Reduction of repolarization reserve unmasks the proarrhythmic role of endogenous late Na\(^+\) current in the heart

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The plateau and duration of the cardiac action potential (AP) depends on a delicate balance of multiple ion channel currents, including inward (peak and late Na\(^+\) and Ca\(^{2+}\)) and outward [transient outward (I\(_{\text{to}}\)], inward-rectifier (I\(_{\text{K1}}\)) and delayed-rectifier (I\(_{\text{Kr}}\)) K\(^+\) currents. A decrease in outward or an increase in inward current prolong the duration of the AP (and its electrophysiologic surrogate, the QT interval) and are associated with an increased incidence of polymorphic ventricular tachycardia (torsade de pointes, TdP). The concept of repolarization reserve suggests that there is normally a reserve of repolarizing current to protect against excessive QT interval prolongation and ventricular arrhythmia. Reduction of repolarization reserve has been associated with an increased risk of arrhythmogenesis in patients with inherited long QT syndromes and/or structural heart disease, and in patients treated with QT-prolonging drugs (21–23, 31). Factors that either increase inward currents or decrease outward currents during repolarization of the cardiac AP are associated with reduced repolarization reserve and are arrhythmogenic.

A rapid, inward Na\(^+\) current (I\(_{\text{Na}}\)) flowing through voltage-gated Na\(^+\) channels accounts for the upstroke of the cardiac AP. This I\(_{\text{Na}}\) normally inactivates rapidly and is thus thought to exert minimal influence on AP duration (APD), except perhaps in Purkinje fibers (5, 10). However, late reopenings of Na\(^+\) channels during the plateau of the AP lead to a small 30- to 60-pA physiological, persistent late I\(_{\text{Na}}\) in ventricular myocytes (7, 13, 38). During the plateau of the AP, when membrane resistance is high and the total membrane current is small, this endogenous late I\(_{\text{Na}}\) may have a significant effect to prolong the APD, especially when K\(^+\) current (I\(_{\text{K}}\)) is reduced. The significance of this effect is not clear, but basic and clinical research results suggest that simultaneous enhancement of late I\(_{\text{Na}}\) [e.g., caused by sea anemone toxin II (ATX-II)] and reduction of I\(_{\text{K}}\) is markedly proarrhythmic (6, 15, 17, 35, 36).

We hypothesized that even the normal endogenous (nonenhanced) physiological late I\(_{\text{Na}}\) may be proarrrhythmic when I\(_{\text{K}}\) is inhibited and net repolarizing current, and therefore repolarization reserve, is decreased. Therefore, to “unmask” the potential proarrhythmic role of endogenous late I\(_{\text{Na}}\) when I\(_{\text{K}}\) is reduced, we exposed rabbit hearts to proarrhythmic concentrations of inhibitors of the rapidly activating I\(_{\text{Kr}}\), I\(_{\text{to}}\), and I\(_{\text{K1}}\) currents and then determined the effects of late I\(_{\text{Na}}\) inhibitors on ventricular repolarization and arrhythmic activity. Tetrodotoxin (TTX) and ranolazine were used to inhibit late I\(_{\text{Na}}\). TTX is a selective inhibitor of voltage-gated Na\(^+\) channels and inhibits both peak and late I\(_{\text{Na}}\). Ranolazine is a selective inhibitor of late I\(_{\text{Na}}\) relative to peak I\(_{\text{Na}}\), but the selectivity of ranolazine for inhibition of late I\(_{\text{Na}}\) relative to inhibition of human ether-a-go-go-related gene (HERG) K\(^+\) current (I\(_{\text{HERG}}\)) is only twofold. The mechanism explaining the effect of ranolazine to antagonize arrhythmias caused by I\(_{\text{Kr}}\) inhibition (27, 32, 36, 37) still needs clarification. We therefore determined the nature of the functional antagonism of effects of E-4031 by ranolazine that were observed in hearts in this study, by comparing the effects of ranolazine and E-4031 on I\(_{\text{HERG}}\) in human embryonic kidney (HEK 293) cells expressing either wild-type HERG channels or HERG channels with drug-binding site mutations.

