Arrhythmogenic left atrial cellular electrophysiology in a murine genetic long QT syndrome model

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Aims
Increasing evidence indicates that congenital long QT syndromes (LQTSs) promote atrial fibrillation. The atrial action potential (AP) has a short plateau, and whether LQTS atrial cardiomyocytes generate triggered activity via early afterdepolarizations (EADs) is unclear. Atrial cellular arrhythmia mechanisms have not been defined in congenital LQTS. Therefore, we studied atrial cardiomyocyte electrophysiology in mice with an LQTS3 SCN5A inactivation-impairing mutation (∆KPQ heterozygotes).

Methods and results
Peak and late Na+ current (INaP and INaL) were measured with whole-cell patch clamp in left atrial (LA) cardiomyocytes. APs were recorded in multicellular LA preparations with floating microelectrodes. INaL was increased by 110% in LA cardiomyocytes of ∆KPQ mice, whereas INaP was unchanged. AP duration (APD) was prolonged over all frequencies in ∆KPQ mice, but particularly at lower frequencies [e.g. APD90 at 0.5 Hz: 197 ± 8 ms vs. wild-type (WT) 82 ± 2 ms, P < 0.001]. EADs occurred at 0.5 Hz in 10/18 ∆KPQ (56%) vs. 1/10 WT (10%) atria (P < 0.05). EADs immediately preceded premature APs in other LA regions, suggesting triggered activity. Ranolazine preferentially inhibited INaL (50% inhibitory concentration: 12.5 vs. 151.8 μM for INaP) in ∆KPQ myocytes. At 10 μM, ranolazine shortened APD (e.g. APD90 at 0.5 Hz to 122 ± 4 ms, P = 0.01) without changing APD in WT and suppressed EAD occurrence and triggered activity (from 10/18 to 1/9 preparations, 11%, P < 0.05).

Conclusion
This study implicates increased INaL in excessive atrial APD prolongation and arrhythmic EAD occurrence in a congenital LQTS3 mouse model. Our observations provide the first direct demonstration of atrial EADs and triggered activity in a genetically defined animal model of human LQTS and have potential clinically-relevant mechanistic and therapeutic implications.

Keywords
Arrhythmia (mechanisms) • Atrial fibrillation • Late Na current • Antiarrhythmic drugs • Cellular electrophysiology

1. Introduction
Atrial fibrillation (AF) is the most frequent sustained arrhythmia observed in clinical practice and the underlying mechanisms are incompletely understood.1 Genetic factors are increasingly recognized to be significant contributors. Patients with long QT syndrome (LQTS) often present with atrial arrhythmias, particularly AF.2–4 At the ventricular level, early afterdepolarizations (EADs) induce torsade de pointes tachyarhythmias, with underlying mechanisms having been demonstrated in murine models.5,6 Atrial cardiomyocytes have much shorter plateaus and slower phase 3 repolarization than ventricular cells, and their ability to generate EADs and EAD-related triggered activity is less clear.

Several recent studies have examined atrial arrhythmia predisposition in mouse models of congenital LQTS. Dautova et al.7 showed...
that Langendorff-perfused hearts from mice heterozygous for the ΔKPQ knock-in LQT3 mutation are resistant to atrial arrhythmia induction by programmed electrical stimulation, while older LQT3 mice may have a higher chance than wild-type (WT) mice of developing atrial arrhythmias.6 We recently reported that protocols generating rapid rate alterations induce atrial arrhythmias significantly more often in ΔKPQ vs. WT mice in vivo,9 suggestive of afterdepolarization-dependent arrhythmogenic mechanisms in this model, similar to prior observations at the ventricular level.5,6 None of these studies examined the atrial cellular phenotype of ΔKPQ mice. Furthermore, the range of heart rates that could be assessed was limited by the very rapid intrinsic sinus node rate of the mouse. We designed the present study to evaluate the cellular electrophysiological properties of ΔKPQ mice, with particular emphasis on: (i) action potential (AP) changes, including susceptibility to EADs; (ii) changes in Na+ current (I Na) properties; and (iii) effects of the late I Na (I NaL) blocker ranolazine on I Na and AP properties.

