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ORIGINAL RESEARCH - PRECLINICAL

Junctional Ectopic Tachycardia Caused by Junctophilin-2 Expression Silencing Is Selectively Sensitive to Ryanodine Receptor Blockade

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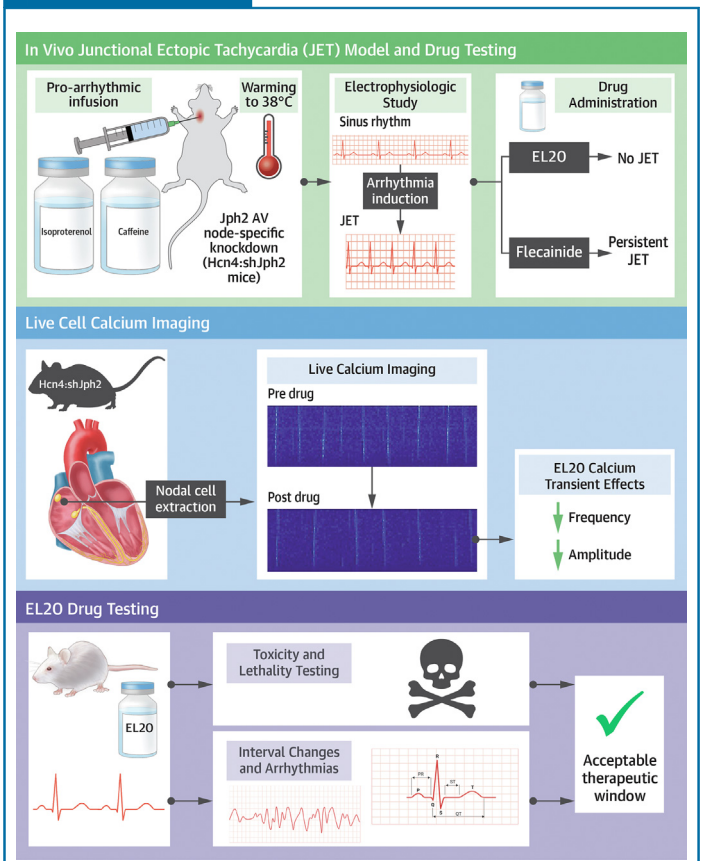
VISUAL ABSTRACT



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HIGHLIGHTS

- **Reduced expression of Jph2 in nodal cells is associated with inducible JET in mice.**
- **EL20, an RyR2 receptor blocker, rapidly terminates JET through reduction of sarcoplasmic reticulum Ca^{2+} leak.**
- **EL20 may have clinical efficacy in the treatment of JET.**

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ABBREVIATIONS AND ACRONYMS

AV = atrioventricular

EP = electrophysiology

HR = heart rate

JET = junctional ectopic tachycardia

Jph2 = junctophilin-2

LCR = local Ca^{2+} release

RyR2 = ryanodine receptor type 2

SR = sarcoplasmic reticulum

SUMMARY

Junctional ectopic tachycardia (JET) is a potentially fatal cardiac arrhythmia. Hcn4:shJph2 mice serve as a model of nodal arrhythmias driven by ryanodine type 2 receptor (RyR2)–mediated Ca^{2+} leak. EL20 is a small molecule that blocks RyR2 Ca^{2+} leak. In a novel in vivo model of JET, Hcn4:shJph2 mice demonstrated rapid conversion of JET to sinus rhythm with infusion of EL20. Primary atrioventricular nodal cells demonstrated increased Ca^{2+} transient oscillation frequency and increased RyR2-mediated stored Ca^{2+} leak which was normalized by EL20. EL20 was found to be rapidly degraded in mouse and human plasma, making it a potential novel therapy for JET. (J Am Coll Cardiol Basic Trans Science 2023;8:1577–1588)

Junctional ectopic tachycardia (JET) is an incessant tachyarrhythmia that originates from the compact atrioventricular (AV) node, usually with AV dissociation. JET is a common complication after surgery for congenital heart disease and contributes significantly to postoperative morbidity and mortality in children.[1,2] Conversion to sinus rhythm and controlling the junctional rate in JET can be difficult. Various antiarrhythmic treatments, including amiodarone, flecainide, propranolol, procainamide, and sotalol have not proven to be completely effective, even at high doses.[3] The underlying pathophysiologic mechanism of JET is unknown. It has been hypothesized that extrinsic mechanical force, increased temperature, and a high catecholamine state increase the automaticity of the AV node; however, the molecular alterations that underlie JET are unclear.

The cardiac pacemaker, which comprises the sinoatrial node and the AV node, sets and modulates the heart rate. Overall, nodal cell automaticity is thought to be generated by a "2-clock model," wherein a "membrane voltage clock" and an intracellular "calcium clock" functionally couple to drive membrane depolarization and subsequent generation of an action potential.[4] The membrane voltage clock is primarily formed by the hyperpolarization-activated current (I_f) via hyperpolarization-activated cyclic nucleotide-gated cation channel 4 (Hcn4). The calcium clock is defined by spontaneous local Ca^{2+} release (LCR) from the sarcoplasmic reticulum (SR) store within the nodal cell through the opening of the ryanodine receptor type 2 (RyR2).[5,6] Various factors contribute to spontaneous LCR, including hyperactivity of RyR2 and increased SR Ca^{2+} stores.[7] However, whether SR-mediated Ca^{2+} leak is sufficient to drive this automaticity is still being explored. If it is, it would provide a molecular target for therapeutic intervention to treat JET.

Jph2-encoded junctophilin-2 (Jph2) is a structural protein that establishes cardiac dyads, maintains normal excitation-contraction coupling, and regulates Ca^{2+} release from the SR through direct interaction with RyR2 in the contractile myocytes of the heart.[8-10] Jph2 down-regulation has been shown to increase Ca^{2+} leak from local cardiomyocyte SR stores, leading to atrial fibrillation, cardiomyopathy, and heart failure.[11-13] We have previously demonstrated that Jph2 knockdown in murine nodal cells causes increased automaticity of nodal cells owing to Ca^{2+} leak from the SR in Hcn4:shJph2 mice.[14] These findings support a potential pharmacologic target for correction of Ca^{2+} dysregulation.[15] Recently, a novel compound, 2-(diethylamino) ethyl 4-(butylamino)-2-methoxybenzoate (EL20) has been shown to decrease Ca^{2+} leak from the SR through targeting RyR2 in ventricular cardiac myocytes.[16] The effectiveness of EL20 in terminating nodal arrhythmias, and the role of RyR2 blockade as a therapeutic target in nodal disease, remains unknown.

In the present study, we demonstrate that nodal-specific knockdown of Jph2 expression in Hcn4:shJph2 mice results in an inducible AV nodal

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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arrhythmia consistent with JET that can be rapidly terminated by EL20. Furthermore, EL20 decreases LCR by inhibiting RyR2 release of Ca^{2+}, and blockade of I_f does not terminate JET or reduce Ca^{2+} leak in this model. EL20 demonstrated a reasonable therapeutic window in mice and was rapidly metabolized in serum. Our findings suggest that targeting RyR2-mediated nodal Ca^{2+} leak may be a therapeutic target for the treatment of JET.

