Late Sodium Current Contributes to the Reverse Rate-Dependent Effect of $I_{Kr}$ Inhibition on Ventricular Repolarization

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**Background**—The reverse rate dependence (RRD) of actions of $I_{Kr}$-blocking drugs to increase the action potential duration (APD) and beat-to-beat variability of repolarization (BVR) of APD is proarrhythmic. We determined whether inhibition of endogenous, physiological late Na$^+$ current (late $I_{Na}$) attenuates the RRD and proarrhythmic effect of $I_{Kr}$ inhibition.

**Methods and Results**—Duration of the monophasic APD (MAPD) was measured from female rabbit hearts paced at cycle lengths from 400 to 2000 milliseconds, and BVR was calculated. In the absence of a drug, duration of monophasic action potential at 90% completion of repolarization (MAPD$_{90}$) and BVR increased as the cycle length was increased from 400 to 2000 milliseconds ($n=36$ and 26; $P<0.01$). Both E-4031 (20 nmol/L) and d-sotalol (10 μmol/L) increased MAPD$_{90}$ and BVR at all stimulation rates, and the increase was greater at slower than at faster pacing rates ($n=19, 11, 12$ and 7, respectively; $P<0.01$). Tetrodotoxin (1 μmol/L) and ranolazine significantly attenuated the RRD of MAPD$_{90}$, reduced BVR ($P<0.01$), and abolished torsade de pointes in hearts treated with either 20 nmol/L E-4031 or 10 μmol/L d-sotalol. Endogenous late $I_{Na}$ in cardiomyocytes stimulated at cycle lengths from 500 to 4000 milliseconds was greater at slower than at faster stimulation rates, and rapidly decreased during the first several beats at faster but not at slower rates ($n=8$; $P<0.01$). In a computational model, simulated RRD of APD caused by E-4031 and d-sotalol was attenuated when late $I_{Na}$ was inhibited.

**Conclusion**—Endogenous late $I_{Na}$ contributes to the RRD of $I_{Kr}$ inhibitor–induced increases in APD and BVR and to bradycardia-related ventricular arrhythmias. (Circulation. 2011;123:1713-1720.)

**Key Words:** action potentials ▪ arrhythmias, cardiac ▪ torsades de pointes ▪ electrophysiology ▪ ion channels ▪ long QT syndrome

**Clinical Perspective on p 1720**

The mechanisms underlying the RRD of physiological and drug-induced enhanced RRD have not been clearly identified. In mammalian hearts in physiological conditions, $I_{Kr}$ and $I_{Ks}$ are major determinants of APD, and $I_{Ks}$ is increased at fast heart rates. An increase in $I_{Ks}$ at faster heart rates due either to incomplete deactivation of current or to accumulation of extracellular K$^+$ is considered a possible mechanism of physiological RRD. In addition, the time and voltage dependencies of both drug binding and disassociation from channels are presumed to be causes of drug-induced enhance-

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ment of RRD. However, none of these mechanisms is fully supported by experimental and/or clinical evidence. Furthermore, an increased beat-to-beat variability of repolarization (BVR) is also associated with drug-induced ventricular tachycardia, and the mechanism responsible for the increased BVR and its rate dependency has not been identified.

The endogenous (or physiological), slowly inactivating component of the inward tetrodotoxin-sensitive Na$^+$ current (late INa) in the heart is normally small. This current is proarrhythmic when enhanced or when cardiac repolarization reserve is reduced by IKr blockers. To the best of our knowledge, the role of late INa in RRD of APD has not been investigated previously. We hypothesized that physiological late INa enhances the RRD and BVR of APD prolongation caused by IKr inhibitors in the heart, perhaps because the magnitude of late INa itself may be use dependent. In this study, the contribution of late INa to the RRD of the IKr inhibitors sotalol and E-4031 was determined in rabbit isolated hearts and myocytes. Tetrodotoxin (1 μmol/L) and ranolazine (10 μmol/L) were used to provide selective inhibition of INa relative to other ion currents and selective inhibition of late INa relative to peak INa, respectively. The contribution of late INa to RRD of APD was also simulated in a computational model of a rabbit ventricular myocyte AP.