MATERIALS AND METHODS

Female Rabbit Isolated Heart Model

The use of animals in this investigation conformed to the “Guide for the Care and Use of Laboratory Animals” published by the United States National Institutes of Health (NIH publication no. 85-23,
revised 1996), and animal use was approved by the Institutional Animal Care and Use Committee of CV Therapeutics (Palo Alto, CA). New Zealand White female rabbits weighing 2.5–3.5 kg were used in this study. Female rabbit hearts were isolated, perfused by the method of Langendorff, and instrumented for recording of electrical activity as previously described by Wu et al. (34, 35). Hearts were submerged in a temperature-controlled bath at 37°C during experiments. Monophasic action potential (MAP) and pseudo 12-lead electrocardiogram (ECG) records were obtained, and transmural dispersion of repolarization (TDR) and beat-to-beat variability of repolarization (BVR) were determined as described by Wu et al. (34, 35). Drug effects on ECG parameters such as the duration of the T wave from Tpeak to Tend (Tpeak-Tend) and the QT and QRS intervals were determined by superimposing 12-lead ECG signals recorded from the heart during steady-state control and treatment periods.

Determination of Concentration-Response Relationships for Effects of E-4031, TTX, and Ranolazine on Ventricular Arrhythmias and Electrophysiological Parameters

Hearts were exposed to increasing concentrations of E-4031 (1–60 nM), TTX (0.1–10 μM), and ranolazine (1–100 μM) in a cumulative manner, allowing 7–15 min between increases in drug concentration to facilitate the recording of a steady-state maximal effect. Determination of the Anti-arrhythmic Effects of TTX and Ranolazine on Ventricular Arrhythmias in the Presence of E-4031

Hearts were perfused with E-4031 (60 nM) for 20 min to induce arrhythmias and then exposed to either TTX (0.3, 0.6, 1, and 3 μM) or ranolazine (5, 10, and 30 μM) in the continued presence of 60 nM E-4031. Electrophysiological parameters were monitored continuously for the occurrence of ectopic ventricular beats (EVBs), early afterdepolarizations (EADs), and ventricular tachycardias (VT). Post-drug exposure values of MAP duration (MAPD) were obtained when drug washout was completed. An EVB was defined as a spontaneous beat occurring earlier than the next paced beat. VT was defined as a sequence of three or more consecutive EVBs at a rate exceeding the pacing rate of 1 Hz. A TdP was defined as a VT wherein the morphology of the QRS complex in a 12-channel ECG record were continuously changing. An EAD was defined as a positive depolarization during phase 2 or 3 of an MAP signal.

Repeated 3-s pauses in ventricular electrical stimulation were used to induce pause-triggered EVBs, EADs, and VT in the absence and presence of drugs. Pause-triggered EADs and ventricular arrhythmias were defined as EAD, EVBs, or VT that occurred within the first three beats after ventricular pacing was resumed.

Determination of the Effects of E-4031, TTX, and Ranolazine on Peak and Late I Na and I HERG in HEK 293 Cells Expressing Either SCN5A or HERG K+ Channels

Heterologous expression of SCN5A Na+ and HERG K+ channels. HEK 293 cells stably expressing the human heart Na+ channel (hH1a; Na1.5) clone of SCN5A gene (α-subunit) or the HERG K+ channels (wild-type) were maintained as described previously (35). With the use of site-directed mutagenesis, two HERG drug-binding mutations, Y652A and F656A, were generated and were stably expressed in HEK 293 cells. Recordings of peak and late I Na and I HERG with whole cell voltage-clamp technique. Whole cell I Na and I HERG were recorded using an Axopatch 200B amplifier (Axon Instruments, Sunnyvale,
CA), pCLAMP 9.0 software (Axon Instruments) was used to generate voltage-clamp protocols and acquire data. Patch pipettes were pulled from borosilicate glass (World Precision Instruments, Sarasota, FL) using a DMZ Universal puller (Dagan, Minneapolis, MN). All patch-clamp experiments were performed at a room temperature of 19 ± 1°C.