METHODS

HUMAN SUBJECTS. This study was approved by the Duke University School of Medicine and Baylor College of Medicine institutional review boards. All other methods are detailed in the Supplemental Methods.

TRANSGENIC MICE. All mice were treated in accordance with the Baylor College of Medicine and Duke University School of Medicine Institutional Animal Care and Use Committees. Details are included in the Supplemental Methods

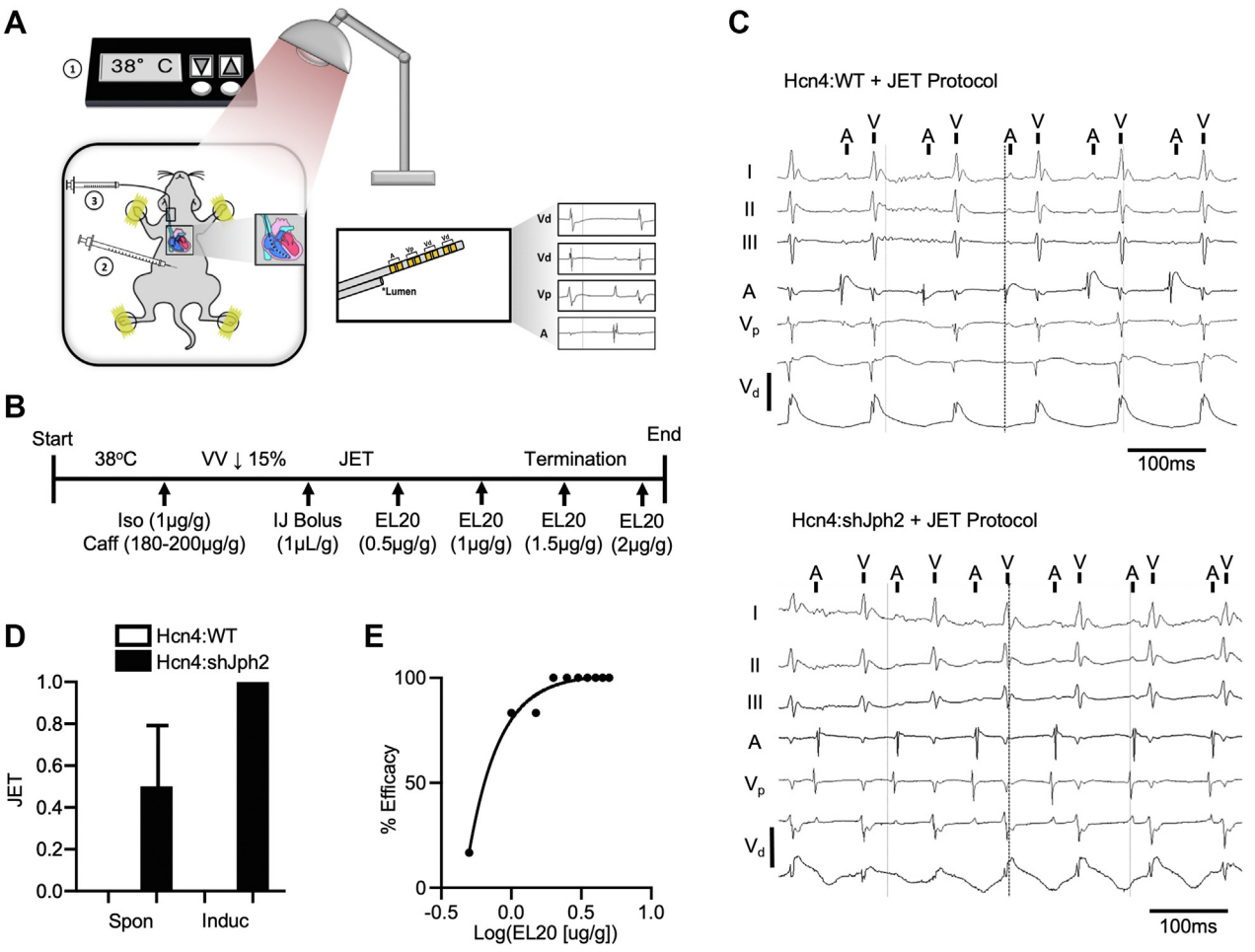
STATISTICAL ANALYSIS. Data were expressed as mean ± SEM or n (%) with 95% CI unless otherwise specified. Normal (gaussian) distribution tests were applied to each data set from different groups with multiple methods including: 1) d'Agostino-Pearson omnibus normality test; 2) Anderson-Darling test; 3) Shapiro-Wilk normality test; and 4) and Kolmogorov-Smirnov normality test with Dallal-Wilkinson-Lilliefor corrected *P* value before between-group and within-group comparisons. Comparisons before and after dosage within the experimental and control groups were made by means of paired Student's *t*-test for continuous variables and McNemar's test for categoric variables. Comparisons between groups were made with the use of unpaired Student's *t*-test or Fisher's exact test. Prism 8.0 (GraphPad) was used for analysis, and a *P* value <0.05 was considered to be statistically significant.

RESULTS

Hcn4:shJph2 MICE DEMONSTRATED INDUCIBLE JET THAT CAN BE CONVERTED TO SINUS RHYTHM BY EL20. Our previous work has established a nodal cell-specific expression-silencing murine model of Jph2 knock-down with the use of tamoxifen-sensitive nodal-specific Cre (Hcn4:shJph2 mice). Compared with tamoxifen-treated control mice (Hcn4:WT mice), knockdown mice demonstrated an approximately 40% reduction of Jph2 transcript and protein expression.[14] These mice demonstrate no arrhythmias at rest nor inducible arrhythmias with intracardiac electrical stimulation, but rapid infusion of isoproterenol in ex vivo Langendorff perfused hearts consistently triggered a rapid AV-dissociated tachycardia; thus, we hypothesized that these mice could serve as an in vivo model of JET.[14] Initially, central venous isoproterenol and/or epinephrine injection via a customized lumened electrophysiologic catheter did not consistently trigger AV-dissociated tachycardia in vivo. Given the well established literature identifying temperature, mechanical "stretch" of the heart, and increased catecholamines as risk factors for the development of postoperative JET in children,[17,18] we sought to establish a novel methodology to simulate JET in mice (**Figures 1A and 1B**). The detailed protocol is described in Supplemental Figure 1. None of the Hcn4:shJph2 mice (n = 6) or control mice (n = 11) exhibited JET at baseline or in nonstressed conditions. Half of Hcn4:shJph2 mice (n = 3) had an accelerated junctional rhythm at baseline, with similar atrial and ventricular rates compared with mice in normal sinus rhythm (n = 3). After warming of mice to 38°C, intraperitoneal injection of caffeine (180-200 μg/g) and isoproterenol (1 μg/g), and an intravenous (IV) bolus of normal saline solution (1 μL/g), JET was induced in 100% of the Hcn4:shJph2 mice (n = 6). No control mice (n = 11) demonstrated JET under these conditions (**Figures 1C and 1D**, Supplemental Figure 2, Videos 1 and 2). We found that central IV bolus of 0.5 μg/g EL20 resulted in conversion of JET (rhythm control) in 17% of the knockdown mice. Bolus of 1.0 μg/g EL20 converted 67% of mice, and 17% of mice required a 2.0 μg/g EL20 bolus. All mice were rhythm controlled with 2.0 μg/g or less of EL20. Rhythm conversion was rapid, an average 25.2 ± 4.5 seconds after the last dose of EL20. Mice were observed for 5 to 10 minutes after conversion, and no JET recurrence was observed (**Figure 1E**, Video 3). These findings yielded a calculated median effective dose (ED_{50}) of 0.72 μg/g for EL20. We further explored: 1) atrial and ventricular rates at baseline (before induction); 2) respective rates after our induction protocol; and 3) respective rates after conversion to sinus rhythm and found an expected decrease in both atrial and ventricular rates while in JET, followed by return to baseline rates after cardioversion to sinus rhythm. These are detailed in Supplemental Figures 3 and 4. Taken together, these results indicate that Hcn4:shJph2 can serve as an inducible model of JET and that EL20 has a high degree of efficacy in the termination of JET in this model.

