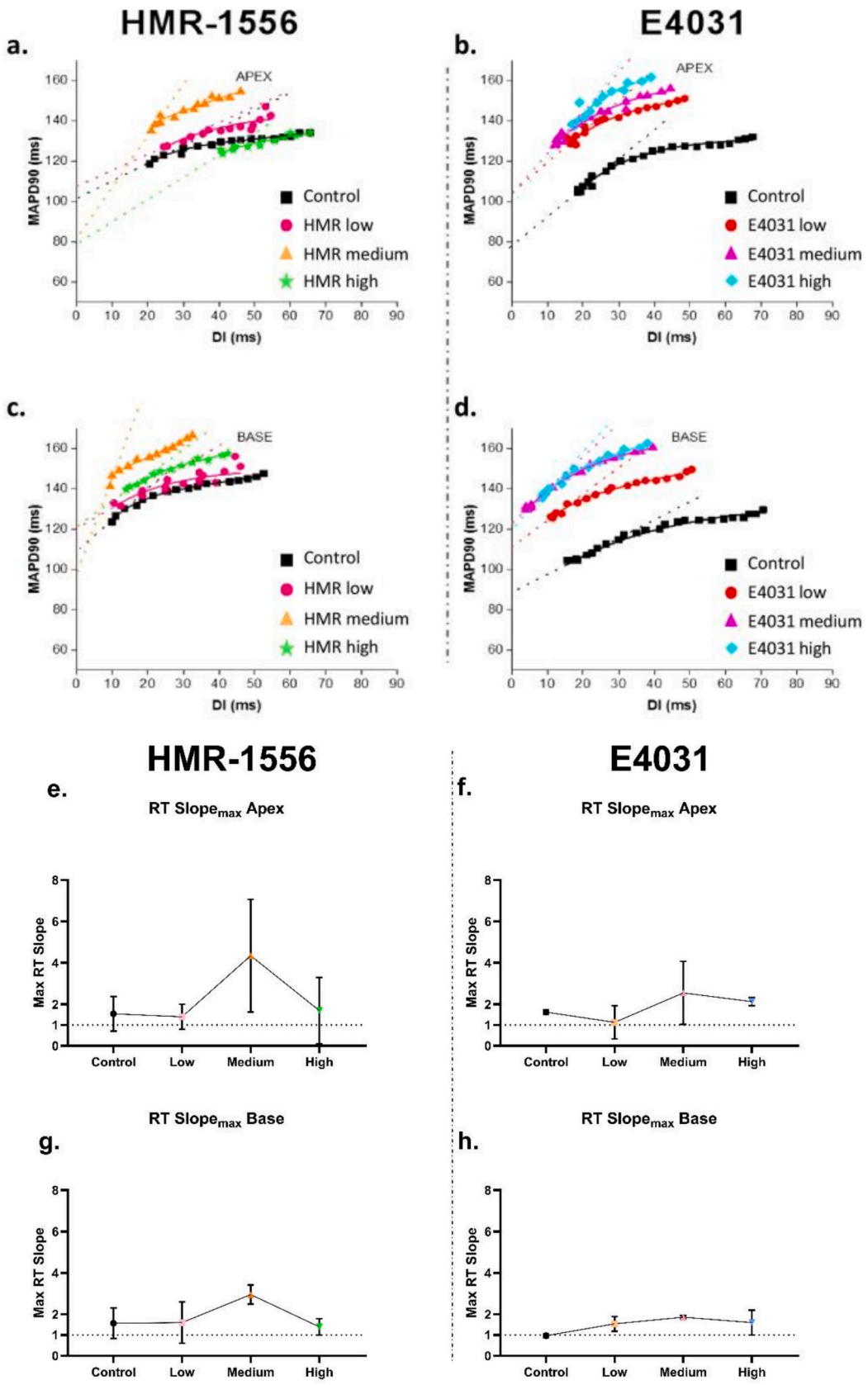

Fig. 4. Effects of HMR-1556 and E4031 on Monophasic Action Potential Duration Restitution. A-D: Representative restitution curves for control conditions and with HMR-1556 (low: 0.1 μM, medium: 0.5 μM, high: 1.0 μM) and E4031 (low: 0.02 μM, medium: 0.05 μM, high: 0.10 μM) fitted with exponential functions (solid lines, see methods). Dotted lines represent the maximum gradient of the restitution curves. *E*-F: Mean (± SEM) RT Slope$_{max}$ plotted during control conditions and with HMR-1556 (low: 0.1 μM, medium: 0.5 μM, high: 1.0 μM) and E4031 (low: 0.02 μM, medium: 0.05 μM, high: 0.10 μM) at the apex (E&F) and the base (G&H). Data are mean ± SEM, $n = 4$ animals. $p > 0.05$ (NS), one way ANOVA and paired *t*-tests.