For recording of \( I_{Na} \), cells were superfused with Tyrode solution containing (in mM): 140 NaCl, 4.0 KCl, 1.8 CaCl\(_2\), 0.75 MgCl\(_2\), and 5 HEPES (pH adjusted to 7.4 with NaOH). Pipette resistance was 1–2 M\( \Omega \) when filled with a pipette (internal) solution containing (in mM): 20 CsCl, 120 CsF, 2 EGTA, and 5 HEPES (pH adjusted to 7.4 with CsOH). Membrane potentials were not corrected for junction potentials that arise between the pipette and bath solutions. For recording of \( I_{HERG} \), cells were superfused with Tyrode solution containing (in mM): 137 NaCl, 4.0 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 10 glucose, and 10 HEPES; pH was adjusted to 7.4 with NaOH. Pipettes were filled with a solution containing (in mM): 130 KCl, 1 MgCl\(_2\), 5 MgATP, and 5 EGTA; pH was adjusted to 7.4 with KOH. In all recordings, 75–80% of the series resistance compensation was achieved, yielding a maximum voltage error of ~5 mV. To measure the tonic block (i.e., block of current that is independent of the frequency of channel activity) by ranolazine or TTX of peak \( I_{Na} \), 24-ms depolarizing steps to ~20 mV from a holding potential of ~140 mV were applied to cells at a rate of 0.1 Hz. To measure the effect of ranolazine or TTX on late \( I_{Na} \), the normally small late \( I_{Na} \) (~68.4 ± 14.7 pA, \( n = 8 \) cells) was augmented by exposure of cells to 3 nM ATX-II, and the effect of ranolazine or TTX to reduce the augmented late \( I_{Na} \) was determined in the presence of 3 nM ATX-II. Late \( I_{Na} \) was defined as the magnitude of \( I_{Na} \) between 650 and 700 ms after application (at a rate of 0.1 Hz) of a 700-ms depolarizing step to ~20 mV from a holding potential of ~140 mV. \( I_{HERG} \) was activated with a step to 20 mV, and tail current was recorded after a subsequent step to ~50 mV. The voltage protocol was repeated at 15-s intervals.

To validate the use of TTX and ranolazine as selective inhibitors of \( I_{Na} \), specifically late \( I_{Na} \), the concentration-response relationships for these agents to inhibit peak and late \( I_{Na} \), and \( I_{HERG} \) were determined in preliminary experiments. HEK 293 cells expressing either human Na\(_v\)1.5 (SCN5A) Na\(^+\) channels or HERG K\(^+\) channels were used for these experiments. TTX concentration dependently inhibited peak and late \( I_{Na} \) (augmented by ATX-II) but not \( I_{HERG} \) (Fig. 1, A and B). The values for drug concentration that causes 50% block (IC\(_{50}\)) and the Hill number (\( n_{H} \); in parentheses) for the inhibitions by TTX of peak (tonic block) and late \( I_{Na} \) were 5.99 ± 0.36 \( \mu \)M (1.1 ± 0.1) and 0.53 ± 0.09 \( \mu \)M (1.3 ± 0.2), respectively (\( n = 3 \) and 4 cells, respectively; Fig. 1C). Ranolazine inhibited peak and late \( I_{Na} \), and also inhibited \( I_{HERG} \) (Fig. 1, D and E). Values of IC\(_{50}\) and \( n_{H} \) (in parentheses) for the inhibitions by ranolazine of peak \( I_{Na} \), late \( I_{Na} \), and \( I_{HERG} \) were 428.10 ± 33.07 \( \mu \)M (1.63 ± 0.18), 6.86 ± 1.64 \( \mu \)M

\[ \text{Fig. 2. Effects of E-4031 (A and B), TTX (C and D), and Ran (E and F) to increase the durations of monophasic action potentials (MAPD\(_{90}\)) recorded from epicardial and endocardial regions of the left ventricle of the rabbit isolated heart. A, C, and E, original recordings of monophasic action potentials (MAPs) recorded from the epicardial surface of the left ventricular wall before (control) and after administration of 30 nM E-4031, 1 \( \mu \)M TTX, and 10 \( \mu \)M Ran, respectively. B, D, and F, concentration-response relationships for effects of E-4031 (B, \( n = 38 \) and 32 hearts), TTX (D, \( n = 8 \) hearts) and Ran (E, \( n = 23 \) and 16 hearts) on epicardial and endocardial MAPD\(_{90}\). *P < 0.05–0.001 compared with control; †torsade de pointes ventricular tachycardia (TdP) occurred at or above the concentration indicated.} \]
(0.71 ± 0.12), and 14.44 ± 3.58 μM (1.01 ± 0.11), respectively (n = 4–8 cells each; Fig. 1F).