FLECAINIDE IS INEFFECTIVE IN TERMINATING JET IN Hcn4:shJph2 MICE AND MAY BE FATAL AT HIGHER DOSES. Because EL20 was shown to reduce Ca^{2+} leak from RyR2, we next sought to compare its efficacy against a clinically available antiarrhythmic with a similar mechanism. Flecainide is a potent,

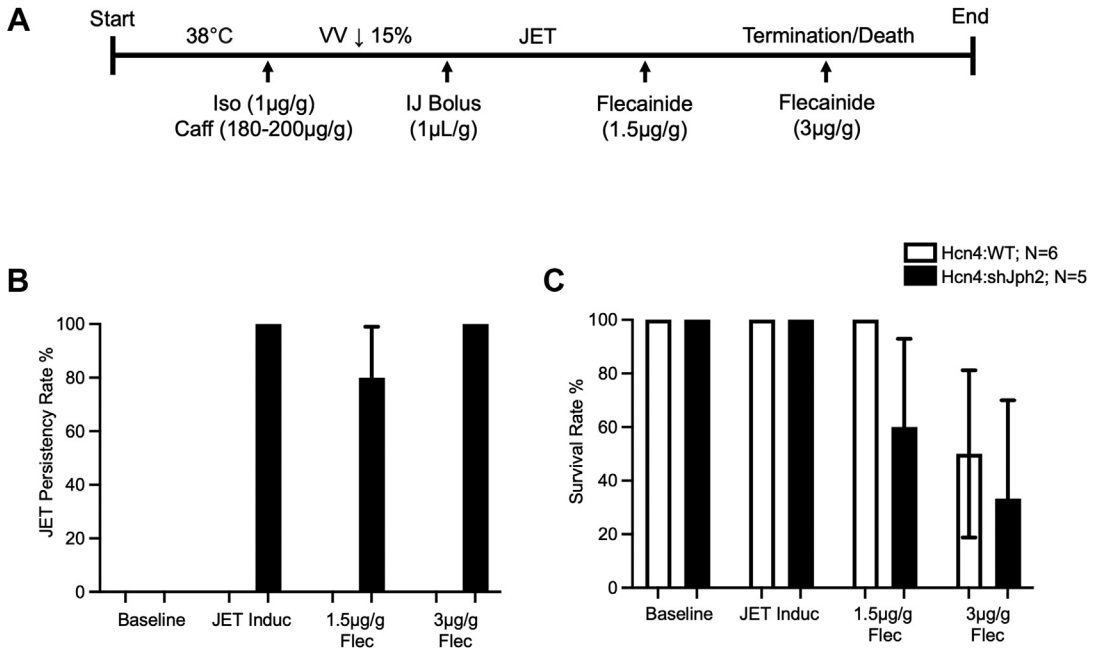
FIGURE 1 **Induction of JET in a Mouse Model and Efficacy of Conversion of JET to Sinus Rhythm by EL20**



(A) Schematic of the experimental setup used to induce junctional ectopic tachycardia (JET) in vivo. 1) body temperature raised to 38°C; 2) intraperitoneal injection with isoproterenol (Iso) (1 μg/g) and caffeine (Caff) (180-200 μg/g); and 3) internal jugular injection of a normal saline solution bolus (1 μL/g). (B) Protocol for JET induction and EL20 pharmacologic termination. VV = interval between 2 ventricular depolarizations. (C) Surface and intracardiac electrocardiographic recordings from control mice after the JET induction protocol (top) and from Hcn4:shJph2 mice (bottom). (D) Bar graph demonstrating the prevalence of 50% spontaneous junctional rhythm and 100% inducible rate of JET in Hcn4:shJph2 mice (black bars) compared with control mice (white bars). Error bars represent 95% CIs. (E) Dose-response curve demonstrating the efficacy of rhythm control with EL20 administration, with each point representing the cumulative percentage of mice converted at those respective EL20 doses. IJ = internal jugular.

though likely indirect, RyR2 blocker used to treat arrhythmogenic conditions such as catecholaminergic polymorphic ventricular tachycardia.[19,20] To test the efficacy of flecainide in our in vivo model, control Hcn4:WT (n = 6) and Hcn4:shJph2 (n = 6) mice underwent our JET protocol followed by central IV boluses of flecainide (Figure 2A). JET was induced in all Hcn4:shJph2 mice and none of the control Hcn4:WT mice. One Hcn4:shJph2 mouse died due to hemodynamic compromise from JET before flecainide administration. Among the surviving mice, we found that flecainide terminated JET in only 1 Hcn4:shJph2 mouse at a dose of 1.5 μg/g (Figure 2B). Conversely, flecainide administration in this dose range was associated with high toxicity. Two Hcn4:shJph2 mice died after the 1.5 μg/g dose, and 2 died after the 3 μg/g dose. Three control Hcn4:WT mice died after the 3 μg/g dose (Figure 2C). This suggests that failure to convert JET by flecainide, or toxicity itself, may have led to mice death. Our results show that EL20 is more tolerable and more effective in terminating JET in our in vivo model.