**Methods**

**Female Rabbit Isolated Heart Model**

The use of animals in this investigation conformed to *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication No. 85–23). Animal use was approved by the Institutional Animal Care and Use Committee of Gilead Sciences (Palo Alto, CA) and by the Administration of Regulation of Laboratory Animals (Hubei Province, China). Hearts from New Zealand White female rabbits weighing 2.5 to 3.5 kg were isolated, perfused by the Langendorff method, and instrumented as previously described by Wu et al. Monophasic action potentials from the left ventricular epicardium and pseudo 12-lead ECGs were recorded. The atrioventricular nodal area was thermally ablated to produce complete atrioventricular block, and hearts were paced at increasing cycle lengths (CLs) of 400, 500, 667, 1000, and 2000 milliseconds for 3 to 4 minutes at each rate. After control (no drug) measurements were recorded, hearts were exposed to either 20 nmol/L E-4031 or 10% variability. Statistical Analyses

Data were plotted and analyzed with Prism version 5 (Graph Pad Software, San Diego, CA) and expressed as mean±SEM. The
significance of differences in measures before and after interventions in the same hearts was determined by repeated measures 1-way ANOVA followed by the Student-Newman-Keuls test. When treatment values were obtained at different rates from different groups of hearts, 2-way ANOVA of repeated measures was used. A paired or unpaired Student t test was used to determine the statistical difference between values of 2 means obtained from the same or different experiments, respectively.

**Results**

**Relationships Between Pacing Heart Rate and Monophasic Action Potential and Beat-to-Beat Variability of Repolarization in Control Hearts**

In the absence of drug (control), MAPD$_{90}$ and BVR were significantly increased in an RRD manner from 148.7 ± 1.67 and 0.20 ± 0.01 to 192.9 ± 3.1 and 0.31 ± 0.01 milliseconds (n = 36 and 26; P < 0.01; Figures 1 through 3, open symbols) as the pacing CL was prolonged from 400 to 2000 milliseconds.

Tetrodotoxin (1 μmol/L) alone did not change MAPD$_{90}$ or BVR at any tested pacing rate (n = 7 and 6; P > 0.05 vs control; Figure 1). The QRS interval was prolonged by 1 μmol/L tetrodotoxin by 2.4 ± 0.3 and 1.7 ± 0.5 milliseconds at CLs of 400 and 2000 milliseconds (n = 7; P < 0.05 vs control at the same rate; Figure 1A, inset).

**Reverse Rate-Dependent Effects of IK$_r$ Inhibitors to Increase Monophasic Action Potential and Beat-to-Beat Variability of Repolarization**

E-4031 (20 nmol/L) and d-sotalol (10 μmol/L) significantly prolonged MAPD$_{90}$ (n = 19 and 11; Figure 2) and increased BVR (n = 12 and 7; Figure 3) at all stimulation rates (P < 0.05 to 0.01), and the increase was greater at longer CL (2000 milliseconds; 26.2 ± 2.2 and 0.04 ± 0.02 milliseconds for E-4031, 17.3 ± 66.9 and 0.07 ± 0.02 for d-sotalol) than at shorter CL (400 milliseconds; 82.6 ± 9.2 and 0.56 ± 0.08 milliseconds for E-4031,
Reversal by Tetrodotoxin and Ranolazine of the Reverse Rate-Dependent Effects of I\textsubscript{K} Inhibitors

Tetrodotoxin (1 μmol/L) significantly (P<0.05) attenuated the rate-dependent increases in MAPD\textsubscript{90} and BVR in the presence of either E-4031 (n=9 and 7; Figures 2A and 3A) or d-sotalol (n=11 and 7; Figures 2C and 3C) by 36±10% and 68±17% and by 24±8% and 46±17% at a CL of 667 milliseconds (150 bpm) and by 63±5% and 75±7% and by 49±5% and 51±12%, at a CL of 2000 milliseconds (30 bpm), respectively.