Statistical Analyses

All data are reported as means ± SE. The concentration-response relationships were analyzed using Prism version 5 (GraphPad Software, San Diego, CA). When control and treatment values were obtained from the same hearts, the significance of differences of measures before and after interventions was determined by repeated-measure one-way ANOVA followed by the Student-Newman-Keul’s test. A paired or unpaired Student’s t-test was used to determine the statistical difference between values of two means obtained from the same or different experiments, respectively. For categorical data, a Chi square test was used.

Ion current data were analyzed using Clampfit and Microcal Origin (MicroCal, Northampton, MA) software. Values of peak and late I_{Na}, and peak tail I_{HERG} were normalized to the respective control values and are plotted as relative currents. Concentration–response relations were fitted using the Hill equation, \( I_{drug}/I_{control} = 1/[1 + (D/DIC50)^n] \), where \( I_{drug}/I_{control} \) is fractional block and D is drug concentration.

Materials

E-4031, 4-aminopyridine (4-AP), and BaCl2 were purchased from Sigma Chemical (St Louis, MO). TTX and ATX-II were purchased from the Department of Bio-Organic Chemistry at CV Therapeutics (Palo Alto, CA). HEK 293 cells stably expressing the human heart SCN5A channels that were originally developed in the laboratory of Dr. Craig T. January were purchased from CytoMx (Cambridge, UK) and from the University of Wisconsin (Madison, WI), respectively.

RESULTS

E-4031 and Ranolazine, but not TTX, Prolonged MAPD$_{90}$ in Female Rabbit Isolated Hearts

E-4031 (1–60 nM) caused concentration-dependent prolongations of epi- and endocardial MAPD$_{90}$ by up to 41 ± 3 and 64 ± 6%, respectively (n = 40, P < 0.001; Figs. 2, A and B, 3, and 4, A and B), and increased TDR from 20 ± 4 to 74 ± 10 ms (n = 39, P < 0.001; Fig. 4C), BVR from 0.24 ± 0.02 to 0.75 ± 0.05 ms (n = 18, P < 0.01; Fig. 4D), Tpeak–Tend from 34 ± 1 to 102 ± 6 ms (n = 38, P < 0.01; Fig. 4E) and QT interval from 249 ± 4 to 429 ± 15 ms (n = 21, P < 0.01; data not shown). TTX (0.1–10 μM) alone did not cause significant changes in either epi- or endo-MAPD$_{90}$ (Fig. 2, C and D) or QT interval compared with control (185 ± 6 vs. 184 ± 6, 204 ± 5 vs. 203 ± 6, and 253 ± 6 vs. 260 ± 5 ms, respectively, each P > 0.05, n = 8). Ranolazine (0.1–100 μM) prolonged epi- and endocardial MAPD$_{90}$ by up to 25 ± 2 and 26 ± 3%, respectively (n = 23 and 16, P < 0.01; Fig. 2, E and F) and QT interval from 249 ± 13 to 286 ± 13 ms (n = 5, P < 0.01, not shown) without increasing the TDR (21 ± 4 ms in control and 22 ± 5 ms with 100 μM ranolazine, n = 16, P > 0.05).

Effects of TTX and Ranolazine on MAPD Prolongation and Arrhythmic Activities of E-4031

In the presence of 60 nM E-4031, both TTX (0.6–3 μM, n = 18; Figs. 3, A and B, and 4, A and B) and ranolazine (5–30 μM, n = 19; Figs. 3, C and D, and 4, A and B) significantly decreased values of epi- and endocardial MAPD$_{90}$. Both TTX and ranolazine also significantly reduced TDR, BVR, Tpeak–Tend (Fig. 4, C–E), and the QT interval (n = 9–20, P < 0.01 and 0.001; data not shown) in the presence of 60 nM E-4031, in a concentration-dependent manner. However, although TdT was not observed in hearts exposed to 60 nM E-4031 in the presence of either 3 μM TTX or 30 μM ranolazine, neither TTX nor ranolazine was able to fully reverse the effects of 60 nM E-4031 on other measures of proarhythmic activity (Fig. 4, A–E).