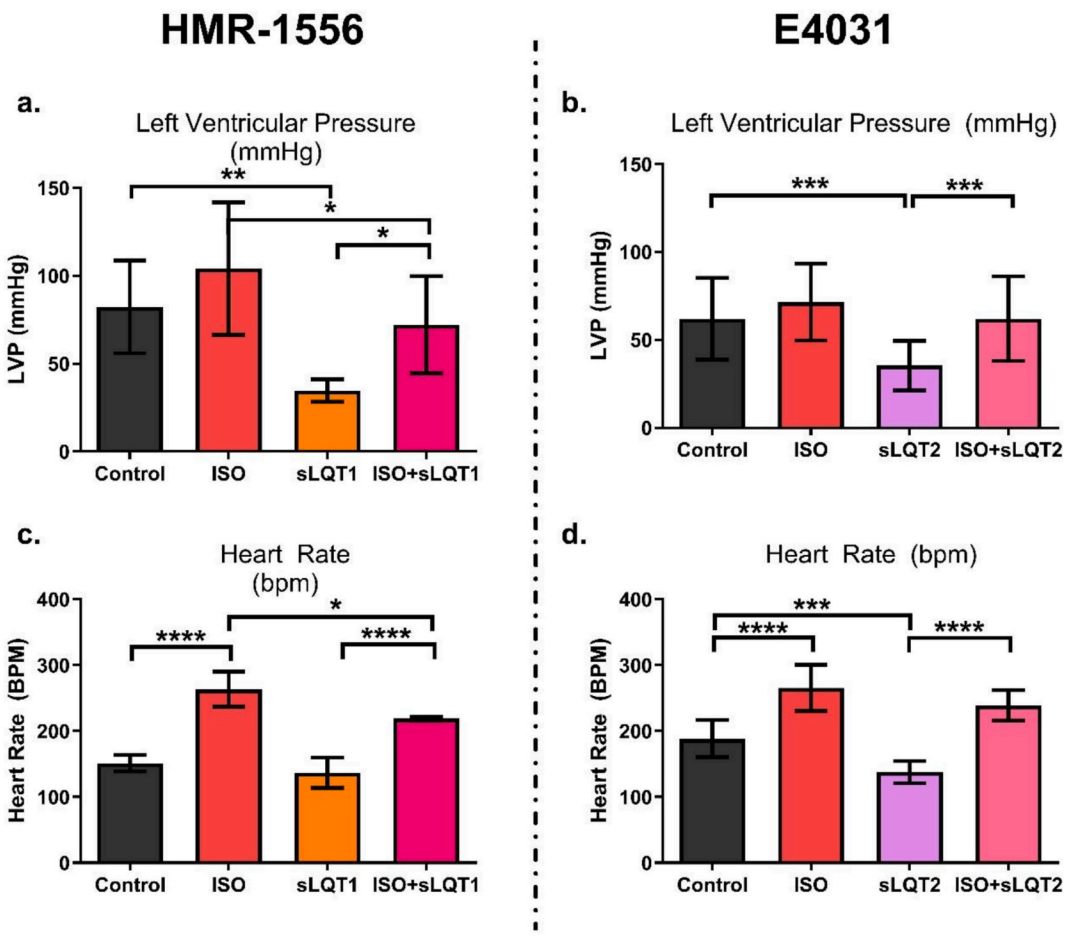

Fig. 5. Effects of β-adrenergic stimulation using isoprenaline (ISO) on Left Ventricular Pressure (LVP) and Heart Rate (HR) during sLQT1&2. Left ventricular pressure (LVP) and heart rate (HR) data for sLQT1 (0.5 μM HMR-1556) is shown on the left and for sLQT2 (0.05 μM E4031) is shown on the right in control conditions and in the presence of isoprenaline (ISO). Data is mean ± SEM, $n = 12$ animals. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; two way ANOVA.

analysed, showing dichotomous results of β-adrenergic activation in sLQT1 and sLQT2 compared to their respective controls (Fig. 8E & F).

3.2.5. *Summary of β-adrenergic stimulation of sLQTS*

ISO had elevating effects on heart rate and left ventricular pressure in control conditions as well as sLQT1 and sLQT2; and $MAPD_{90}$ was shortened in the presence of ISO in all conditions. $\%\Delta MAPD_{90}$ during adrenergic activation in sLQT1 was smaller compared to control and the change observed in sLQT2. Analysis of changes between apex and base $MAPD_{90}$ yielded no significance.

In presence of ISO, %ΔERP was found to be greater in sLQT1 compared to control changes; in contrast with the marginal %ΔERP in sLQT2 in regards to its respective control. VFT dropped significantly in presence of ISO in both sLQT1&2. Restitution slope gradient was decreased in sLQT1 upon β-adrenergic activation, but increased in sLQT2; significant change in RT $Slope_{max}$ when comparing the two models.

4. Discussion

The aim of this study was to firstly create pharmacological models for LQT1 & 2 by using HMR-1556 and E4031 respectively; and secondly to investigate the effects of β-adrenergic receptor stimulation in LQT1 & 2.

4.1. *Effects of HMR-1556 & E4031 on physiological parameters*

4.1.1. *Effects of HMR-1556 & E4031 on HR and LVP (sinus rhythm)*

E4031 caused significant bradycardia whereas the decrease in heart rate observed with HMR-1556 was not significant as also reported in existing literature (Sanguinetti et al., 1995; Gögelein et al., 2000). I_{Ks} and I_{Kr} are expressed in the sinoatrial node and contribute to repolarisation phase of the pacemaker action potential as well as the atrial and ventricular action potentials (Bertaso et al., 2002; Schram et al., 2002). IKr is well known to contribute an outward, slowly decreasing current during the diastolic depolarisation. It is the slow deactivation kinetics of I_{Kr} that are important for timing the contribution to the diastolic depolarisation. Simply blocking I_{Kr} would be expected to accelerate the diastolic depolarisation by reducing the amount of outward relative to inward current, which is the opposite to what was observed. One possibility is that E4031 affects the time dependent kinetics of I_{Kr} channel deactivation, slowing deactivation. To our knowledge, the mechanistic basis behind this has yet to be described. I_{Ks} has a less important role in the timing of the diastolic depolarisation and this is consistent with the effects of HMR-1556 on heart rate.