The tetrodotoxin-sensitive component of MAPD and BVR was minimal in control hearts (Figures 2D and 3D), but was significantly increased in the presence of either E-4031 or d-sotalol, especially at the slower pacing rate of 30 bpm (Figures 2D and 3D). In the presence of 20 nmol/L E-4031, the late I\textsubscript{IK} inhibitor ranolazine (10 μmol/L) also attenuated the RRD of MAPD and BVR, especially at the longer pacing CLs (n=10 and 6; P<0.05; Figures 1B and 3B), despite the fact that ranolazine is also reported to block I\textsubscript{Kr} and late I\textsubscript{INa}.25

Values of the QT interval, Tpeak-Tend, and the index of diastolic interval, known as the restitution curve,26 is similar to the dependence of MAPD on the duration of the pacing cycle length (CL) was increased from 400 to 2000 milliseconds in the absence (control) to 0.158±0.017 and from 0.112±0.024 to 0.157±0.023, respectively (P<0.05; Figure 4). Tetrodotoxin (1 μmol/L) reduced the increases in MAPD and BVR in the continued presence of either E-4031 or d-sotalol to 35.7±4.7 and 0.22±0.08 milliseconds (P<0.05) and 28.0±2.2 and 0.08±0.02 milliseconds (P<0.05), respectively.

The plot of the dependence of MAPD on the duration of the diastolic interval, known as the restitution curve,26 is similar to the dependence of MAPD on CL that is shown in Figure 2. Inhibition of I\textsubscript{Kr} by either E-4031 or d-sotalol increased the steepness of the slope of the restitution curve from 0.103±0.021 (control) to 0.158±0.017 and from 0.112±0.024 to 0.157±0.023, respectively (P<0.05; Figure 4). Tetrodotoxin (1 μmol/L) flattened the restitution curves in the continuous presence of either E-4031 (Figure 4A) or d-sotalol (Figure 4B) and decreased the slope of the curves to 0.109±0.029 and 0.131±0.028, respectively (P<0.05).

Simulated Effect of Late I\textsubscript{INa} Inhibition on Reverse Rate Dependence of APD\textsubscript{90}

We used the Shannon-Bers rabbit ventricular AP model to recapitulate our MAPD\textsubscript{90} results from rabbit hearts. Repre-
sentative AP traces are shown in Figure 5D. The control APD was 252 milliseconds at a CL of 2000 milliseconds and 185 milliseconds at a CL of 400 milliseconds (Figure 5A, open circles). The rate-dependent properties of late $I_{Na}$ contributed to APD adaptation in that the simulated current amplitude was larger at slow versus fast pacing rates (not shown). When the effect of tetrodotoxin was simulated by $G_{Na,L}$ reductions was larger at slow versus fast pacing rates (not shown). When the effect of tetrodotoxin was simulated by $G_{Na,L}$ reductions was larger at slow versus fast pacing rates (not shown). When the effect of tetrodotoxin was simulated by $G_{Na,L}$ reductions was larger at slow versus fast pacing rates (not shown). When the effect of tetrodotoxin was simulated by $G_{Na,L}$ reductions was larger at slow versus fast pacing rates (not shown). When the effect of tetrodotoxin was simulated by $G_{Na,L}$ reductions was larger at slow versus fast pacing rates (not shown). When the effect of tetrodotoxin was simulated by $G_{Na,L}$ reductions was larger at slow versus fast pacing rates (not shown).

$G_{Na,L}$ was decreased (Figure 5). APD adaptation induced by $G_{Kr}$ reduction was more pronounced at longer CLs (1000 and 2000 milliseconds), especially when simulating complete late $I_{Na}$ block by 20 nmol/L E-4031. Tetrodotoxin shortened APD$_{90}$ from pulses 1, 2, 5, 10, and 20. The depolarization pulse protocol to elicit late $I_{Na}$ is shown in the inset.

