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Late sodium current contributes to the reverse rate-dependent effect of I_{Kr} inhibition on ventricular repolarization

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Abstract

Background—The reverse rate dependence (RRD) of actions of I_{Kr}-blocking drugs to increase action potential duration (APD) and beat-to-beat variability (BVR) of APD is proarrhythmic. Therefore we determined if 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 action potential (MAPD) was measured from female rabbit hearts paced at cycle lengths from 400 to 2000 ms and BVR was calculated. In the absence of drug, $MAPD_{90}$ and BVR increased as the cycle length was increased from 400 to 2000 ms (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 and 11, 12 and 7, respectively, $p<0.01$). TTX (1 μmol/L) significantly attenuated the RRD of $MAPD_{90}$, reduced BVR, ($p<0.01$), and abolished torsade de pointes (TdP) in 5 of 6 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 ms was greater at slower than at faster stimulation rates, and rapidly decreased during the first several beats at faster but not at slower rates ($p<0.01$, n=8). In a computational model, simulated RRD of APD caused by E-4031 and d-sotalol was attenuated when late I_{Na} was inhibited.

Conclusions—Endogenous late I_{Na} contributes to the RRD of I_{Kr} inhibitor-induced increases in APD and BVR and to bradycardia-related ventricular arrhythmias.

Keywords

Late sodium current; reverse rate dependence; action potential duration; beat-to-beat variability; rabbit heart

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Introduction

Reverse rate (or frequency or use)-dependence (RRD) of action potential duration (APD) is an adaptive property of the normal heart wherein the APD shortens as heart rate increases.[1] Drug effects may also have a reverse rate-dependency, i.e., the effect of drug to prolong APD may be greater at slow vs. fast heart rates. Drug RRD is associated with ventricular proarrhythmic activity.[1, 2] Inhibitions of rapidly-activating delayed rectifier K^+ current (I_{Kr}) or inward rectifier K^+ current (I_{K1}),[3] and enhancement of L-type Ca^{2+}-inward current (I_{CaL})[4] have been reported to prolong APD in a RRD manner,[5, 6] whereas inhibition of I_{Ks} by chromanol 293B prolonged APD in a frequency-independent manner.[3, 7]. RRD of APD caused by drugs that inhibit I_{Kr} is considered to be an undesired response because it results in a reduction of drug efficacy during tachycardia and may lead to proarrhythmic activity when the tachycardia is terminated. [1,8]

The mechanisms underlying the RRD of physiological and drug-induced enhanced RRD have not been clearly identified.[5] 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.[9] 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.[10-12] Also, the time- and voltage-dependencies of both drug binding and disassociation from channels are presumed to be causes of drug-induced enhancement of RRD.[13, 14] However, none of these mechanisms is fully supported by experimental and/or clinical evidence.[5] Furthermore, an increased beat-to-beat variability of repolarization (BVR) is also associated with drug-induced ventricular tachycardia[15-19] and the mechanism responsible for the increased BVR and its rate dependency have not been identified.

The endogenous (or physiological) slowly-inactivating component of the inward tetrodotoxin (TTX)-sensitive Na^+ current (late I_{Na}) in the heart is normally small.[20] This current is proarrhythmic when enhanced or when cardiac repolarization reserve is reduced by I_{Kr} blockers.[16-20] To our knowledge, the role of late I_{Na} in RRD of APD has not been previously investigated. We hypothesized that physiological late I_{Na} enhances the RRD and BVR of APD prolongation caused by I_{Kr} inhibitors in the heart, perhaps because the magnitude of late I_{Na} itself may be use-dependent.[20] In this study, the contribution of late I_{Na} to RRD of the I_{Kr} inhibitors sotalol and E-4031 was determined in rabbit isolated hearts and myocytes. TTX (1 μmol/L) and ranolazine (10 μmol/L) were used to provide selective inhibition of I_{Na} relative to other ion currents, and selective inhibition of late I_{Na} relative to peak I_{Na}, respectively.[19] The contribution of late I_{Na} 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” (NIH publication No. 85-23). Animal use was approved by the Institutional Animal Care and Use Committee of Gilead Sciences (Palo Alto, CA, USA) and by the Administration of Regulation of Laboratory Animals (Hubei Province, China). Hearts