2. Methods

2.1 Animal model

Experiments were performed on age-matched pairs of adult (21–52 week) male LQTS3 mice with a heterozygous ΔKPQ-SCNSA knock-in mutation on a Swiss Agouti background and WT littermates as described previously.5 The genotypes of each animal were not revealed to investigators during the performance of experiments, and analyses of experimental data were performed blinded to the genotype (ΔKPQ-SCNSA or WT); genotypes were revealed for statistical analysis and data presentation. Animal care procedures followed National Institutes of Health guidelines (http://oacu.od.nih.gov/training/index.htm) and were approved by the Animal Research Ethics Committee of the Montreal Heart Institute. Mice were heparinized (100 IU ip) and euthanized by cervical dislocation by the Animal Research Ethics Committee of the Montreal Heart Institute.

2.2 Cell isolation

Hearts were removed and perfused at 37°C in storage solution10 at 4°C. Epicardial APs were recorded with borosilicate glass microelectrodes (resistances 10–20 MΩ) inserted into isolated, enzymatically dissociated LA free wall samples (~4 mm diameter) were superfused with Krebs–Henseleit solution containing (mM): 120 NaCl, 4 KCl, 1.2 NaH2PO4, 1.2 MgSO4, 25 NaHCO3, 1.25 CaCl2, and 5.5 glucose (95% O2 –5% CO2, pH 7.4) at 36°C. Epicardial APs were recorded with borosilicate glass microelectrodes (resistances 10–20 MΩ when filled with 3 M KCl) coupled to an Axoclamp-2B amplifier (Axon Instruments, Foster City, CA, USA), digitized with a Digidata-1200 series A/D-converter, and displayed with Axotape 2.0 software (10 kHz sampling). Preparations were stimulated with 2 ms 1.5 × diastolic threshold current square wave pulses through bipolar Teflon-coated silver electrodes. Current output averaged 0.65 ± 0.12 mA at baseline vs. 1.07 ± 0.37 mA in the presence of ranolazine (P < 0.05). After 15 min of pacing for equilibration, preparations were paced successively at increasing frequencies from 0.5 to 10 Hz, with 200 beats at each frequency to ensure steady state prior to recording. AP duration (APD) was analysed with a custom-made Matlab algorithm, with each value based on the average of five consecutive APs at four different regions of the preparation. The APs shown in the figures were obtained by averaging values from individual mean AP values in all the experiments.

2.4 Patch-clamp experiments

Borosilicate glass microelectrode pipettes (tip resistances 1.5–2.5 MΩ) were used to record whole-cell I Na at room temperature (22 ± 0.5°C) with an Axopatch-200 amplifier (Axon). Membrane currents were low-pass filtered at 5 kHz and sampled at 100 kHz (first 4 ms) and 12.5 kHz (last 196 ms). Pipette solutions contained (mM): 50 Cs aspartate, 60 CsCl, 5 Na2ATP, 11 EGTA, 10 HEPES, 1 CaCl2, and 1 MgCl2 (pH 7.2, CsOH). The external solution contained (mM): 130 NaCl, 1.8 CaCl2, 5 CsCl, 1.2 MgCl2, 10 HEPES, and 5 glucose (pH 7.4, CsOH). L-type Ca2+-current was blocked with 0.1 mM CdCl2. Drugs were applied with a fast perfusion system that replaced cell superfusate within 500 ms. Peak I Na (I NaP) and I NaL were defined on based on 30 μM tetrodotoxin (TTX)-sensitive currents (Tocris-Bioscience, Ellisville, MO, USA). TTX-sensitive current values before and after ranolazine (Sigma) superfusion were obtained with the protocol shown in Supplementary material online, Figure S1, to establish I Na inhibition by ranolazine in a precise fashion. The criteria for satisfactory voltage control for I NaP recordings were: (i) smooth I–V curve, without abrupt jumps on the negative-slope branch; (ii) maximum current observed around 30 mV positive to the voltage threshold (Supplementary material online, Figure S2).