Figure 6. Representative recordings of the rate (2.0, 1.5, 1.0, 0.5, and 0.25 Hz) dependent changes in late Na$^+$ current (late $I_{Na}$) in a single rabbit ventricular myocyte. Traces of $I_{Na}$ at different stimulation cycle lengths (CL; in milliseconds) in the absence (control; A) and presence (1 μmol/L in B; 4 μmol/L in C) of tetrodotoxin (TTX) from pulses 1, 2, 5, 10, and 20. The depolarization pulse protocol to elicit late $I_{Na}$ is shown in the inset.

**Inhibition of Endogenous Late $I_{Na}$ Abolished Slow Rate–Related Torsades de Pointes in the Presence of E-4031 and d-Sotalol**

Early afterdepolarizations, extraventricular beats, and episodes of torsade de pointes were observed when the CL was increased from 400 to 2000 milliseconds. Similar arrhythmic activities were observed in 6 of 11 hearts (55%) treated with d-sotalol. These results are consistent with a previous report of the proarrhythmic effects of QT-prolonging drugs. Early afterdepolarizations, extraventricular beats, and episodes of torsade de pointes caused by 10 nmol/L E-4031 were abolished by 1 μmol/L tetrodotoxin and 10 μmol/L ranolazine in 5 of 6 hearts (83%; Figure 2B, inset) and 8 of 8 hearts (100%; Figure 2B, inset), respectively. Similarly, 1 μmol/L tetrodotoxin abolished torsade de pointes caused by 10 μmol/L d-sotalol in 5 of 6 hearts (83%; Figure 2C, inset).

**Effect of Stimulation Rate on Magnitude of Endogenous Late $I_{Na}$ in Rabbit Ventricular Myocytes**

The magnitude of late $I_{Na}$ in rabbit isolated cardiomyocytes decreased with increasing stimulation rate (CL from 4000 to 500 milliseconds) and with use (ie, from first pulse to the 20th pulse; see representative traces in Figure 6 and summary data in Figure 7). The amplitude of late $I_{Na}$ recorded during the first pulse of each pulse train was similar: 48.1±3.8, 48.2±3.8, 48.1±4.3, 48.2±4.3, and 47.9±4.1 pA (n=8; P=NS; Figures 7 and 8). Tetrodotoxin (1 and 4 μmol/L) caused a significant decrease in late $I_{Na}$ at all stimulation rates as the rate–late $I_{Na}$ response relationship was shifted down in parallel (P<0.01 for each rate; Figures 6 through 8). The steady-state amplitudes of late $I_{Na}$ recorded from cells paced at 2.0, 1.5, 1.0, 0.5, and 0.25 Hz were $-18.8±3.9$, $-24.3±3.8$, $-32.0±4.2$, and $-41.4±4.4$ pA, respectively (n=8; P<0.05 to 0.01). Tetrodotoxin at concentrations of 1 and 4 μmol/L attenuated late $I_{Na}$ at all stimulating rates (Figures 6 through 8).

The amplitude of late $I_{Na}$ during a train of 20 pulses decreased from the first to the 20th pulse; the decrease was
greater when the stimulation rate was increased (see Figure 8D for summary data). Compared with pulse 1, the amplitude of late INa at pulse 20 was significantly decreased to 19.8 ± 4.0 (Figure 7A), 22.6 ± 4.0 (Figure 7B), 25.2 ± 4.4 pA (Figure 7C), and 32.4 ± 4.4 pA (Figure 7D) at CLs of 500, 667, 1000, and 2000 Hz, respectively (n = 8; P < 0.05), but did not change at CL of 4000 milliseconds (42.5 ± 4.6 pA; n = 8; P = NS; Figure 7E). A higher rate of stimulation was associated with a faster decrease in late INa. The greatest decrease in late INa occurred during the first few pulses at each rate (Figure 7).