Data in the present study show a decrease in LVP with increasing concentrations of HMR-1556 and E4031; a trend also demonstrated by Gögolein et al. (Gögelein et al., 2000) in guinea pigs. This phenomenon can be explained by the force frequency relationship; an inotropic effect that is caused by an accumulation of intracellular Ca^{2+} at high heart rates and is present in both human and guinea pig hearts. This increase in $[Ca^{2+}]_i$ promotes an increased force of contraction; therefore, the

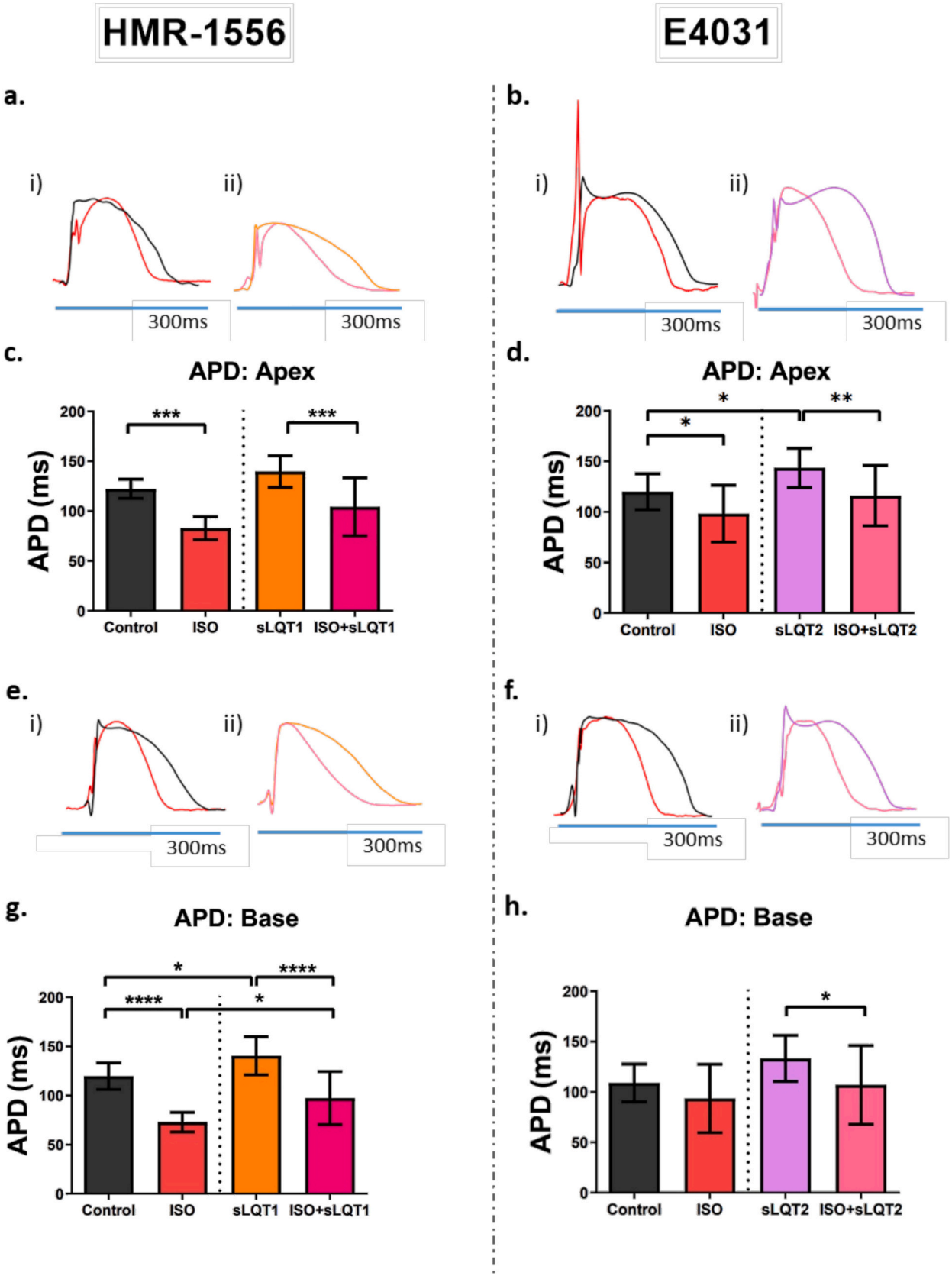

Fig. 6. Effects of β-adrenergic stimulation using isoprenaline (ISO) on Monophasic action Potential Duration ($MAPD_{90}$) during sLQT1&2. Monophasic action Potential Duration ($MAPD_{90}$) data when pacing at 200 ms for sLQT1 (0.5 μM HMR-1556) is shown on the left and for sLQT2 (0.05 μM E4031) is shown on the right in control conditions and in the presence of isoprenaline (ISO) at the apex (A-D) and the base (E-F). Raw MAP traces showing control vs ISO in A(i) and B(i) at the apex and E(i) and F(i) at the base, and sLQTS vs ISO + LQTS in A(ii)(apex) and E(ii)(base) for sLQTS1 and B(ii)(apex) and F(ii)(base) for sLQTS2. Trace colours match with the conditions on the bar graphs. Data is mean ± SEM, n = 12 animals. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; two way ANOVA.