FIGURE 2 Flecainide Is Minimally Effective in Terminating JET in Hcn4:shJph2 Mice

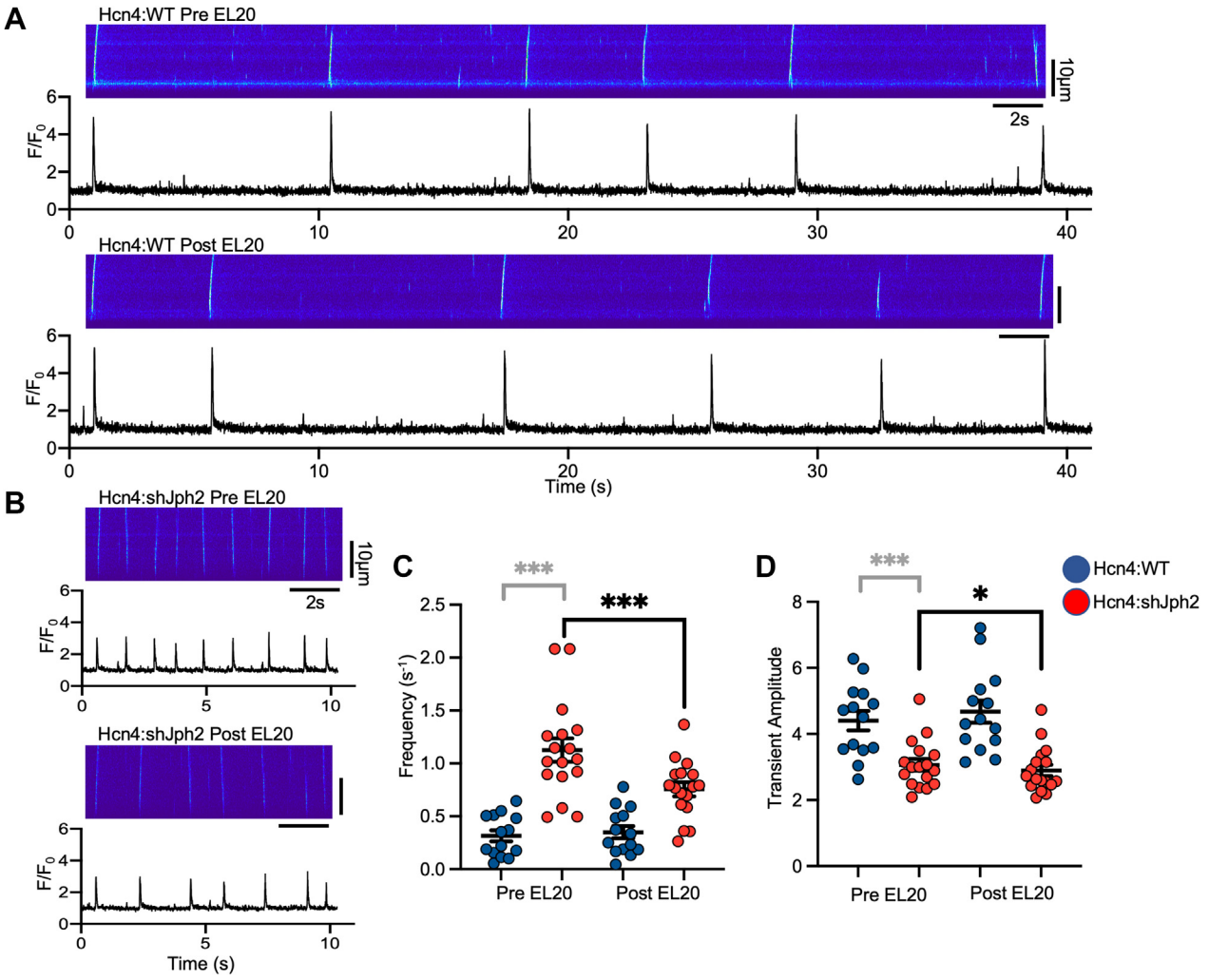


(A) Protocol for JET induction and flecainide pharmacologic termination. (B) Bar graph demonstrating the percentage of JET persistency with flecainide usage in Hcn4:shJph2 mice (black bars; n = 5) compared with Hcn4:WT mice (white bars; n = 6). (C) Bar graph demonstrating the survival rate with flecainide application in Hcn4:shJph2 mice (black bars; n = 5) compared with Hcn4:WT mice (white bars; n = 6). Error bars represent 95% CIs. Abbreviations as in Figure 1.

EL20 REDUCES THE INCREASED FREQUENCY OF CA^{2+} TRANSIENTS IN Hcn4:shJph2 NODAL CELLS. We previously found that Jph2 knockdown increases nodal cell automaticity by increasing intracellular Ca^{2+} leak from the SR, which drives increased nodal automaticity.[14] Given our finding that EL20 rapidly terminated tachycardia in vivo, we next sought to evaluate whether increased frequency of spontaneous nodal cell depolarizations could be selectively blunted by EL20 at the single-cell level. To do this, we conducted live cell Ca^{2+} imaging with the fluorescent Ca^{2+} dye Cal-520 on isolated single nodal cells from Hcn4:shJph2 and Hcn4:WT control mice, and applied 0.5 μmol/L EL20. In keeping with our previous findings, Hcn4:shJph2 demonstrated a 3-fold increase in Ca^{2+} transient frequency and a 30% decrease in transient amplitude compared with control (both $P < 0.001$) (Figure 3). Application of EL20 reduced this increased transient frequency by 33% ($P < 0.001$) (Figure 3C). Conversely, Hcn4:WT cells demonstrated no significant change in Ca^{2+} transient frequency with application of EL20 ($P = 0.11$) (Figure 3C). Hcn4:shJph2 nodal cells demonstrated reduced Ca^{2+} transient amplitude of 5% compared with minimal decrease in control cells with application of EL20 ($P < 0.050$) (Figure 3D). These findings suggest that increased nodal cell Ca^{2+} transient frequency can be reduced by EL20 in Hcn4:shJph2 nodal cells.

IVABRADINE DOES NOT ALTER CA^{2+} TRANSIENT FREQUENCY IN Hcn4:shJph2 NODAL CELLS. Ivabradine is a promising clinical antiarrhythmic drug which decreases heart rate by reduction of I_f in nodal cells and is mainly used in the management of inappropriate sinus tachycardia.[21] We next set out to investigate the effect of ivabradine on Ca^{2+}-driven automaticity, which we hypothesized would be insensitive to treatment. As anticipated, 100 μmol/L ivabradine decreased the frequency of Ca^{2+} transients in control nodal cells ($P < 0.050$) (Figures 4A and 4C). Conversely, in Hcn4:shJph2 nodal cells, ivabradine had a minimal effect on Ca^{2+} transient frequency (Figures 4B and 4C). Specifically, ivabradine caused no change in the frequency and amplitude of Ca^{2+} transients of Hcn4:shJph2 nodal cells ($P > 0.050$), while decreasing the frequency of Ca^{2+} transients in wild-type nodal cells by 30% ($P < 0.050$) (Figures 4C and 4D). These results further support the

FIGURE 3 **EL20 Reduces the Increased Frequency of Ca^{2+} Transients in Hcn4:shJph2 Nodal Cells Compared With Control**