Discussion

The findings in this study suggest that physiological late INa may play an important role in the enhanced RRD of APD and BVR caused by QT-prolonging agents that inhibit IKr. RRD of MAPD and BVR was demonstrated with female rabbit isolated hearts, and RRD of APD was simulated in a computer model. In the continuous presence of IKr inhibitors, tetrodotoxin (1 μmol/L) and ranolazine (10 μmol/L) reduced the RRD of MAPD and BVR induced by IKr inhibitors and abolished slow rate–related episodes of torsade de pointes in the presence of IKr inhibitors. Indeed, the amplitude of endogenous late INa was larger at slower than faster heart rates. Because tetrodotoxin and ranolazine are known to block cardiac late INa (NaV1.5) at concentrations of 1 and 10 μmol/L, respectively,19,20 these results suggest that inhibition of late INa may diminish the RRD of the APD/QT interval prolongation and BVR and therefore may antagonize the slow rate– and pause–triggered ventricular arrhythmias that may be caused by disease– or drug–induced inhibition of IKr.
Reverse rate dependence is an implicit property of the heart that reflects the underlying rate dependence of individual cardiac ion channel currents,5,27 and thus their contribution to membrane resistance during the AP plateau. In this study, the magnitude of late $I_{Na}$ was also found to be RRD. Thus, reduction of the RRD inward late $I_{Na}$ by tetrodotoxin or ranolazine offset the reduction of outward $K^{+}$ current by RRD $I_{Kr}$-blocking drugs. Inhibition of late $I_{Na}$ by either 1 $\mu$mol/L tetrodotoxin or 10 $\mu$mol/L ranolazine reduced the RRD of MAPD in hearts treated with the $I_{Kr}$ blockers E-4031 and d-sotalol (Figures 2 and 3). Simulated data from a computer model of rabbit heart electric activity (including changes in intracellular Na$^{+}$, Ca$^{2+}$, and late $I_{Na}$) recapitulated the RRD of APD in the presence of $I_{Kr}$ blockers and predicted that a reduction in $I_{Kr}$ accentuates the RRD of APD. Thus, both experimental data and model predictions provide evidence that the RRD of effects of E-4031 and d-sotalol were reduced when late $I_{Na}$ was inhibited and that the contribution of late $I_{Na}$ to prolongation of ventricular repolarization was greater at slower heart rates. The latter result is consistent with a previous study of LQT3 AKPQ mutant Na$^{+}$ channels expressed in HEK293 cells, wherein it was reported that the amplitude of $\Delta$APD late $I_{Na}$ was strongly rate dependent.28

Increased BVR is proarrhythmic and has been associated with interventions that either block $I_{Kr}$ or augment late $I_{Na}$, especially at slower heart rates.15 Late $I_{Na}$ blockade with 1 $\mu$mol/L tetrodotoxin did not significantly alter baseline RRD of BVR in rabbit hearts but attenuated the increased RRD of BVR during administration of E-4031 and d-sotalol. Although inhibition of physiological late $I_{Na}$ by tetrodotoxin and lidocaine is sufficient to shorten APD in normal cardiac tissue,19,20,29 our findings suggest that late $I_{Na}$ contributes more to BVR when repolarization reserve is reduced and the net transmembrane current is small (ie, during the long AP plateau in the presence of an $I_{Kr}$ inhibitor) and support previous reports that endogenous late $I_{Na}$ is proarrhythmic in hearts with reduced repolarization reserve.16–20 We speculate that a reduction in a depolarizing current during the AP plateau shortens the vulnerable phase, in which stochastic fluctuations in ion channel or transporter currents or local Ca$^{2+}$ concentrations can have a significant impact on the duration of ventricular repolarization.15 Indeed, Ca$^{2+}$-dependent mechanisms are thought to underlie BVR because exogenous intracellular Ca$^{2+}$ buffering suppresses it.30