from New Zealand White female rabbits weighing 2.5-3.5 kg were isolated, perfused by the method of Langendorff, and instrumented as previously described by Wu et al.[17] Monophasic action potentials (MAPs) from the left ventricular epicardium and pseudo 12-lead electrocardiograms (ECGs) were recorded. The AV 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 ms for 3-4 minutes at each rate. After control (no drug) measurements were recorded, hearts were exposed to either 20 nmol/L E-4031 or 10 μmol/L d-sotalol in the absence and presence of 1 μmol/L TTX, allowing 20 min between increases in drug concentration to record a steady-state effect. The stimulation protocol at CL from 400 to 2000 ms was repeated before (control) and after each drug treatment.

BVR was determined by analysis of 30 consecutive stimulated beats using the following equation: $\Sigma|MAPD_{n+1}\text{-}MAPD_n|/[30\times\sqrt{2}]$, as described by Wu et al.[16-18] The slope of the restitution curves was calculated based on the fast slope portion (at stimulation CL of 400, 500 and 667 ms) of the relationship between the $MAPD_{90}$ and diastolic interval.

Whole cell patch clamp study

Rabbit left ventricular cardiomyocytes were isolated enzymatically as prescribed previously.[21] Only cardiomyocytes with smooth and glossy edges and surfaces, clear transverse striations, and that could be stably voltage-clamped for 8 min without significant contraction were used. Seal resistance was above 1GΩ with less than 10 % variability. Currents were obtained with a patch-clamp amplifier (EPC-10, Heka Electronic, Lambrecht, Pfalz, Germany), filtered at 1 kHz and digitized at 10 kHz. During recording of late I_{Na}, the bath solution contained (in mmol/L):135 NaCl, 5.4 $CsCl_2$, 1.8 $CaCl_2$, 1 $MgCl_2$, 0.3 $BaCl_2$, 0.33 NaH_2PO_4, 10 glucose, 10 HEPES, 0.001 nicardipine, pH 7.3. The patch pipette solution contained (in mmol/L):120 $CsCl_2$, 1.0 $CaCl_2$, 5 $MgCl_2$, 5 Na_2ATP, 10 TEACl, 11 EGTA, 10 HEPES (pH 7.3, adjusted with CsOH). All experiments were carried out at a temperature of 23±1 °C.

To elicit a late I_{Na} in a single cell, twenty 300-ms depolarizing pulses to -20 mV from a holding potential of -90 mV were applied at CLs of 500, 667, 1000, 2000 and 4000 ms using a serial repeated-measurement protocol, with 3 min between each change of stimulation CL, during which membrane potential was held at -90 mV. In cardiomyocytes isolated from 8 rabbit hearts, the amplitude of late I_{Na} was measured at 200 ms after initiation of the depolarization step before (control, no drug) and 3 min after exposure of a myocyte to 1 and 4 μmol/L TTX, respectively.

Rabbit ventricular AP simulations

The rabbit ventricular AP was simulated using the Shannon-Bers model.[2223] Simulation of the late I_{Na} was added to the original model using a Hodgkin-Huxley formalism as done by Hund et al.[24]

$$I_{NaL}=\overline{G}_{NaL}\cdot m_L^3\cdot h_L\cdot(V-E_{Na})$$

Late I_{Na} activation (gating variable m) mimics the activation of the fast I_{Na}, whereas inactivation (gating variable h) was formulated as follow:

$$h_{L,\infty} = \frac{1}{1+exp((V_m+91)/6.1)}$$
$$\tau_{hL}=600ms$$

Late I_{Na} maximal conductance ($G_{Na,L}$) was set to 0.012 mS/μF (i.e., 0.075% of the fast I_{Na} conductance) to simulate a small endogenous late I_{Na} in rabbit ventricular myocytes. Model differential equations were implemented in Matlab (Mathworks Inc., Natick, MA, U.S.A.) and solved numerically using a variable order solver (ode15s). The digital cell was stimulated with a current pulse (9.5 A/F, 5 ms) at various CLs from 400 to 2000 ms, and APD was measured as the interval between AP upstroke and 90% repolarization level (APD_{90}).