2.5 Data analysis

Clampfit 9.0 (Axon), Matlab 7.10 (R2010a), Excel 2003 (Microsoft), and GraphPad Prism 5.01 were used for data analysis. Student’s t-tests were used for comparisons with two groups only and two-way ANOVA with Bonferroni-adjusted t-test was used to compare repeated measures. The occurrence rates of EADs were compared between groups with Fisher’s exact test. All analyses were two-tailed, and P < 0.05 was considered to be statistically significant. Group data are presented as mean ± SEM.

3. Results

3.1 Na+ current changes

There was no evidence of changes in cell morphology with ΔKPQ, and the mean cell capacitances were similar in both groups: WT (84.1 ± 5.3 pF; n = 25/5, cells/hearts) vs. ΔKPQ (80.0 ± 3.1 pF; n = 39/8). Figure 1A shows averaged TTX-sensitive I NaP recordings based on electronic averages of all recordings obtained from WT and ΔKPQ cardiomyocytes. Peak currents and overall morphologies are similar. Figure 1B shows averaged currents during the full 200 ms depolarizing pulse on an expanded current scale, illustrating the larger I NaP in ΔKPQ. I NaL was defined for each cell as the mean TTX-sensitive current between 150 and 200 ms following depolarization. The mean I NaP and I NaL data are shown in Figure 1C and D. ΔKPQ had no effect on peak currents but increased late-current density about three-fold.

3.2 AP changes

Figure 2A shows the average superimposed APs from ΔKPQ and WT hearts recorded at different frequencies. Because we used an isolated
LA preparation that exhibited very little spontaneous automaticity, we were able to record APs over a wide range of frequencies. Figure 2B shows the overall mean ± SEM APD90 results. ΔKPQ atria had significantly longer APDs over the full range of frequencies tested, with the largest increases seen at slower rates.

EADs occurred frequently in ΔKPQ atria under bradycardic conditions (Figure 3). Figure 3 shows typical single (Figure 3A) and multiple (Figure 3B) EADs. EADs developed in 10 of 18 (56%) LA preparations at 0.5 Hz, but only one of 10 (10%) WT preparations (P < 0.05; Figure 3C). EADs were rarer at 1 Hz and never observed at higher frequencies (Supplementary material online, Figure S3).

Figure 4A shows a preparation from a ΔKPQ mouse in which simultaneous recordings were obtained with two microelectrodes at the locations shown. Recordings during extrasystolic activity are shown in Figure 4B. Cell 1 clearly showed a gradual transition from a prolonged plateau to an EAD, whereas cell 2 showed the upstroke of an extrasystolic AP occurring with a clear but short delay after the EAD upstroke in cell 1. Such activity was seen typically in ΔKPQ hearts and suggests EAD-induced triggered activity. The upstroke amplitude of the triggered AP depended on the degree of repolarization at the time of activation from the EAD, reflecting the voltage dependency of hNa, as illustrated by the four recordings from cell 2 (AP recordings 1–4) in Figure 4C. Figure 4D shows recurrent EADs and triggered responses in a bigeminal fashion.