The increased density of late $I_{Na}$ recorded from rabbit cardiomyocytes paced at slow versus fast rates is consistent with the RRD of MAPD and BVR in isolated hearts and in the computational model.17 The increase in late $I_{Na}$ at slower heart rates may be attributed to decreased inactivation of Na$^{+}$ channels at these slow rates,30 because the greatest decrease in late $I_{Na}$ was found during the first several beats when the pacing rate was increased (Figure 7). In the computer model, APD adaptation (ie, shortening) to an increase in pacing rate was linked to increased intracellular Na$^{+}$ accumulation at fast rates and its consequences to increased outward Na$^{+}$ pump and NCX currents.27,31 A potential positive feedback between increased late $I_{Na}$ and reduced $I_{Kr}$ can explain the effect of an enhanced late $I_{Na}$ to cause arrhythmias when repolarization reserve is reduced.19 An increase in late $I_{Na}$ may also cause changes in Ca$^{2+}$ handling that alter APD adaptation by multiple mechanisms.20,27,32

The observation that late $I_{Na}$ plays an important role in the enhanced RRD of $I_{Kr}$ inhibitors may explain previous experimental and clinical findings that RRD is greater in Purkinje fibers and midmyocardial cells, where the late $I_{Na}$ is greater than in epicardial cells,33 and that $I_{Kr}$-blocking drugs such as amiodarone and ranolazine that also block late $I_{Na}$ have no RRD and no or minimal proarrhythmic risk.16,17,34 On the other hand, because of the synergistic effect of increased late $I_{Na}$ and inhibition of $I_{Kr}$ to increase APD and BVR,17,35 an increase in late $I_{Na}$ as occurs in the ischemic heart and other structure heart diseases,36 can potentiate the proarrhythmic effect of an $I_{Kr}$ blocker.

Conclusions

Endogenous physiological late $I_{Na}$ plays an important role in the RRD of APD and BVR caused by $I_{Kr}$ inhibitors. Inhibition of endogenous late $I_{Na}$ may diminish the rate-dependent prolongation of the APD/QT interval by either drugs or pathological conditions that decrease $I_{Kr}$, and may decrease the occurrence of slow rate- or pause-triggered cardiac arrhythmias. If late $I_{Na}$ were to be exacerbated by pathological conditions, it is possible that its impact to increase the RRD of $I_{Kr}$ blocking drugs would also be augmented.

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Disclosures

Dr Wu, Li, Shroyock, and Belardinelli are employees of Gilead Sciences. Dr Ma received a research grant from Gilead. The other authors report no conflicts.

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**CLINICAL PERSPECTIVE**

The reverse rate-dependent effect of drugs, especially those that inhibit I_{Ks} to prolong ventricular repolarization, has long been recognized as an important proarrhythmic risk factor. We hypothesize that inhibition of the small physiological late Na\(^+\) current (late I_{Na}) will reduce reverse rate dependence associated with I_{Kr}-blocking drugs. Late I_{Na} is greater, and reverse rate dependence of APD/QT interval is prominent in patients with structural heart diseases (heart failure, myocardial ischemia, etc), especially after treatment with an I_{Kr}-inhibiting drug. In this study, the amplitude of endogenous or physiological late I_{Na} in myocytes was increased as the frequency of stimulation slowed. Inhibition of late I_{Na} by tetrodotoxin or ranolazine diminished the reverse rate dependence of action potential duration prolongation and beat-to-beat variability of repolarization caused by I_{Kr} inhibitors in isolated hearts. Results of computer simulations of the effect of I_{Na} block in the absence and presence of late I_{Na} block were consistent with the results of the experimental studies. The findings can explain, at least in part, why drugs that inhibit both I_{Kr} and late I_{Na} (ie, amiodarone and ranolazine) have no reverse rate-dependent effect and little or no proarrhythmic activity, whereas proarrhythmic activity associated with more selective I_{Kr} blockers is exacerbated when late I_{Na} is increased. This concept may be used to explain the occurrence of slow rate– or pause-triggered cardiac arrhythmias, and may be relevant in the choice of treatment drug(s) in patients with compromised repolarization reserve resulting from increased late I_{Na} or decreased I_{Kr}, such as patients with heart failure.
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