Spontaneous or 3-s pause-triggered EADs, frequent EVBs, and episodes of TdP were induced in 20 out of 25 (80%) hearts treated with 60 nM E-4031 (Figs. 4F and 5, B and E). Both TTX (0.6–3 μM) and ranolazine (5–30 μM)
concentration dependently abolished the spontaneous and 3-s pause-triggered episodes of TdP caused by 60 nM E-4031 (Figs. 4 and 5, C and F). The effects of TTX (data not shown) and ranolazine were reversible, and their washout in the presence of E-4031 resulted in a reappearance of TdP (Fig. 5G).

Exposure of hearts to TTX at concentrations of 3, 10, and 30 μM (but not at 0.3 or 0.6 μM) resulted in increases of the QRS interval by 5 ± 1, 29 ± 6, and 67 ± 10 ms (n = 12, P < 0.01; data not shown), respectively. Hence, the effects of TTX to shorten MAPD₉₀ in presence of E-4031 at concentrations ≤1 μM were unlikely to be due to the inhibition of peak $I_{Na}$. Neither E-4031 nor ranolazine increased the QRS interval at concentrations used in these experiments.

**Effects of the Late $I_{Na}$ Inhibitor TTX to Attenuate MAPD Prolongation and Arrhythmic Activity Caused by 4-AP Plus E-4031, or by BaCl₂**

To further demonstrate that inhibition of physiological late $I_{Na}$ reduces the proarrhythmic effects of $I_K$ inhibitors, hearts were treated with the K⁺ channel inhibitors 4-AP, E-4031, or BaCl₂ in the absence and presence of the late $I_{Na}$ inhibitor, TTX. 4-AP is a selective $I_{Na}$ inhibitor at low concentrations (9, 11). Both 4-AP (100 μM) and a low concentration of E-4031

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**Fig. 4.** Effects of TTX and Ran on electrophysiological parameters and incidence of TdP in presence of 60 nM E-4031. Each bar indicates a mean value of determinations (n = 9–21 hearts) of epi- and endocardial MAPD₉₀ (A and B), transmural dispersion of repolarization (TDR) of MAPD₉₀ (MAPD₉₀ Endo – MAPD₉₀ Epi; C), beat-to-beat variability (BVR) of MAPD₉₀ (D), duration of the T wave from Tpeak to Tend (Tpeak-Tend; E), and incidence of TdP (F) in the presence of 60 nM E-4031 alone (0 drug) or with either TTX (open bars) or Ran (filled bars). *P < 0.05 and **P < 0.01 compared with 60 nM E-4031 alone.
(30 nM) caused significant prolongations of epi-MAPD from 196 ± 5 and 183 ± 3 ms to 216 ± 5 and 224 ± 6 ms (n = 14 and 20, P < 0.01; Fig. 6A), respectively, without causing TdP. The combination of 4-AP (100 μM) and E-4031 (30 nM) caused a much greater increase in MAPD to 310 ± 7 ms, and caused TdP in all hearts studied (n = 8). In the presence of 4-AP (100 μM) and E-4031 (30 nM), TTX shortened the MAPD and decreased Tpeak-Tend in a concentration-dependent manner (P < 0.01, Fig. 6B). Although frequent episodes of TdP were observed in the presence of 4-AP (100 μM) plus E-4031 (30 nM), no episodes of TdP were observed in the presence of these agents when 0.3–3 μM TTX was also present (Fig. 6B).

Ranolazine Shared a Binding Domain with E-4031 in HERG Channels

E-4031 and ranolazine inhibit IHERG with high and low potencies, respectively (4, 20, 30), and prolong the duration of the AP (Fig. 2). The functional antagonism by ranolazine of the effects of E-4031 to increase MAPD (Figs. 3 and 4) and markers of proarrhythmic activity (Fig. 4, C and D), and to cause TdP (Fig. 4F), could be the result of the reduction by ranolazine of late INa, but it could also be due to displacement by ranolazine of E-4031 from the HERG channel. To address the latter, antagonism by each drug of the effect of the other to inhibit IHERG was investigated. In preparations of HEK 293...
cells stably expressing HERG K⁺ channels, superfusion of either 300 μM ranolazine (Fig. 7A and inset) or 0.3 μM E-4031 (Fig. 7B and inset) caused a rapid and nearly complete reduction in \( I_{\text{HERG}} \) amplitude. The recovery of \( I_{\text{HERG}} \) was rapid and complete after washout of ranolazine (Fig. 7A). In contrast, the recovery of \( I_{\text{HERG}} \) after washout of E-4031 was negligible (Fig. 7B). The amplitude of peak tail \( I_{\text{HERG}} \) after 9 min washout of E-4031 was only 15.6 ± 5.7% of the predrug amplitude (Fig. 7B and inset). To determine if ranolazine and E-4031 shared a similar binding domain to cause inhibition of \( I_{\text{HERG}} \), cells were