Based on the results of measurements of late I_{Na} in rabbit cardiomyocytes, applications of 1 and 4 μmol/L TTX were simulated by reducing G_{NaL} by 47 and 100 %, respectively. G_{Kr} was reduced by 55% to simulate the effect of 20 nmol/L E-4031 and by 45% to simulate the effect of 10 μmol/L d-sotalol application.

Statistical analyses

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

Results

Relationships between pacing heart rate and MAPD or BVR in control hearts

In the absence of drug (control), $MAPD_{90}$ and BVR were significantly increased in a reverse rate-dependent manner from 148.7±1.67 and 0.20±0.01 to 192.9±3.1 and 0.31±0.01 ms (n=36 and 26, $p<0.01$, Figs. 1-3, open symbols) as the pacing CL was prolonged from 400 ms to 2000 ms.

TTX (1 μmol/L) alone did not change $MAPD_{90}$ or BVR at any tested pacing rate (n=7 and 6, compared to control, $p>0.05$, Fig. 1). The QRS interval was prolonged by 1 μmol/L TTX by 2.4±0.3 and 1.7±0.5 ms at CL of 400 and 2,000 ms (n=7, compared to control at same rate, $p<0.05$, inset in Fig. 1A).

RRD effects of I_{Kr} inhibitors to increase MAPD and BVR

E-4031 (20 nmol/L) and d-sotalol (10 μmol/L) significantly prolonged $MAPD_{90}$ (n=19 and 11, Fig. 2) and increased BVR (n=12 and 7, Fig. 3) at all stimulation rates ($p<0.05$-0.01) and

the increase was greater at longer (2000 ms, 26.2±2.2 and 0.04±0.02 ms for E-4031, 17.3±66.9 and 0.07±0.02 for d-sotalol) than at shorter CLs (400 ms, 82.6±9.2 and 0.56±0.08 ms for E-4031, 66.9±9.2 and 0.19±0.02 for d-sotalol, $p<0.01$); i.e., the actions of E-4031 and d-sotalol on MAPD and BVR were reverse rate-dependent.

Reversal by TTX and ranolazine of the RRD effects of I_K inhibitors

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

The TTX-sensitive component of MAPD and BVR was minimal in control hearts (Figs. 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 (Figs. 2D and 3D).

In the presence of 20 nmol/L E-4031, the late I_{Na} inhibitor ranolazine (Ran, 10 μmol/L) also attenuated the RRD of MAPD and BVR, especially at the longer pacing CLs (n=10 and 6, $p<0.05$, Figs. 1B and 3B), in spite of the fact that ranolazine is also reported to block I_{Kr} as well as late I_{Na}.[25]

Values of QT interval, Tpeak-Tend, and the index of Tpeak-Tend/QT interval had similar RRD as did MAPD and BVR (supplemental figure), and the effects of TTX and ranolazine on these parameters were also similar to the effects of TTX and ranolazine on the rate dependence of MAPD (supplemental figure).

When CL was increased from 500 to 1000 ms (i.e., when stimulation rate was decreased for 120 to 60 beats per minute), MAPD and BVR were increased by 20.6±1.2 (n=36) and 0.04±0.01 ms (n=26, $p<0.01$) in normal hearts (no drug). In the presence of E-4031 and d-sotalol, MAPD and BVR were further increased to 45.0±4.7 and 0.22±0.04 ms (n=19 and 12, respectively, $p<0.01$), and 38.1±2.4 and 0.11±0.02 ms (n=11 and 7, $p<0.05$), respectively. TTX (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 ms ($p<0.05$), and 28.0±2.2 and 0.08±0.02 ms ($p<0.05$), respectively.