3.3 Effects of ranolazine

To assess the role of hNa in EADs and triggered activity of ΔKPQ hearts, we studied the effects of ranolazine, a selective hNa blocker.11 The blue and lavender lines in Figure 5 show ranolazine concentration–response curves for ranolazine inhibition of hNa in WT and ΔKPQ, respectively, in ΔKPQ cells. Ranolazine-induced current inhibition was significantly greater for hNa in WT myocytes than in ΔKPQ myocytes for 10 μM (hNa: 43.5 ± 4.2% and hNa: 10.3 ± 3.6% inhibition; P < 0.001) and 100 μM ranolazine (hNa: 87.1 ± 0.9% and hNa: 33.6 ± 3.2% inhibition; P < 0.001). The 50% inhibitory concentration (IC50) was 12.5 μM (95% confidence limits 9.9–15.4 μM) for hNa vs. 151.8 μM (95% confidence limits 113.9–202.3 μM) for hNa, representing ~12-fold greater potency for hNa. The concentration–response relationship for hNa inhibition in WT cells, IC50 121.2 μM (95% confidence limits 81.1–181.3 μM), was similar to that for ΔKPQ. The hNa inhibitory potency could not be tested accurately in WT cells because of the very small size of the current.

Figure 6 shows the hNa inhibitory effects of ranolazine at the concentration selected for assessment of effects on APs, 10 μM. Figure 6A and B illustrates the effects on peak and late currents, respectively, in one cell. Overall, 10 μM ranolazine reduced the mean hNa by 10.3% (Figure 6C), whereas the mean hNa was decreased by 43.5% (Figure 6D). The difference in hNa between WT and ΔKPQ cells was substantially reduced by ranolazine, with ΔKPQ cells having a value 126% greater than WT in the absence of the drug vs. 29% larger than WT in the presence of 10 μM ranolazine.

The effect of ranolazine was assessed in parallel experiments with both WT and ΔKPQ preparations, in which the drug (10 μM) was administered in the bath solution over the same time course as parallel drug-free experiments. In the presence of the drug, the AP shape of ΔKPQ cells was more similar to that of WT cell APs than to APs recorded from drug-free ΔKPQ cells (Figure 2A). Ranolazine produced much smaller effects in WT cells (statistically non-significant; Figure 2B) than in ΔKPQ cells, reflecting the relatively small amplitude...
Figure 2 APD prolongation in multicellular preparations. Isolated LA from WT mice without (n = 10) and with (n = 5) ranolazine, and ΔKPO mice without (n = 18) and with (n = 8) 10 μM ranolazine (Ran), were constantly superfused (7.5 mL/min) with Tyrode’s solution at 36°C and paced over a range of frequencies. APs were recorded in multicellular LA preparations with fine-tipped floating microelectrode techniques. The APs shown in (A) at 0.5, 1, 2, 5, 6.7, and 10 Hz are based on an average of steady-state APs in all cells recorded. (B) Mean ± SEM APD values at 90% repolarization (APD90) show APD prolongation as a function of pacing frequency. ***P < 0.001, **P < 0.01, *P < 0.05 (n = 18, ΔKPO) vs. WT (n = 10); †P < 0.05; ‡P = 0.06 for ΔKPO vs. ΔKPO-Ran (n = 8) (two-way ANOVA).

Figure 3 Presentation and occurrence rate of EADs in mouse atrial preparations. In ΔKPO atria, single (A) and multiple (B) EADs occurred at 0.5 Hz. (C) EAD occurrence during 0.5 and 1 Hz pacing. EAD occurrence was quantified as percentage of preparations for which more than 1% of APs showed EADs (numbers of preparations with EADs are the numerator and total number of preparations the denominator). *P < 0.05 (Fisher’s exact test). EAD results at all frequencies are shown in Supplementary material online, Figure S3.
of the target current (I_{NaL}) in WT cells. APD_{90} was significantly shorter in D{\textsubscript{KPQ}} cells superfused with 10 \textmu{}M ranolazine than in drug-free D{\textsubscript{KPQ}} cells, with the largest changes observed at the slowest stimulation frequencies (Figure 2B). Consistent with the notion that EADs and triggered activity in D{\textsubscript{KPQ}} atria are due to enhanced I_{NaL}, ranolazine suppressed EADs and triggered activity in D{\textsubscript{KPQ}} hearts (Figure 3; P, 0.05). As previously noted with TTX effects on quinidine-induced EADs and in contrast to L-type Ca^{2+}-channel blocker effects, ranolazine suppressed EADs by reducing APD to values at which EADs did not occur (in either WT cells or D{\textsubscript{KPQ}} cells at faster frequencies), suggesting that EADs were suppressed by reversing APD prolongation that allows EAD formation rather than by suppressing the depolarizing mechanism underlying the EAD itself.