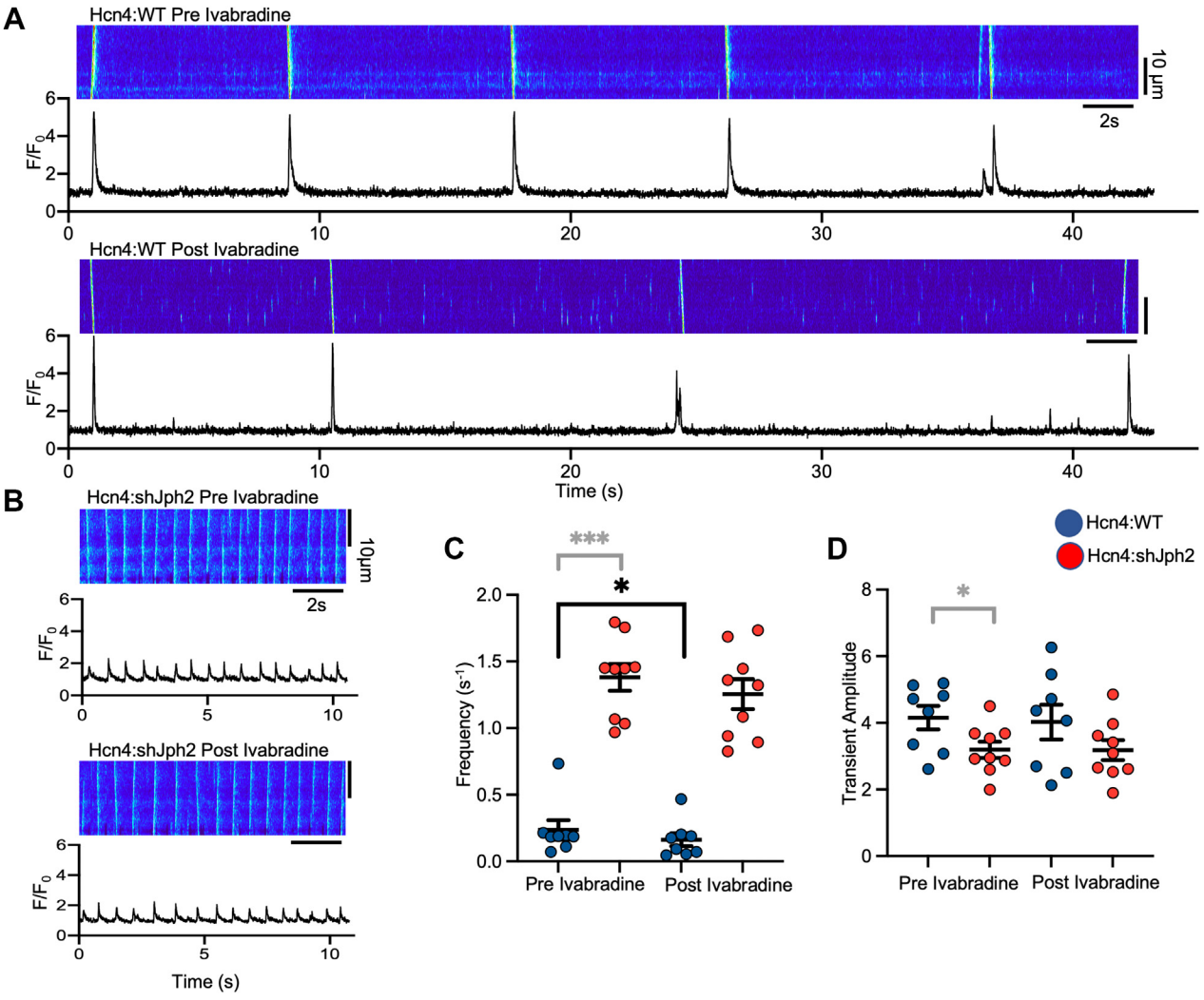


(A) Representative confocal line scan image and corresponding Ca^{2+} fluorescent tracings in an Hcn4:WT nodal cell before and after EL20 administration. (B) Representative confocal line scan image and corresponding Ca^{2+} fluorescent tracings in an Hcn4:shJph2 nodal cell before and after EL20 administration. (C and D) Dot plots demonstrating effect of EL20 (0.5 μmol/L) on frequency and amplitude of spontaneous Ca^{2+} transients in Hcn4:shJph2 nodal cells (n = 17; red circles) compared with control Hcn4:WT cells (n = 14; blue circles). Data points depicted represent individual cells derived from 4 Hcn4:WT mice and 4 Hcn4:shJph2 mice. Data are presented as mean ± SEM. A paired Student's *t*-test or McNemar's test was applied to compute *P* values between before and after dosage within the Hcn4:WT or Hcn4:shJph2 group, respectively, indicated by black brackets. An unpaired Student's *t*-test or Fisher's exact test was used to calculate *P* values between the Hcn4:WT and Hcn4:shJph2 groups before dosage, indicated by gray brackets. *$P < 0.050$; ***$P < 0.001$. F/F_0 = fluorescence (F) was normalized to baseline (F_0), and $Fmax/F_0$ represented amplitude.

conclusion that Jph2 knockdown in nodal cells results in uncoupling of the membrane voltage clock from the intracellular Ca^{2+} clock that drives automaticity in this model.

EL20 REDUCED THE SIZE AND FREQUENCY OF CA^{2+} SPARKS IN Hcn4:shJph2 NODAL CELLS. To investigate the mechanism of EL20-mediated reduction in rapid nodal cell automaticity, and to test the hypothesis that this reduction is achieved through correction of increased Ca^{2+} leak from the SR, we compared the Ca^{2+} sparks between Hcn4:shJph2 and control nodal cells (Figures 5A and 5B). There was no significant difference in Ca^{2+} sparks in wild-type nodal cells treated with EL20. In contrast, EL20 treatment in Hcn4:shJph2 nodal cells resulted in significant reductions in Ca^{2+} spark parameters, including 10% decrease in amplitude ($P < 0.010$), 5% decrease in full width at half-max ($P < 0.001$), and 2%

FIGURE 4 **Ivabradine Does Not Reduce the Increased Oscillation of Ca^{2+} Transients in Hcn4:shJph2 Nodal Cells Compared With Control**