The plot of the dependence of MAPD on the duration of the diastolic interval is known as the restitution curve,[26] and is similar to the dependence of MAPD on CL that is shown in Fig. 2. Inhibition of I_{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 ($p<0.05$, Fig. 4). TTX (1 μmol/L) flattened the restitution curves in the continuous presence of either E-4031 (Fig. 4A) or d-sotalol (Fig. 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_{Na} inhibition on RRD of APD_{90}

We used the Shannon-Bers rabbit ventricular AP model to recapitulate our $MAPD_{90}$ results from rabbit hearts. Representative AP traces are shown in Figure 5D. The control APD was

252 ms at a CL of 2000 ms, and 185 ms at a CL of 400 ms (Fig 5A, open circles). The rate-dependent properties of late l_{Na} contributed to APD adaptation, as the simulated current amplitude was larger at slow vs. fast pacing rates (not shown). When the effect of TTX was simulated by $G_{Na,L}$ reductions of 47 % (1 μmol/L TTX) and 100 % (4 μmol/L TTX), APD_{90} was decreased (Fig 5). APD adaptation to change of stimulation rate in the absence and presence of 1 μmol/L TTX was not markedly different (APD_{90} shortened by ~23 and ~27% with and without TTX respectively, when decreasing the CL from 2000 to 400 ms). TTX shortened APD_{90} more prominently at longer CLs (1000 and 2000 ms), especially when simulating complete late I_{Na} block by 4 μmol/L TTX (Fig. 5A).

Simulation of I_{Kr} block by E-4031 predicted a marked prolongation of the rabbit ventricular AP showing clear RRD (Fig. 5B, squares), being larger at 2000 than at 400 ms CL (100 vs. 32 ms). When late I_{Na} inhibition was simultaneously simulated, the model predicted a reduction of RRD in the presence of E-4031 (Fig. 5B, closed circles). Similar results were found when I_{Kr} block by d-sotalol was simulated (Fig. 5C), i.e., APD prolongation induced by G_{Kr} reduction was more pronounced at slower than at faster heart rates, and this effect was markedly reduced when 47 and 100 % block of late I_{Na} was simulated.

Inhibition of endogenous late I_{Na} abolished slow rate-related TdP in the presence of E-4031 and d-sotalol

Neither early afterdepolarizations (EAD), extra-ventricular beats (EVB) nor TdP was observed in 28 control hearts at any tested pacing rate. In contrast, in 15 of 17 (88 %) hearts treated with 20 nmol/L E-4031, EAD, frequent EVBs and episodes of TdP were observed when CL was increased from 400 to 2000 ms. Similar arrhythmic activities were observed 6 of 11 (55 %) hearts treated with d-sotalol. These results are consistent with a previous report of the proarrhythmic effects of QT prolonging drugs. EADs, EVBs and episodes of TdP caused by 20 nmol/L E-4031 were abolished by 1 μmol/L TTX and 10 μmol/L ranolazine in 5 of 6 (83 % Fig. 2A, inset) and 8 of 8 (100 %, Fig. 2B, inset) hearts, respectively. Similarly, 1 μmol/L TTX abolished TdP caused by 10 μmol/L d-sotalol in 5 of 6 hearts (83 %, Fig. 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 ms) and with use (i.e., from first pulse to 20^{th} pulse) (see representative traces in Fig. 6 and summary data in Fig. 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, Figs.7, 8). TTX (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, Figs. 6-8). 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, $p<0.05$-0.01). TTX at concentrations of 1 and 4 μmol/L attenuated late I_{Na} at all stimulating rates (Figs. 6-8).