4. Discussion

In this study, we characterized the LA cellular phenotype of a murine model of LQTS3. The mutation increased I_{NaL} and prolonged APD, with the greatest prolongation evident at low frequencies. APD prolongation at low rates was associated with a propensity for EADs. The response to ranolazine at a concentration highly selective for I_{NaL} inhibition confirmed the role of enhanced I_{NaL} in repolarization delay and arrhythmogenesis. This study demonstrates that atrial APD prolongation secondary to increased I_{NaL} caused by impaired I_{Na} inactivation gate function is sufficient to provoke EADs, and suggests that ranolazine may be a valid therapeutic intervention to prevent atrial arrhythmias in this setting.

4.1 Atrial repolarization delay, EADs, and AF

The atrial AP typically has a much shorter plateau and more gradual phase 3 repolarization than ventricular AP,\textsuperscript{13} and there has been some doubt about the occurrence and importance of EADs in atrial cardiomyocytes. Atrial arrhythmias with apparent EAD morphology on monophasic AP recordings have been demonstrated in response to intravenous administration of Cs\textsuperscript{+}, a K\textsuperscript{+}-current blocker;\textsuperscript{14} however, Cs\textsuperscript{+} may also induce arrhythmias in association with hyperkalaemia and abnormal automaticity.\textsuperscript{15} There has been increasing evidence of a predilection to AF in LQTS patients.\textsuperscript{2–4} In addition, gene variants with loss of K\textsuperscript{+}-channel function have been associated with AF. The first such example was a KCNE1 gene polymorphism that reduces slow delayed rectifier current (I_{Kr}) and \alpha-subunit KCNQ1 membrane expression.\textsuperscript{16} Mathematical modelling predicted the occurrence of EADs when I_{Kr} and I_{K1} were decreased in the presence of reduced net outward current resulting from reduced I_{Ks}.\textsuperscript{16} Subsequently, Olson et al. reported a KCNA5 (ultrarapid delayed rectifier K\textsuperscript{+}-current, I_{Kur}) mutation in a lone AF family,\textsuperscript{17} with atrial EADs produced in human atrial cardiomyocytes by blocking the corresponding current with low concentrations of 4-aminopyridine\textsuperscript{18} and stimulating \beta-adrenergic receptors. More recently, EADs have been
Figure 6 Response of \(I_{\text{NaP}}\) and \(I_{\text{NaL}}\) at ranolazine concentration selected for AP-response assessment in ΔKPQ. Measurements were performed under the same conditions as in the baseline \(I_{\text{Na}}\) measurements (Figure 1). The drug application protocol is illustrated in detail in Supplementary material online, Figure S1. (A and B) Examples of the effect of 10 μM ranolazine on \(I_{\text{NaP}}\) (A) and \(I_{\text{NaL}}\) (B). Mean ± SEM data are shown in (C) and (D) (ΔKPQ: \(n = 6/5\), cells/hearts). Vertical axes show values both in terms of current density (left sides) and values relative to WT mean values (right side) (dotted line = 100%). **\(P < 0.01\) and *\(P < 0.05\) (paired Student’s t-test).
demonstrated in guinea pig atrial cardiomyocytes exposed to the $I_{Na}$ inactivation inhibiting toxin, ATX-II.9

Studies of atrial arrhythmogenesis in engineered models of LQTS have been limited to date, and no cellular work has been reported in the literature. Recent studies in ΔKPQ mouse hearts suggest that pacing protocols that allowed for transient pauses provoke atrial arrhythmias. An important limitation of these studies in isolated perfused hearts is the restricted range of rates that can be studied because of rapid intrinsic automaticity from the mouse sinoatrial node. In the Blana et al. study of ΔKPQ hearts, a slight but statistically significant rate-dependent monophasic APD prolongation, reversible by Na$^+$-channel block with flecainide, was documented. In the present study, we were able to investigate systematically a wide range of frequencies because of the very low spontaneous firing rate of isolated mouse LA preparations. This allowed us to demonstrate marked APD prolongation and frequent occurrence of EADs at slow atrial rates.