![Graph A](image1.png)

**Fig. 6.** Effects of TTX on epicardial MAPD90 (open bars), Tpeak-Tend (filled bars), and incidence of TdP (%TdP) in the absence and presence of either 100 μM 4-aminopyridine (4-AP) plus 30 nM E-4031 (A) or 30 μM BaCl₂ (B). BaCl₂ (30 μM) did not induce TdP. Each bar indicates a mean of determinations from 6–8 hearts. *P < 0.01 compared with control. **P < 0.05 compared with either 4-AP + E-4031 or BaCl₂.

![Graph B](image2.png)

**Fig. 7.** Effects of Ran and E-4031 alone and sequentially applied to reduce the amplitude of \( I_{\text{HERG}} \). A–D: relative amplitude of peak tail \( I_{\text{HERG}} \) vs. time. Horizontal line(s) indicate periods of drug exposure or washout. A and B: relative peak tail \( I_{\text{HERG}} \) amplitude during exposures to either 300 μM Ran for 7.5 min (A) or to 0.3 μM E-4031 for 2.5 min (B), followed by drug washout. Insets: sample current records in control (a), the presence of 300 μM Ran or 0.3 μM E-4031 (b and c), and after washout (d). Scale in inset: horizontal and vertical bars represent 2 s and 250 pA, respectively. C and D: peak tail \( I_{\text{HERG}} \) amplitude during exposures to Ran alone and then Ran + E-4031 or to E-4031 alone and then E-4031 + Ran, respectively, followed by washout of both drugs.

![Graph C](image3.png)

![Graph D](image4.png)
exposed to 300 μM ranolazine, and then to E-4031 (0.3 μM) in the continued presence of ranolazine, after which both drugs were washed out (Fig. 7C). The recovery of \( I_{\text{HERG}} \) was nearly complete after washout of both drugs, since the peak tail \( I_{\text{HERG}} \) amplitude recovered to \( 91.9 \pm 2.1\% \) of control \((n = 3\) cells, \( P < 0.05 \) compared with E-4031 alone; Fig. 7C). When the order of exposure to ranolazine and E-4031 was reversed and cells were exposed first to 0.3 μM E-4031, and then to 300 μM ranolazine in the continued presence of E-4031, there was minimal recovery in peak tail \( I_{\text{HERG}} \) amplitude after washout of both drugs, to only \( 10.7 \pm 2.3\% \) \((n = 3\) cells) of control (Fig. 7D). The result that saturating the HERG drug-binding site with ranolazine prevented E-4031 from having an effect suggests that binding of ranolazine and E-4031 to HERG was not concurrent, but that the binding of one prevented the binding of the other. However, although ranolazine acutely reversed the inhibition \( I_{\text{HERG}} \) by inhibiting late \( I_{\text{Na}} \)\(^{+}\) in the brief duration of experiments \((e.g., 10\text{ min in Fig. 7})\), it did not acutely reverse the inhibitory effect of E-4031 on \( I_{\text{HERG}} \), suggesting that it did not displace E-4031 binding.