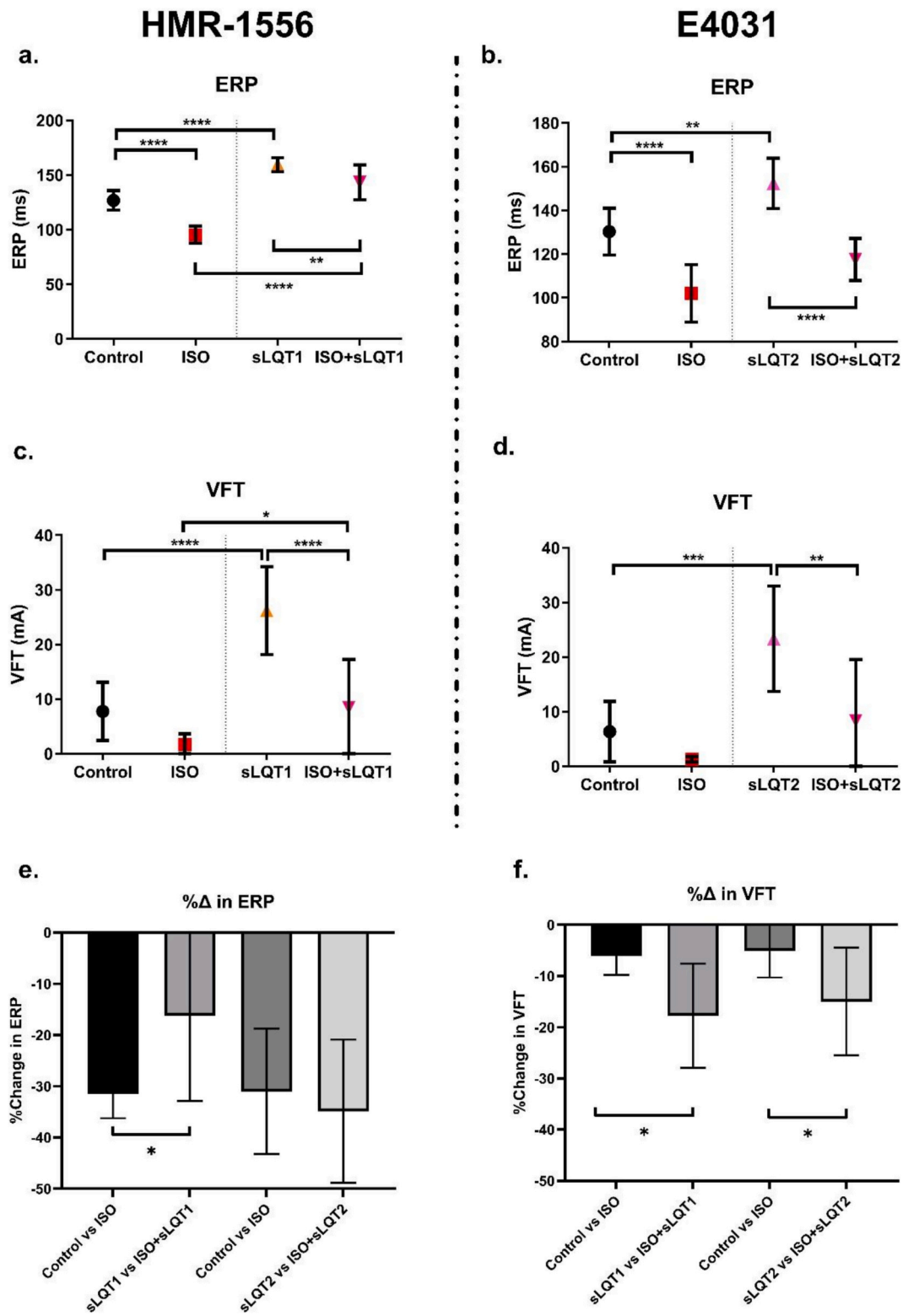

Fig. 7. Effects of β-adrenergic stimulation using isoprenaline (ISO) on Effective Refractory Period (ERP) and Ventricular Fibrillation Threshold (VFT) during sLQT1&2. Effective refractory period (ERP) (a and b) and ventricular fibrillation threshold (VFT) (c and d) data for sLQT1 (0.5 μM HMR-1556) is shown on the left and for sLQT2 (0.05 μM E4031) is shown on the right in control conditions and in the presence of isoprenaline (ISO). Percentage change in ERP (e) and percentage change in VFT (f) data are also shown. Data is mean ± SEM, $n = 9$ animals for sLQT1 and $n = 7$ animals for sLQT2. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; two way ANOVA (a-d), unpaired t-test (e-f).