4.1.1 Underlying mechanisms
Enhanced $I_{Na}$ was likely the factor leading to atrial EADs and triggered activity in our ΔKPQ mice. We directly demonstrated enhanced $I_{Na}$ in ΔKPQ atrial cardiomyocytes, and administration of ranolazine at concentrations we showed to be highly selective for $I_{Na}$ reversed both APD prolongation and EAD susceptibility. It is known that $I_{Na}$ is rate-dependent, being enhanced at slow rates. The rate dependence of $I_{Na}$ likely accounts for the bradycardia dependence of left atrial APD prolongation in ΔKPQ mice.

The properties of arrhythmogenesis we saw in the present study are very similar to those previously described in ventricular tissue from ΔKPQ hearts.9 Ventricular cardiomyocytes showed frequency-dependent APD prolongation and EADs similar to what we saw in atrial tissue. Unlike our findings, EADs at the ventricular level also occur upon sudden rate acceleration, possibly reflecting atrial–ventricular differences in plateau ionic currents.

4.2 Ranolazine and EAD suppression
Ranolazine was effective in suppressing spontaneous EADs and triggered activity in ΔKPQ atrial cardiomyocytes. In our ΔKPQ atrial myocytes, we noted a 12-fold difference in the $IC_{50}$ of $I_{Na}$ vs. $I_{NaP}$ ($IC_{50}$ of 12.5 μM for $I_{Na}$ and 151.8 μM for $I_{NaP}$). A similar study on ventricular cardiomyocytes of LQTS3 mice revealed a nine-fold selectivity for $I_{Na}$. This relatively small discrepancy may be due to differences in the molecular basis of atrial vs. ventricular Na$^+$-channels.21 The selectivity of ranolazine for $I_{Na}$ vs. $I_{NaP}$ is greater than that of a variety of other Na$^+$-channel blockers like mexiletine, with an $IC_{50}$ of 2.1 μM for $I_{Na}$ and 6.5 μM for $I_{NaP}$, lidocaine (89 and 205–516 μM, respectively)22,24 and flecainide (19 and 48–80 μM).24,25 The results of the present study indicate that ranolazine may be particularly relevant for the treatment of AF in patients with LQT3 physiology and suggest that it may be potentially useful in other clinical situations in which atrial APD prolongation is a suspected cause of AF.

4.3 Novelty and potential significance
These findings constitute to our knowledge the first detailed study of the atrial cellular electrophysiological consequences of genetically defined LQTS. The mechanisms underlying AF are known to be complex and the role of specific potential mechanistic contributors remains poorly understood.26 The development of EADs may be a mechanistic link between the ion-channel impairment induced by the mutation and the clinically evident atrial arrhythmias in LQTS patients. Our findings may also be relevant to acquired forms of AF associated with an increased $I_{Na}$ such as AF occurring in congestive heart failure.27 This physiology may be particularly important in atrial cardiomyocytes, in which increased $I_{Na}$ enhances APD, cellular Ca$^{2+}$ content, and diastolic intracellular Ca$^{2+}$ levels.28 Similarly, patch-clamp studies in atrial cardiomyocytes from AF patients have shown enhanced $I_{Na}$ and suppression of both $I_{Na}$ and atrial arrhythmogenesis by 10 μM ranolazine, suggesting that the pathophysiology described here may contribute to AF initiation in some cases of the more common acquired forms of the arrhythmia.29 Our data add support to the notion that ranolazine may suppress arrhythmias in AF patients by acting on $I_{Na}$ and that enhanced $I_{Na}$ in AF patients may be more than just an epiphenomenon.30