To further elucidate the binding sites for ranolazine and E-4031 in the HERG channel, the effects of both compounds to inhibit \( I_{\text{HERG}} \) in cells with mutant HERG \( \text{K}^{+} \) channels \((Y652\text{ or } F656A)\) \((17)\) were studied. The concentration-response relationships for both ranolazine and E-4031 to inhibit \( I_{\text{HERG}} \) in cells with mutant HERG channels were shifted to the right by >10-fold (Fig. 8). Ranolazine at concentrations as high as 300 μM caused only \(~33\% \) \((n = 4\) cells) and 26% \((n = 4\) cells) block of \( I_{\text{HERG}} \) in cells expressing Y652A and F656A mutant channels, respectively, whereas wild-type HERG channels were nearly completely blocked by 100 μM ranolazine (Fig. 8A). Similarly, the IC\(_{50}\) values of 0.36 and 0.56 μM for E-4031 to block \( I_{\text{HERG}} \) in cells expressing mutant channels Y652A \((n = 4\) cells, each) and F656A \((n = 4\) cells, each) were significantly greater than the IC\(_{50}\) value for E-4031 to block \( I_{\text{HERG}} \) in cells expressing wild-type channels \((15.8\text{ nM}; \text{Fig. 8B})\). These data suggest the possibility that ranolazine and E-4031 compete for a common site in the HERG channel pore. However, ranolazine could not displace the tightly bound E-4031 during the brief duration of experiments \((e.g., 10\text{ min in Fig. 7})\). Thus the data are consistent with the hypothesis that ranolazine antagonized the effect of E-4031 by inhibiting late \( I_{\text{Na}} \).

**DISCUSSION**

The main finding of this study is that endogenous \((\text{physiological})\) late \( I_{\text{Na}} \), although small in amplitude \((8–10\) μM) modulates ventricular repolarization, APD, and arrhythmogenesis when repolarization reserve was decreased by treatments of rabbit hearts with 4-AP, E-4031, or BaCl\(_2\) to decrease \( I_{\text{Kr}} \), \( I_{\text{K1}} \), and \( I_{\text{K1}} \), respectively. Either TTX at low \((\leq 1\) μM) or ranolazine at therapeutic \((2–10\) μM) concentrations significantly decreased MAPD\(_{90}\), TDR, BVR, Tpeak–Tend, and QT interval, and abolished the EADs, EVBs, and TdP caused by the \( \text{K}^{+} \) inhibitor E-4031. Ranolazine or TTX partially reversed the prolongations of MAPD and arrhythmogenesis caused by the high-affinity and potent \( \text{K}^{+} \) inhibitor E-4031 \((\text{Figs. 3–5})\). TTX shortened the MAPD caused by either a combination of the \( I_{\text{Na}} \) inhibitor 4-AP with E-4031, or the \( I_{\text{K1}} \) inhibitor BaCl\(_2\) \((30\) μM) \((\text{Fig. 6})\). The results suggest that endogenous late \( I_{\text{Na}} \) contributes to prolongation of the APD and is arrhythmogenic in hearts with decreased repolarization reserve, and that inhibition of this small current reduces arrhythmogenic activity. This finding may explain observations that \( \text{Na}^{+} \) channel inhibitors are able to reverse the proarrhythmic, electrophysiological effects of various \( I_{\text{K}} \) inhibitors on the heart \((1, 19)\).

Ranolazine is a selective inhibitor of late vs. peak \( I_{\text{Na}} \) \((4)\). In HEK 293 cells, ranolazine inhibited late \( I_{\text{Na}} \) and \( I_{\text{Kr}} \) with potencies of \( 6.9 \pm 1.6 \) and \( 14.4 \pm 3.4\) μM \((\text{Fig. 1F})\); these values are similar to those reported in a study of canine ventricular myocytes \((4)\). Thus the anti-arrhythmic effect of ranolazine in experiments with E-4031 in this study could potentially be due either to reduction of late \( I_{\text{Na}} \), or to displacement of the \( I_{\text{Kr}} \) inhibitor E-4031 from its binding site in the HERG channel pore. Electrophysiological studies of HERG channels expressed in HEK 293 cells indicated that ranolazine and E-4031 shared the same binding domain to inhibit \( I_{\text{HERG}} \) but that ranolazine could not competitively displace E-4031 \((\text{Fig. 7})\). This finding suggests that the effect of ranolazine to reverse actions of E-4031 \((\text{Figs. 3–5})\) is the result of inhibition of late \( I_{\text{Na}} \) by ranolazine, rather than displacement of E-4031 from the HERG channel protein.

TTX inhibited \( \text{Nav}1.5 \) late \( I_{\text{Na}} \) at lower concentrations than it inhibited \( \text{Nav}1.5 \) peak \( I_{\text{Na}} \) in this study, with potencies of \( 0.5 \pm 0.1 \) and \( 6.0 \pm 0.4\) μM, respectively \((\text{Fig. 1C})\). Thus, at

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**Fig. 8.** Concentration-response relationships for inhibition by Ran (A) and E-4031 (B) of peak tail \( I_{\text{HERG}} \) in HEK 293 cells expressing wild-type (WT, ○), Y652A (●), and F656A (▲) HERG channels. Relative \( I_{\text{HERG}} \) was calculated as the magnitude of peak tail \( I_{\text{HERG}} \) at \(-50\) (WT) and \(-120\) (Y652A and F656A) mV in the presence of drug relative to control (absence of drug). Symbols represent means ± SE of measurements in 4 cells.