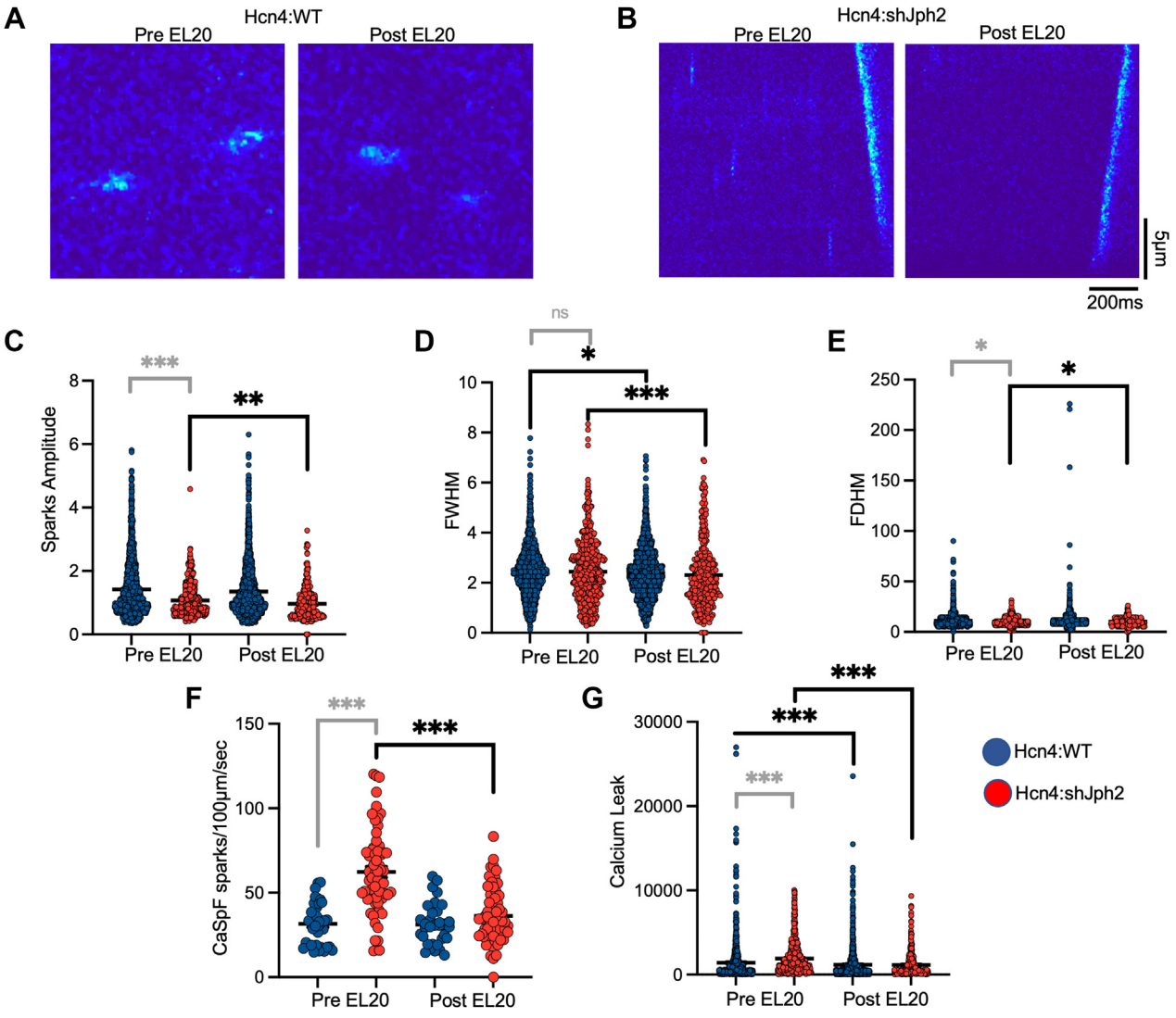


(A) Representative confocal line scan image and corresponding Ca^{2+} fluorescent tracings in an Hcn4:WT nodal cell before and after ivabradine administration. (B) Representative confocal line scan image and corresponding Ca^{2+} fluorescent tracings in an Hcn4:shJph2 nodal cell preivabradine and postivabradine administration. (C and D) Dot plots demonstrating effect of ivabradine (100 μmol/L) frequency and amplitude of spontaneous Ca^{2+} transients in Hcn4:shJph2 nodal cells (n = 9; red circles) compared with control Hcn4:WT cells (n = 8; blue circles). Data points depicted represent individual cells derived from 4 Hcn4:WT mice and 4 Hcn4:shJph2 mice. Data are presented as mean ± SEM. A paired Student's *t*-test or McNemar's test was applied to compute *P* values between before and after dosage within the Hcn4:WT or Hcn4:shJph2 group, respectively, indicated by a black bracket. An unpaired Student's *t*-test or Fisher's exact test was used to calculate *P* values between the Hcn4:WT and Hcn4:shJph2 groups before dosage, indicated by gray brackets. *$P < 0.050$, ***$P < 0.001$. F/F_O = fluorescence (F) was normalized to baseline (F_O), and $Fmax/F_O$ represented amplitude.

decrease in full duration at half-max ($P < 0.050$) (Figures 5C to 5E). Although these spark parameters were relatively modest, we noted a marked 40% reduction in Ca^{2+} spark frequency ($P < 0.001$) compared with wild-type cells (Figure 5F). Overall, EL20 reduced approximately 40% of the aberrant Ca^{2+} leak ($P < 0.001$) in Hcn4:shJph2 nodal cells, compared with only 15% reduction ($P < 0.001$) in wild-type cells (Figure 5G). Taken together, these findings suggest that EL20 decreases the size and frequency of Ca^{2+} sparks and diminishes excessive SR Ca^{2+} leak secondary to dysfunctional RyR2 in Jph2-knockdown Hcn4:shJph2 nodal cells.

EL20 HAS A REASONABLE THERAPEUTIC WINDOW AND IS RAPIDLY DEGRADED IN MURINE AND HUMAN PLASMA. Given the efficacy of EL20 in terminating JET in both in vivo and isolated single cells from Hcn4:shJph2 mice, we next explored the side-effect