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

Discussion

The findings in this study suggest that physiological late I_{Na} may play an important role in the enhanced RRD of APD and BVR caused by QT-prolonging agents that inhibit I_{Kr}. RRD of MAPD and BVR were demonstrated using female rabbit isolated hearts, and RRD of APD was simulated in a computer model. In the continuous presence of I_{Kr} inhibitors, TTX (1 μmol/L) and ranolazine (10 μmol/L) reduced the RRD of MAPD and BVR induced by I_{Kr} inhibitors, and abolished slow rate-related episodes of TdP in the presence of I_{Kr} inhibitors. Indeed, the amplitude of endogenous late I_{Na} was larger at slower than faster heart rates. Because TTX and ranolazine are known to block cardiac late I_{Na} (NaV1.5) at concentrations of 1 and 10 μmol/L, respectively, [19, 20] these results suggest that inhibition of late I_{Na} 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 I_{Kr}.

RRD 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 reverse rate-dependent. Thus, reduction of the reverse rate-dependent inward late I_{Na} by TTX or ranolazine "offset" reduction of outward K^+ current by reverse rate-dependent I_{Kr}-blocking drugs. Inhibition of late I_{Na} by either 1 μmol/L TTX or 10 μmol/L ranolazine reduced the RRD of MAPD in hearts treated with the I_{Kr} blockers E-4031 and d-sotalol (Figs. 2, 3). Simulated data from a computer model of rabbit heart electrical 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 of 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 ΔKPQ mutant Na^+ channels expressed in HEK293 cells, wherein it was reported that the amplitude of ΔKPQ late I_{Na} was strongly rate-dependent.[28]

Increased BVR is proarrhythmic and has been associated with interventions that either block I_K or augment late I_{Na}, especially at slower heart rates.[15] Late I_{Na} blockade with 1 μmol/L TTX 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 TTX 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 (i.e., 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, 18, 20] We speculate that reduction of 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 significant impact on the duration of ventricular repolarization.[15] Indeed, Ca^{2+}-dependent mechanisms are thought to underlie BVR, as exogenous intracellular Ca^{2+} buffering suppresses it. [30]

The increased density of late I_{Na} recorded from rabbit cardiomyocytes paced at slow vs. 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] as the greatest decrease in late I_{Na} was found during the first several beats when the pacing rate was increased (Fig. 7). In the computer model, APD adaptation (i.e., shortening) to an increase of pacing rate was linked to increased intracellular Na^+ accumulation at fast rates, and its consequences to increase 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 mid-myocardial 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, due to the synergistic effect of increased late I_{Na} and inhibition of I_{Kr} to increase APD and BVR,[17, 35] an increase of 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.

Conclusion

Endogenous physiological late I_{Na} plays an important role in the RRD of APD and BVR caused by I_K 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 it's impact to increase the RRD of I_{Kr} blocking drugs would also be augmented.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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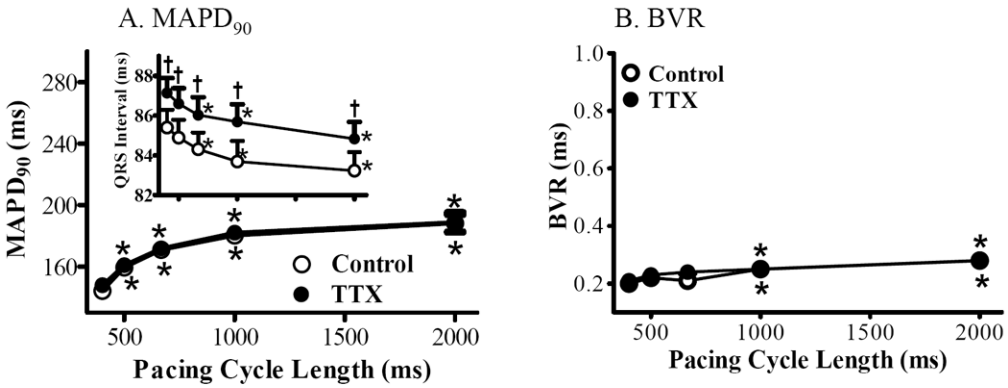

Figure 1.