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concentrations of 0.6–1 μM, TTX is a relatively selective inhibitor of late $I_{Na}$. Therefore, the effects of ranolazine as well as those of TTX (which is not an inhibitor of HERG) to reduce MAPD and arrhythmic activity were due to their reduction of late $I_{Na}$. The results indicate that endogenous late $I_{Na}$ in rabbit hearts with reduced repolarization reserve is an important regulator of both APD and ventricular arrhythmic activity.

Potential causes of reduced repolarization reserve include gene mutations or polymorphisms that enhance late $I_{Na}$ or Ca$^{2+}$ current, mutations in K$^+$ channels that result in decreased outward $I_K$, drugs that affect ion channels (e.g., inhibitors of $I_{Kr}$), electrolyte abnormalities (hypokalemia and hypomagnesia), and structural heart diseases (heart failure and myocardial ischemia) (14, 16, 21, 22, 28, 29). A reduction of net outward current during phase 3 of the ventricular AP (i.e., reduced repolarization reserve) is potentially proarrhythmic, especially when associated with AP triangulation, reverse rate dependency, and increased dispersion of repolarization (3, 12). In a study of cardiomyocytes isolated from hearts of dogs with heart failure and arrhythmias, Maltsev et al. (16) reported that EADs ceased and both APD and BVR returned to normal when late $I_{Na}$ was partially inhibited by TTX or saxitoxin, and when outward current was injected (50 pA × 200 ms) during the AP plateau.Injecting current opposite in direction to late $I_{Na}$ (which is an inward current) did not alter the contribution of peak $I_{Na}$ to the AP upstroke and to Na$^+$ entry, but yet confirmed the effect of a relatively small current to alter repolarization, APD, and arrhythmic activity (16). Because AP prolongation alone may not be a good predictor of proarrhythmnic risk (5), the specific current(s) affected and the nature of the changes precipitated as a result may modify or determine risk. For example, an increase of late $I_{Na}$ is reported to lead to EAD and delayed afterdepolarization (DAD) formation and dispersion of repolarization (34), all of which are proarrhythmic. Potential reasons for the marked proarrhythmic effect of late $I_{Na}$ are that late $I_{Na}$ peaks during phase 3 of the AP when repolarization begins, due to an increase of the driving force for Na$^+$ entry (34), and Na$^+$ entry leads to increased Na$^+$/Ca$^{2+}$ exchange and Ca$^{2+}$ entry, which may lead to Ca$^{2+}$ loading of the sarcoplasmic reticulum, and DADs. In addition, a reduction of repolarization reserve by one mechanism (e.g., reduction of $I_{Kr}$) can sensitize the myocardium to the proarrhythmic effects of drugs or conditions that reduce net outward current by another mechanism (14, 21, 24, 35, 36). Consistent with this principle is our finding that reduction of endogenous (physiological) late $I_{Na}$, which is a small inward current in the absence of disease (i.e., the situation in the present study), reduced arrhythmic activity in the presence of inhibitors of $I_{Ks}$, $I_{Kr}$, and $I_{K1}$. These results suggest that endogenous late $I_{Na}$ can be potentially proarrhythmic in the presence of other factors that reduce repolarization reserve in the ventricular myocardium.

In conclusion, inhibition of late $I_{Na}$ reversed the AP prolongation and arrhythmogenesis caused by inhibitors of multiple K$^+$ channels. Physiological, endogenous late $I_{Na}$ therefore appears to play an important role in controlling repolarization in normal rabbit hearts, and it may be an important modulator of arrhythmogenicity in the heart with reduced repolarization reserve. This finding may explain the observations that late $I_{Na}$ inhibitors (e.g., lidocaine, mexiletine, and ranolazine) were found to be anti-arrhythmic in clinical and experimental long QT syndromes (2, 18, 24–26, 32).

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**Disclosures**

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