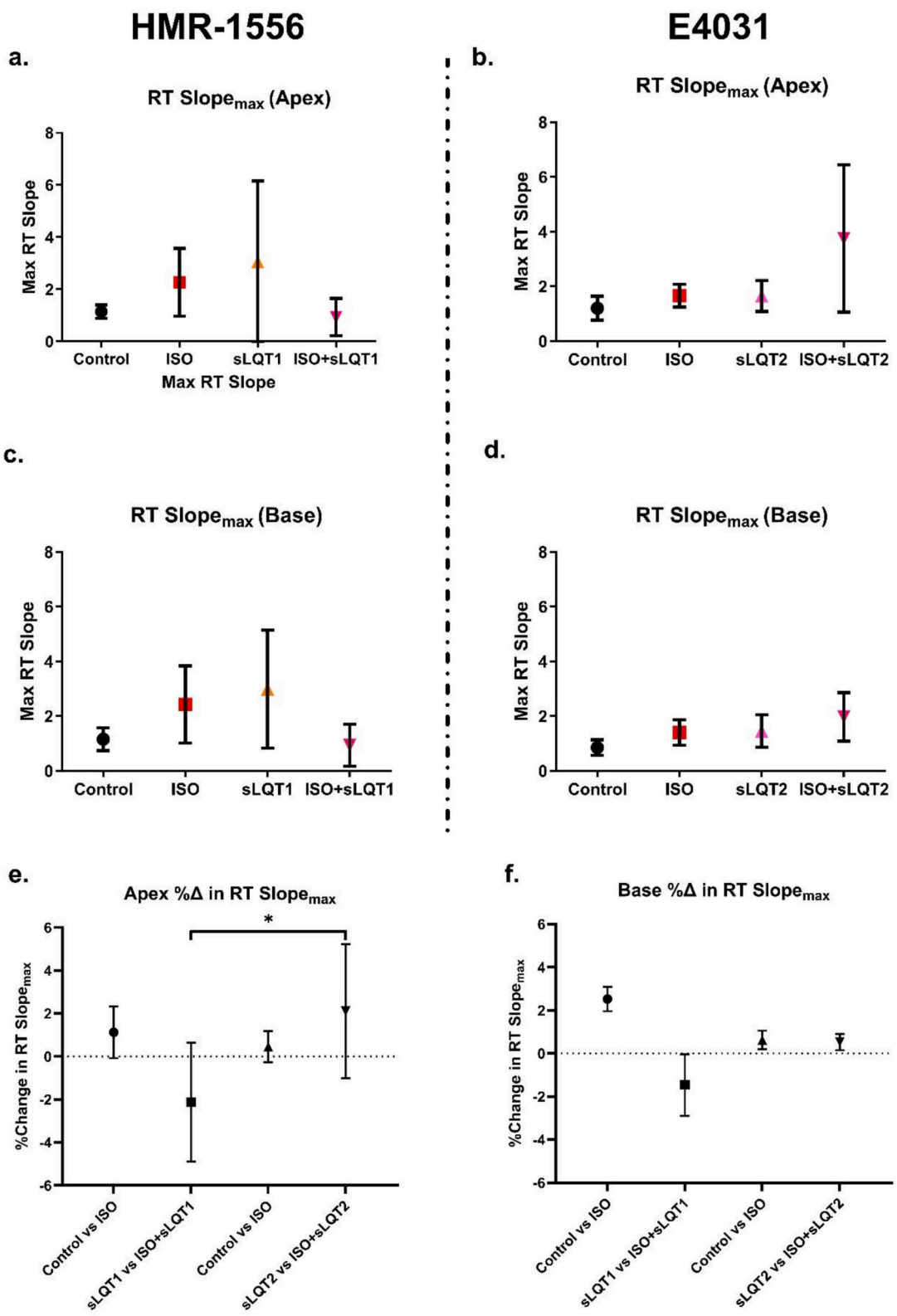

Fig. 8. Effects of β-adrenergic stimulation using isoprenaline (ISO) on Monophasic Action Potential Duration Restitution during sLQT1&2. Max RT slope data for sLQT1 (0.5 μM HMR-1556) at the apex (a) and base (c) is shown on the left and for sLQT2 (0.05 μM E4031) is shown on the right at apex (b) and base (d) in control conditions and in the presence of isoprenaline (ISO). Percentage change in max RT slope is also shown at apex (e) and base (f). Data is mean ± SEM, $n = 6$ animals. *$p < 0.05$; two way ANOVA.

converse takes place in a reduced heart rate, producing a resultant decrease in force of ventricular contraction (Carmeliet, 2004; Bers, 2000). HMR-1556 has also been found to block L-type Ca^{2+} channels at high concentrations (10 μM) causing a negative inotropic response (Gögelein et al., 2000). However, the concentration of HMR-1556 needed to block the Ca2+ channel is much higher than that needed to block I_{Ks}.

4.1.2. Effects of HMR-1556 and E4031 on $MAPD_{90}$

Data obtained in this project show significant prolongation of $MAPD_{90}$ in medium concentrations of HMR-1556 and E4031, an observation that agrees with a wealth of evidence in the literature (Gögelein et al., 2000; Han et al., 2001) confirming the critical role I_{Ks} and I_{Kr} play in the repolarisation of ventricular action potentials. Block of either current would therefore cause a lengthening in $MAPD_{90}$ (Tamargo et al., 2004; Heath and Terrar, 1996; Jost et al., 2005). The response seen with E4031 appeared to be concentration dependent with the similar responses seen for both medium and high concentrations. With HMR-1556, the effects on $MAPD_{90}$ were not concentration dependent with the medium concentration showing the greatest increase in $MAPD_{90}$. This could suggest evidence of block of other currents at high concentrations. However, existing literature suggests that HMR-1556 is a selective and potent inhibitor of I_{Ks} and block of other currents such as I_{to}, I_{sus} and L-type Ca^{2+} channels only occurs at higher concentrations (10 μM) (Schwartz et al., 2001).