Rate-dependent changes of MAPD and BVR in absence (control, ○) and presence of 1 μmol/L TTX (●). $MAPD_{90}$ (A), BVR (B) and QRS interval (A, inset) were measured from rabbit isolated hearts paced at CLs of 400, 500, 667, 1000 and 2000 ms, respectively. *, $p<0.05$ compared with values at pacing CL of 400 ms, $p<0.05$. †, compared with control at same pacing CL, $p<0.05$.

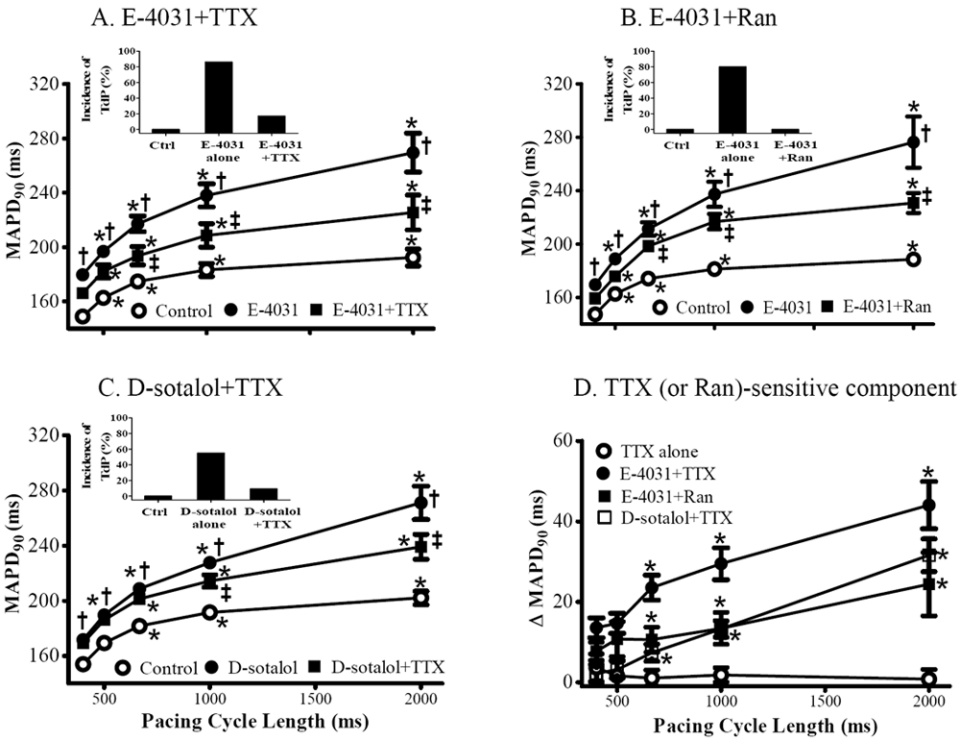

Figure 2.

Effect of TTX and ranolazine (Ran) on the reverse rate-dependent changes of left ventricular epicardial $MAPD_{90}$ caused by I_{Kr} inhibitors. Values of $MAPD_{90}$ were measured in the absence (control, ○) and presence of either E-4031 (20 nmol/L, ●), E-4031 plus TTX (1 μmol/L, ■, A), E-4031 plus Ran (10 μmol/L, B), or d-sotalol (10 μmol/L) plus TTX (C). Each point indicates a mean of 7, 9 and 11 hearts, respectively. The TTX (or Ran) sensitive component of $MAPD_{90}$ prolongation by each intervention is plotted in panel D. Insets represent the incidence of torsade de pointes in control, E-4031 or d-sotalol in absence and presence of TTX or Ran. *, compared to CL of 400 ms, $p<0.05$-0.001; †, Compared to control at the same CL; $p<0.05$-0.001; ‡, compared to either E-4031 (A and B) or d-sotalol (C) alone at the same CL, $p<0.05$-0.001.