4.4 Limitations
There are significant differences between human and murine electrophysiology; especially in terms of the different contribution of $I_{Kr}$ and $I_{Ks}$ to repolarization.31 Nevertheless, mouse models have provided important insights into the genetic control of human electrophysiology, and notably in LQT3.31 Besides its effects on $I_{Na}$, ranolazine can also inhibit other ion currents like $I_{Kr}$ and $I_{Ks}$.32,33 We used ranolazine at concentrations that are relatively selective for $I_{Na}$; however, we cannot exclude an arrhythmic effect from $I_{NaP}$ inhibition or contributions from effects on other ionic processes. The dose–response data provided do not provide insight into the molecular mechanisms of the ranolazine interaction with its Na$^+$-channel-binding site. Much more detailed studies, including a larger range of concentrations than the 1000-fold range that we assessed, would be needed to evaluate evidence for the number of binding sites and positive or negative cooperativity. Ideally, precise definition of ranolazine interactions with the channel would involve site-directed mutagenesis studies to indicate amino-acid residues needed for binding and X-ray crystallography to determine threedimensional structures that control binding. We saw EADs and marked APD prolongation at pacing rates that are similar to bradycardic rhythms in man, but are very slow for the mouse. The fact that we did not observe marked EADs at high heart rates may explain the observation that spontaneous atrial arrhythmias have only rarely been recorded in this mouse model in vivo.

We attributed the antiarrhythmic effect of ranolazine to effects on $I_{Na}$. This explanation is likely because of the much greater effect of ranolazine on $I_{Na}$ than $I_{NaP}$ and because ranolazine reduced APD in ΔKPQ mice to values at which EADs were not seen in control mice. However, we did see a statistically significant (albeit small, ~10%) decrease in $I_{NaP}$ with 10 μM ranolazine, the concentration used for AP studies, so a contribution of $I_{NaP}$ inhibition to the drug’s antiarrhythmic actions cannot be totally excluded.

Since APD prolongation and EAD generation in ΔKPQ was most prominent at rates under 2 Hz (Figures 2 and 3; see Supplementary material online, Figure S2), the mechanisms suggested by our results could account for prevention by ranolazine of the triggers for AF initiation (presumably provided by EADs) during sinus rhythm in LQTS patients, but not for arrhythmia termination at the very rapid atrial rates during AF. The efficacy of ranolazine for AF termination would be needed to account for prevention by ranolazine of the triggers for AF initiation (presumably provided by EADs) during sinus rhythm in LQTS patients, but not for arrhythmia termination at the very rapid atrial rates during AF. The efficacy of ranolazine for AF termination must be due to a different mechanism, most likely accumulation of rate-dependent $I_{NaP}$ block typical of fast-unbinding $I_{Na}$ blockers.35

We adjusted stimulus strength under all conditions to be 50% above excitation threshold. Larger stimulus strengths were required...
under ranolazine and we cannot exclude an effect of stimulus strength on repolarization.

4.5 Conclusions
In atrial cardiomyocytes, a genetically enhanced I_{NaL} prolongs the atrial AP and provokes EADs accompanied by triggered activity at low rates, indicating that atrial cardiomyocytes are capable of manifesting these properties which heretofore have only been reported in ventricular cardiomyocytes from congenital LQTS models. These findings are relevant to the cellular mechanisms of atrial arrhythmia in LQTS patients, identify increased findings are relevant to the cellular mechanisms of atrial arrhythmia festing these properties which heretofore have only been reported and P.K.). M.D.L. was supported by an MD-Fellowship of the Boehringer- and S.N.), Deutsche Forschungsgemeinschaft DFG FA 413-3/1 (L.F.); (MOP 44365, S.N.); the Mathematics of Information Technology and Funding

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