There were no significant differences found comparing $MAPD_{90}$ at the apex and base during I_{Ks} and I_{Kr} blockade; this suggests a consistent prolongation of APD at both sites of the heart. This is in agreement with a study by Brunner et al., who used transgenic rabbits to create LQT1 models (Brunner et al., 2008); but different from data from other existing literature. An increased I_{Ks} channel distribution at the base of the myocardium has been reported in other rabbit and human studies (Cheng et al., 1999; Ng et al., 2009), which implies that HMR-1556 should have had a greater prolongation effect at the base than the apex, as demonstrated by Ng et al., who used HMR-1556 to simulate LQT1 pharmacologically and showed a greater APD prolongation at the apex than the base in rabbits (Ng et al., 2009). However, one can argue that the absence of association between regional channel distribution and response to inhibition is linked with the finding that I_{Ks} activity is more pronounced only in presence of sympathetic stimulation (Sanguinetti et al., 1991; Volders et al., 2003) in addition to species differences.

The implied homogenous prolongation of APD in presence of E4031 is also from reports from existing literature. Like the heterogenous distribution of I_{Ks} channels, I_{Kr} channels have also been reported to be present at higher densities at the apex than the base; and studies have demonstrated a greater prolongation of APD in apex than base when I_{Kr} channels were blocked by E4031 in rabbits (Cheng et al., 1999). This could be because of species difference in channel expression (Volders et al., 2003; Varró and Baczkó, 2011), or because the study was carried out on single cells, whereas the current experiments are based on whole hearts, where there is a greater influence of other factors such as gap junctions and repolarisation reserve (Roden, 2008; Viswanathan et al., 1999).

4.1.3. Effects of HMR-1556 and E4031 on ERP and VFT

Both HMR-1556 and E4031 caused a concentration-dependent increase in ERP, as ERP is directly related to the increase in APD. The trend of increased ERP may be explained by the reduced rate of repolarisation when I_{Ks} or I_{Kr} blockade takes place, which implies that a longer duration is required for membrane potential to return to a voltage negative enough for I_{Na} channels to fully recover from their inactivation. This observation is in accord with existing findings by So et al. demonstrated in an in vivo study with HMR-1556 (So et al., 2007a) and by Sanguinetti et al. in guinea pig myocytes with E4031 (Sanguinetti et al., 1991).

HMR-1556 and E4031 also caused a significant increase in VFT in a concentration-dependent manner, which suggests that they have a cardioprotective effect. The findings for HMR-1556 are in accord with several studies that observed the absence of TdP in the sole presence of I_{Kr} blockade (So et al., 2007a; Lengyel et al., 2007; Michael et al., 2007) and with studies suggesting I_{Ks} channel dysfunction is not the sole cause of arrhythmogenesis in LQT1 (Brunner et al., 2008; Shimizu and Antzelevitch, 2000; Shimizu and Antzelevitch, 1998).

The implicated resistance to VF of E4031 is contradicted by clinical reports of LQT2 patients as well as animal studies that show enhanced arrhythmia susceptibility in I_{Kr} channel blockade (Sanguinetti et al., 1995; Michael et al., 2007). This could be due to a limitation in methodology; although the reliability of VFT protocol has been demonstrated in determining VF susceptibility (Ng et al., 2007; el-Sherif et al., 1996), it has been suggested that the protocol is less reliable in studies investigating APD prolongation drugs (Quesada et al., 1993; Jaillon et al., 1980).

However, the decrease in VF susceptibility is supported by data from Lynch et al., who concluded that lengthening of ERP has a protective effect (Lynch Jr. et al., 1990). There are also findings that suggest I_{Kr} blockade does not necessarily cause TdP all the time, because of the concept of repolarisation reserve (Herring et al., 2019; Silva and Rudy, 2005); that blockade of one channel may not manifest unless there are additional lesions or dysfunction, as the heart has multiple mechanisms that contribute to repolarisation and can be modulated to compensate for the loss of a channel to prevent malignant APD prolongation (Silva and Rudy, 2005; Roden and Yang, 2005; Zeng et al., 1995).

4.1.4. Effects of HMR-1556 and E4031 on restitution

HMR-1556 and E4031 increased maximum restitution slope at medium dose, suggesting that there is an increased susceptibility in arrhythmogenesis with I_{Ks} and I_{Kr} blockade, according to the "Restitution Hypothesis". The association of I_{Ks} and I_{Kr} blockade with implied increased arrhythmia vulnerability has been shown in existing studies, as there is an implication of repolarisation abnormality (Tsuji et al., 2006; Tsuji et al., 2000). I_{Kr} blockade is also reported to induce the incidence of electrical alternans and TdP in existing literature (Tsuji et al., 2000; Fossa et al., 2004; Woosley et al., 1993; Heist and Ruskin, 2005; Bischoff et al., 2000), as well as increasing restitution slopes (Yamauchi et al., 2002); although there are mixed findings (Lengyel et al., 2007).

4.2. Effects of ISO on cardiac electrophysiology

4.2.1. Effects of ISO on electrophysiological parameters in baseline conditions, sLQT1 & 2

Perfusion of isoproterenol caused an increase in left ventricular pressure and heart rate due to the inotropic and chronotropic effects of β-adrenergic activation on the heart; and perfusion pressure decrease as it has a vasodilatory effect on coronary vessels. This effect was also mimicked in sLQT1 and 2, as I_{Ks} and I_{Kr} channel blockade does not impede the enhanced intracellular calcium oscillations upon ISO activation.