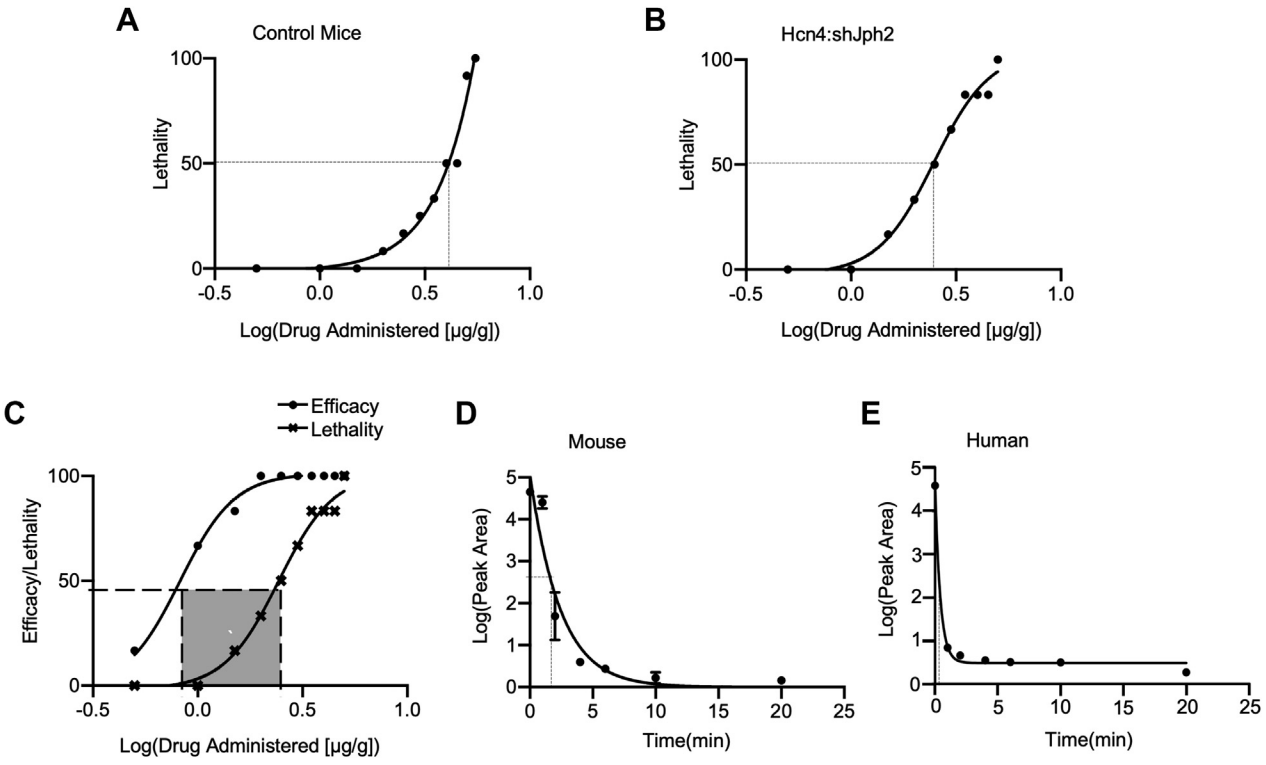
FIGURE 5 EL20 Suppresses Abnormal Sarcoplasmic Reticulum Ca^{2+} Release in Hcn4:shJph2 Nodal Cells



Representative confocal line scan images of Ca^{2+} sparks in (A) Hcn4:WT vs (B) Hcn4:shJph2 nodal cells before and after EL20 administration. Dot plots demonstrating that EL20 reduced (C) Ca^{2+} spark amplitude, (D) full width at half-max (FWHM), (E) full duration at half-max (FDHM), (F) Ca^{2+} spark frequency (CaSpF), and (G) calculated Ca^{2+} leak in Hcn4:shJph2 nodal cells (n = 17; red circles) compared with Hcn4:WT nodal cells (n = 14; blue circles). Data points depicted represent individual cells derived from 4 Hcn4:WT mice and 4 Hcn4:shJph2 mice. Data are presented as mean ± SEM. A paired Student's *t*-test or McNemar's test was applied to compute *P* values between before and after dosage within the Hcn4:WT or Hcn4:shJph2 group, respectively, indicated by black brackets. An unpaired Student's *t*-test or Fisher's exact test was used to calculate *P* values between the Hcn4:WT and Hcn4:shJph2 groups before dosage, indicated by gray brackets. *$P < 0.050$, **$P < 0.010$, ***$P < 0.001$.

profile and lethality dosing of the EL20 compound. In control mice (n = 11), EL20 demonstrated a median lethal dose (LD_{50}) of 3.9 μg/g, and Hcn4:shJph2 mice (n = 6) demonstrated a slightly lower LD_{50} of 2.6 μg/g (Figures 6A and 6B). Furthermore, when combined with dose-responsive curves demonstrating the efficacy of EL20 in breaking JET, we calculated a therapeutic index of EL20 of 2.9 (Figure 6C). In addition to lethality, the effects of EL20 on a number of electrophysiologic parameters of the heart were also measured, including atrial rate, ventricular rate, AV interval duration, QRS duration, and the presence of AV block and severe sinus bradycardia. ED_{50} calculations for AV interval prolongation, AV block, and

FIGURE 6 Lethality, Therapeutic Index, and Plasma Stability of EL20



Lethality dose-response curve for EL20 in (A) control mice and (B) Hcn4:shJph2 mice. (C) Efficacy dose-response curve of junctional ectopic tachycardia termination (dots) and lethality dose-response curve (x's). Median efficacy and lethal doses are demarcated. (D) Plasma decay analysis of EL20 in mouse plasma. (E) Plasma decay analysis of EL20 in human plasma.

sinus bradycardia with junctional escape were done in both control and Hcn4:shJph2 mice (Supplemental Results, Supplemental Figures 5 and 6). ED_{50} values were higher than our ED_{50} for controlling JET, further supporting a reasonable therapeutic window. We ruled out that the observed changes in electrocardiographic parameters were secondary to the volume of fluid delivered (Supplemental Figure 7).

Given the brief period of time during which EL20 converted JET to sinus rhythm in vivo, we hypothesized that the bioavailable active compound is rapidly cleared from the plasma. To test this, we performed EL20 degradation analysis of the compound in mouse and human plasma by mass spectroscopy. We found that EL20 was rapidly metabolized following first-order kinetics with a calculated half-life of 1.64 minutes in mouse and 0.29 minutes in human plasma (**Figures 6D and 6E**). The rapid breakdown in plasma suggests that primary clearance mechanism of EL20 occurs within the plasma rather than by hepatic or renal metabolism.

DISCUSSION

JET can be congenital or, more often, presents after congenital heart surgery. Postoperative JET is the most common hemodynamically significant tachycardia in the postoperative setting and usually occurs within 24 hours following heart surgery.[1] Management of JET is multifactorial and requires a staged therapeutic approach that includes sedation, pain control, avoidance of hyperthermia, and reduction of catecholamine use. The majority of JET occurrences require medical intervention, with the mainstay of therapy for postoperative JET being amiodarone.[22] Unfortunately, amiodarone is associated with a significant failure rate and unfavorable side-effect profile. Often JET requires treatment with multiple pharmacologic agents and occasionally can be fatal.[23,24] In many cases, JET requires both medical and cardiac overdrive pacing interventions to control rhythm.[25] However, even when successfully treated, JET can recur in up to 33% of cases initially treated

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with amiodarone.[26] This challenge is even greater in nonpostoperative congenital JET, where medical therapy is insufficient to achieve complete rhythm control in nearly 90% of patients.[27] Amiodarone blocks a number of ion currents of the cardiac myocyte and nodal tissue. Though principally believed to block repolarizing K^+ currents, amiodarone has been shown to block both ligand- and voltage-gated K^+ channels, including I_{Kr}, I_{Ks}, $I_{K,Na}$, and $I_{K,Ach}$. Amiodarone also blocks I_{to}, I_{Na}, and I_{Ca}, all representing various plasma membrane-limited currents responsible for the generation of the nodal and myocardial action potential.[28] In the present study, we found that by replicating similar predisposing factors to JET development in children, such as high temperature, high stress states with caffeine and isoproterenol, and mechanical stress, we were able to induce JET in our Hcn4:shJph2 mice in vivo. Furthermore, our finding that EL20 has a high degree of efficacy in the treatment of JET in these mice supports the conclusion that targeting an alternative molecular site (SR-mediated Ca^{2+} leak) may be efficacious in the treatment of nodal arrhythmia in humans.