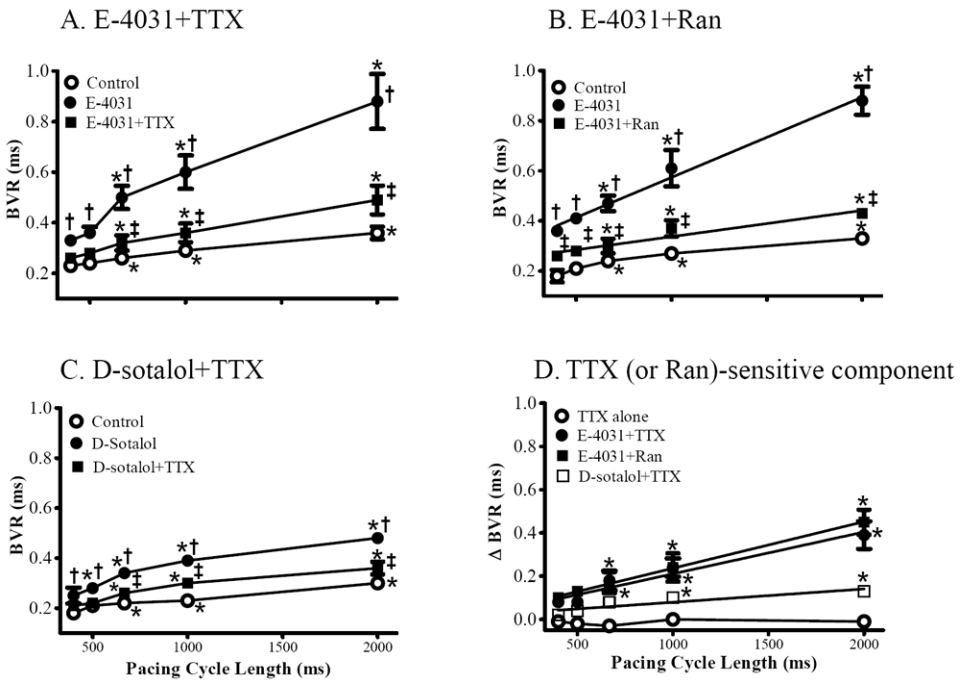

Figure 3.

Effects of TTX and ranolazine (Ran) on the reverse rate-dependent changes of BVR of left ventricular MAPD induced by I_{Kr} inhibitors. BVR was calculated in absence (control, ○) and presence of either E-4031 (20 nmol/L, ●), E-4031 plus TTX (1 μmol/L, ■, A), E-4031 plus Ran (10 μmol/L, B), or d-sotalol (10 μmol/L) plus TTX (C). Each point indicates a mean of 4, 7 and 11 hearts, respectively. The TTX (or Ran) sensitive components of BVR are plotted in panel D. *, compared to CL of 400 ms, $p<0.05$-0.001, $p<0.05$-0.001; †, Compared to control at the same CL; $p<0.05$-0.001; ‡, compared to either E-4031 (A and B) or d-sotalol (C) at same CL, $p<0.05$-0.001.