4.2.2. Effects of ISO on $MAPD_{90}$ in baseline conditions, sLQT1 & 2

ISO had an effect of significantly shortening $MAPD_{90}$; an effect that is keeping with existing literature (Jurkiewicz and Sanguinetti, 1993). The significant changes of action potential duration of sLQT1 and 2 in presence of β-adrenergic activation agrees with findings from existing studies (Han et al., 2001; Volders et al., 2003; Giles et al., 1989).

β-adrenergic stimulation significantly shortened MAPD90 in both baseline conditions and in the presence of simulated LQT1 and LQT2. This observation is consistent with previous studies demonstrating that β-adrenergic activation accelerates ventricular repolarisation through modulation of multiple ionic currents, including enhancement of IKs activity. In the present study, no statistically significant difference in the magnitude of MAPD90 shortening was observed between sLQT1 and

sLQT2 conditions. This may reflect the integrated nature of repolarisation in the intact heart, where multiple ionic currents contribute to action potential duration and may compensate for pharmacological inhibition of individual potassium currents. Some studies have determined I_{Ks} channels as the dominant repolarisation channel in presence of β-adrenergic stimulation (Sanguinetti et al., 1991; Banyasz et al., 2014); and I_{Kr} channels as the dominant repolarisation channel in absence of sympathetic stimulation (Jost et al., 2005). I_{Ks} has been suggested to have a greater role in APD shortening when ventricular repolarisation reserve is limited (Jost et al., 2005). In sLQT1, where I_{Ks} channels are blocked, the main channel for repolarisation in presence of sympathetic stimulation is no longer functional and thus will manifest in a longer APD than baseline; this effect has also been observed by Volders et al. in canines (Volders et al., 2003) and Jost et al. in humans (Jost et al., 2005). In contrast, I_{Kr} blockade has been reported to have a smaller effect in inhibiting the effectiveness of overall repolarisation in sLQT2 (Varró and Baczkó, 2011; Tsuji et al., 2000).

4.2.3. Effects of ISO on ERP and VFT in baseline conditions, sLQT1 & 2

ISO significantly shortened ERP in baseline conditions, sLQT1 and sLQT2. Intriguingly, there is a significant difference in ERP change, in sLQT1 but not in sLQT2. This finding is in agreement with published studies regarding the different roles of I_{Ks} and I_{Kr} in repolarisation during the absence and presence of sympathetic activity; I_{Ks} is reported to decrease refractory period during sympathetic activation, as PKA phosphorylation secondary to adrenergic receptor activation upregulates I_{Ks} activity through the phosphorylation of the subunit (Sanguinetti et al., 1991)– but has a negligible (Thomas et al., 1999) or even an inhibitory effect on I_{Kr} channels (Volders et al., 2003; Karle et al., 2002; Varró et al., 2001).

An interesting observation in the present study was that the effect of β-adrenergic stimulation on ERP differed from that observed for MAPD90 in the sLQT1 condition. While ISO shortened MAPD90 to a similar extent in both control and sLQT1 hearts, the reduction in ERP was smaller in sLQT1 compared with control. This discrepancy may reflect the fact that MAPD90 and ERP represent distinct electrophysiological measurements. MAPD90 reflects local repolarisation at the epicardial recording site, whereas ERP represents the functional refractory period of the myocardium. As such, ERP may be influenced by additional factors including conduction properties, recovery of sodium channel availability, and post-repolarisation refractoriness. Furthermore, the ERP measurement obtained using programmed stimulation may reflect refractoriness within the conduction pathway between the site of stimulation and the recording electrode, rather than solely the local action potential duration at the recording site. These factors may therefore contribute to the differing effects of β-adrenergic stimulation on ERP and MAPD90 observed in the sLQT1 condition.

VFT was also lowered in presence of ISO in each of the conditions, where there is a more significant decrease comparing β-adrenergic activation of sLQT1 with sLQT2; which is in line with the notion that I_{Ks} has a more significant role in repolarisation of the myocardium when sympathetic stimulation is activated. This suggests that the myocardium is more prone to arrhythmia in presence of I_{Ks} blockade than I_{Kr} blockade. This reinforces the implication reported in other studies that I_{Kr} channels have more dominant roles in repolarisation in normal conditions, whereas I_{Ks} channels have a more pronounced role in preventing arrhythmogenesis during sympathetic activation (Han et al., 2001; Bryant et al., 1998).

4.2.4. Effects of ISO on RT slope in baseline conditions, sLQT1 & 2

The RT slope data contradicts data from published literature that I_{Ks} has a larger role than I_{Kr} in repolarisation during sympathetic stimulation. The restitution slopes obtained upon β-adrenergic activation of sLQT1 was significantly flattened, in contrast to the steeper gradient of stimulated sLQT2 RT slopes. This suggests that β-adrenergic activation during I_{Ks} and I_{Kr} blockade reduces and increases arrhythmia susceptibility respectively. The implication that I_{Kr} channels have a greater role in arrhythmogenesis according to the "Restitution Hypothesis" contradicts current evidence that I_{Ks} channel has a more significant role during sympathetic stimulation.

A possible reason for increased RT slope with I_{Kr} blockade is enhancement of spatial and transmural dispersion of repolarisation that already exists in the whole heart, where different regions of the epicardial surface and myocardial layers have been shown to have varied densities of I_K channel expressions (Kawano et al., 2003). When coupled with the finding that sympathetic stimulation changes repolarisation direction (Roden, 2008; Thomas et al., 1999), there is a strong implication that refractoriness may also be enhanced; accounting for the increased susceptibility to arrhythmia.