Identification of an antiarrhythmic agent that has a rapid onset effect, and is quickly cleared from the plasma, suggests favorable pharmacokinetics, particularly for use in the postoperative period. In comparison, because amiodarone is highly lipophilic, it has a prolonged plasma half-life of 3.2 to 79.7 hours, which can increase to up to 100 days in long-term therapy.[29] Amiodarone also exhibits extensive interpatient variability, wide tissue distribution, and slow total body clearance, with a time of onset of 2 to 24 hours.[30] This long half-life is particularly salient, given the relatively severe side-effect profile of the drug. In the acute phase, amiodarone infusion is associated with significant hypotension, necessitating inotropic support or cardiac pacing, severe bradycardia, and chelation of free Ca^{2+}, resulting in further hemodynamic compromise.[23,26] Chronic administration of amiodarone is associated with systemic toxicity to the thyroid, liver, lungs, and eyes.[31] Given these shortcomings regarding efficacy and side-effects, a number of other antiarrhythmic agents have been investigated for the treatment of JET, including flecainide, procainamide, and sotalol.[32,33] However, an efficacious agent with a rapid onset and tolerable adverse event profile has not yet been identified.

Given the limited efficacy of current antiarrhythmic therapies in the acute treatment of JET and other nodal-type arrhythmias, exploration of novel targets amenable to pharmacologic manipulation is critical. Although plasmalemmal-limited ion channels, such as the hyperpolarization-activated current (I_f), have been historically thought to govern nodal cell automaticity, a growing body of evidence has identified intracellular Ca^{2+} as a key modulator of the rate of spontaneous depolarization.[34] Previous work has shown that pre-action potential Ca^{2+} release from the SR via RyR2 drives the action of the Na^+-Ca^{2+} exchanger (NCX) resulting in a net positive charge accumulation within the cytosol. Blockade of NCX has been found to acutely terminate spontaneous depolarization of sinoatrial nodal cells.[34] This balance between direct membrane depolarizing current from ion channels and indirect modulation by intracellular Ca^{2+} through NCX action is perturbed in the setting of altered nodal firing.[35,36]

Our previous results have shown that Jph2 mediates Ca^{2+} homeostasis within nodal cells. In the present study, we provide further evidence of the critical role of RyR2-mediated Ca^{2+} leak causing Ca^{2+} signal uncoupling from I_f in nodal automaticity in the development of JET. EL20 is a novel tetracaine derivative that has been shown to decrease arrhythmia burden in a murine model of catecholaminergic polymorphic ventricular tachycardia by reducing the diastolic leak of SR-stored Ca^{2+} by selective inhibition of RyR2 channels.[16] In our model, EL20 was well tolerated, with no deaths noted in our experiment stage, compared with flecainide and did not cause posttreatment electrocardiographic parameter changes.[16] Although flecainide can block cytosolic to luminal cation flow across RyR2, it cannot directly block the more physiologically relevant luminal to cytosolic cation flow.[37] As such, flecainide likely modulates RyR2 activity indirectly by reducing I_{Na}, which secondarily enhances sarcolemma Ca^{2+} release and reduces the open probability of the RyR2 channel by lowering the cytosolic Ca^{2+} concentration.[38] We hypothesize that the differences seen between EL20 and flecainide are likely due to EL20's direct RyR2 inhibition and thereby its ability to directly suppress nodal specific Ca^{2+} leak.

Our findings are consistent with earlier work and indicate that EL20 is effective at a dosing range of 1-2 μg/g. Moreover, we find that EL20 has a reasonable therapeutic index, although it is nearing the threshold of what may be considered a more narrow therapeutic index, which suggests that future use of this compound may warrant close monitoring for toxicity. Nonetheless, its relatively short half-life may help to offset concerns of toxicity. We also observed differences in pharmacokinetics between the wild-type and Hcn4:shJph2 mice during EL20 administration, such as a left shift in the lethality curve and lower LD_{50}. This is likely due to the hemodynamic

compromise in JET mice and raises the possibility of increased susceptibility to side-effects in a tachycardic state. Finally, although we likely had predominantly AV nodal cells in our single-cell calcium analysis, we cannot exclude the possibility of sinoatrial nodal cells being a small part of the analysis and conclusions. Overall, given the emerging role of abnormal Ca^{2+} homeostasis and signaling in driving automaticity, our findings support targeting SR-mediated Ca^{2+} leak via RyR2 as a novel method for the treatment of nodal arrhythmias.

STUDY LIMITATIONS. This study is limited by use of a narrow spectrum of RyR2-blocking reagents as well as the limited number of existing models of JET. Future studies, leveraging other RyR2 blockers, conducted in larger mammalian models of JET, will be critical to translating these findings to humans. Owing to differential cardiac toxicity following tamoxifen exposure in female mice, only male mice were used in this study. Furthermore, we did not control for multiple comparisons in our group-to-group analyses, and as such, results from our nodal cell experiments should be interpreted with caution. Future work examining both the development of JET and preclinical efficacy and toxicity in mammalian models with inclusion of both sexes is needed.

CONCLUSIONS

We have established an in vivo disease model for JET and identified an association between intracellular Ca^{2+} leak and JET through nodal-specific knockdown of Jph2 expression. SR-mediated Ca^{2+} leak from RyR2 may be a critical target for the treatment of nodal arrhythmias. EL20, a small-molecule inhibitor of RyR2, rapidly terminates JET in these mice and reduces increased Ca^{2+} oscillations and Ca^{2+} leak. Further studies are needed to determine the efficacy of Ca^{2+} modulation and the role of RyR2 inhibition in the treatment of nodal arrhythmias.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The underlying cause of JET is not well understood. JET may be due to calcium leak from the sarcoplasmic reticulum via RyR2 in nodal cell–specific junctophilin-2–knockdown mice. EL20, a novel RyR2 blocker, may represent a therapeutic candidate for converting JET to normal sinus rhythm in vivo.

TRANSLATIONAL OUTLOOK: JET may be due to sarcoplasmic reticulum Ca^{2+} leak from the ryanodine receptor in nodal cells of the heart, and molecular blockade of this leak may be a therapeutic target for the treatment of JET. EL20 is a candidate Ca^{2+} leak blocker with efficacy in treating JET in an in vivo mouse model with rapid metabolism in serum and a wide therapeutic window. If replicated in other models, such as larger mammalian models of JET, then EL20, and RyR2 blockade more generally, may be a therapeutic option or target for the treatment of JET and potentially nodal diseases of the heart.

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KEY WORDS calcium, JET, Jph2, junctional ectopic tachycardia, ryanodine receptor

APPENDIX For a supplemental Methods section, figures, and videos, please see the online version of this paper.