Another explanation relates to the repolarisation reserve hypothesis (Jost et al., 2005), where an increased arrhythmia susceptibility can be explained by changes in repolarisation reserves during I_{Kr} blockade (Roden, 2008; Silva and Rudy, 2005; Biliczki et al., 2002). Current findings that indicate that PKA has an inhibitory effect on I_{Kr} channel in single cells (Karle et al., 2002; Mantravadi et al., 2007; Kiehn et al., 1998), suggesting that the downregulation of I_{Kr} channels takes place physiologically during sympathetic stimulation; thus, during I_{Kr} blockade in sLQT2, the reduced capacity of functional I_{Kr} channels available to offset the inward current for cellular repolarisation may increase arrhythmia susceptibility through processes like intracellular calcium loading (Han et al., 2001; Charpentier et al., 1993). However, as it has been proposed that I_{Kr} channel has a minor role in repolarisation during adrenergic stimulation, one may argue that physiological upregulation of I_{Ks} channel function by sympathetic stimulation (Han et al., 2001; Charpentier et al., 1993) should be able to compensate (Sanguinetti et al., 1991; So et al., 2006).

So et al. presented the notion that compensatory I_{Ks} activation takes place at slower rates, which implies that at higher heart rates that are induced more suddenly, the I_{Ks} channels may not be able to activate quickly enough due to its slower activation kinetics (So et al., 2007a; So et al., 2007b); but this notion is opposed by several experimental studies (Demolombe et al., 2001). Some studies also suggest that sympathetic stimulation may have an attenuating effect on the repolarisation reserve of the heart (Jost et al., 2005), which implies a smaller capacity for compensation to allow effective repolarisation if a component of the delayed rectifier current is impaired.

Some control conditions exhibited fitted RTSlopemax values >1.0, a threshold generally associated with arrhythmogenesis. Importantly, no spontaneous ventricular tachyarrhythmias were observed under baseline conditions, consistent with previous reports of stable electrophysiology (Brack et al., 2013) Small differences in baseline RTSlopemax between HMR/sLQT1 and E4031/sLQT2 groups, as well as between apex and base, likely reflect biological variability and regional differences in repolarization rather than systematic experimental bias.

The data in the present study are supported by observations in clinical reports that imply LQT2 is more prone to arrhythmias than LQT1 – as events in LQT2 patients are reported to be precipitated by sudden auditory stimuli, in contrast to the association of symptomatic manifestation of LQT1 patients with physical exercise (Bischoff et al., 2000). In addition, guinea pig whole hearts were used in this study, in contrast to single cell studies, on which the existing literature findings are mainly based, suggesting that there may be other mechanisms involved in arrhythmogenesis, for instance, derangement in properties of gap junctions and heterogeneous ion channel distributions (Kanno and Saffitz, 2001).

4.3. Limitations

Several limitations should be acknowledged. LQT1 and LQT2 were modelled pharmacologically using HMR-1556 and E4031, which enables selective interrogation of IKs and IKr but does not fully replicate the complexity of congenital disease. β-adrenergic stimulation was

achieved using isoprenaline, a well-established surrogate that does not capture the spatial and temporal dynamics of physiological sympathetic activation.

While hiPSC-derived models offer human-specific systems, they lack key tissue-level properties required for arrhythmogenesis. In contrast, the intact whole heart preserves conduction, electrotonic coupling, and spatial heterogeneity of repolarisation and ion channel expression, which are essential for the initiation and maintenance of re-entrant arrhythmias.

Finally, although findings are interpreted in the context of the restitution hypothesis, this should be considered within a broader mechanistic framework. Steep or altered restitution dynamics are thought to promote electrical instability and wavebreak, which may facilitate the formation and maintenance of re-entrant activity such as rotors and multiple wavelets (Ng et al., 2007; Nash et al., 2006). The present findings are therefore consistent with an integrated view of arrhythmogenesis rather than a single mechanistic pathway.

5. Conclusion

This study has shown for the first time the unexpected dichotomous effect on $MAPD_{90}$ RT slope gradient between the two models of LQTS, which implies a decreased susceptibility to ventricular fibrillation according to the "Restitution Hypothesis" with IKs blockade and the converse with IKr blockade. The findings from this project questions the role of IKs and IKr function in repolarisation during sympathetic stimulation in the whole heart. The correlation with ERP values, on the other hand, may imply the increased susceptibility to arrhythmia in LQT1 and 2 in presence of adrenergic activation is caused by refractoriness as opposed to restitution. Additional work needs to be done to characterise repolarisation reserve in the whole heart by combining both IKs and IKr blockade; in presence of direct sympathetic nerve stimulation.

CRediT authorship contribution statement

Zhia Lim: Writing – original draft, Investigation, Formal analysis, Data curation. **Reshma A. Chauhan:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation. **Bethan Roper-Jones:** Writing – review & editing, Visualization, Validation, Formal analysis. **Emily Allen:** Writing – review & editing, Supervision, Investigation. **John Mitcheson:** Writing – review & editing, Supervision. **Richard H. Clayton:** Writing – review & editing. **Kieran E. Brack:** Writing – review & editing, Supervision, Methodology, Conceptualization. **G. Andre Ng:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have no competing interests to declare that are relevant to the content of this article.

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