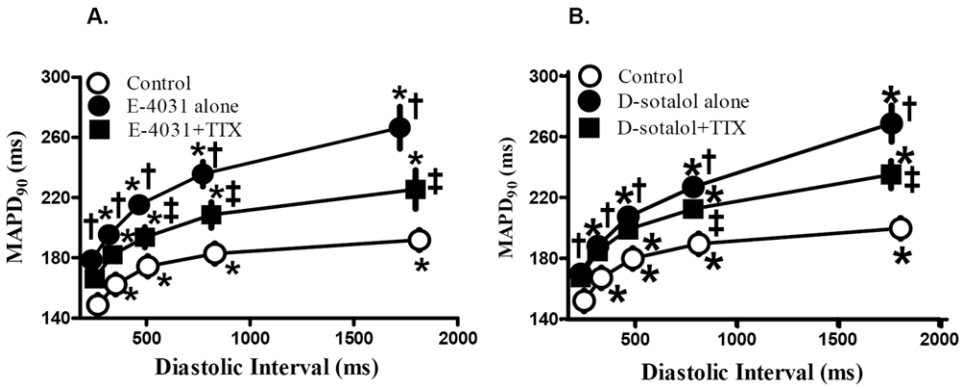

Figure 4.

MAPD restitution curve in control (no drug) and in presence of either drug alone (E-4031 in A or d-sotalol in B), or drug plus 1 μmol/L TTX. *, Compared with CL of 400 ms, $p<0.05$; †, Compared to control at the same CL; $p<0.05$; ‡, compared to either E-4031 (A and B) or d-sotalol (C) at same CL, $p<0.05$.

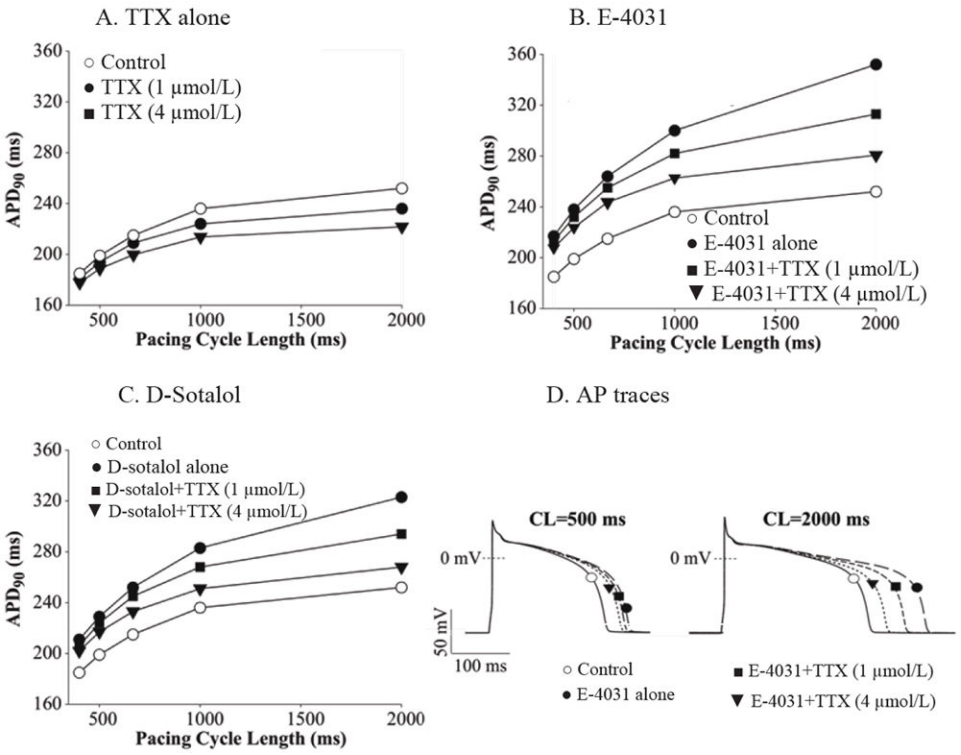

Figure 5.

Simulated effect of TTX on the reverse rate-dependent changes of rabbit action potential duration (APD_{90}). A, APD_{90} prolongation as pacing CL was increased from 400 to 2000 ms in absence (open circles) and presence of TTX (1 and 4 μmol/L). B. RRD of APD_{90}, predicted when reducing I_{Kr} conductance to mimic E-4031 administration (solid circles), was greatly attenuated when late I_{Na} was inhibited by TTX (1 and 4 μmol/L, solid symbols). C. RRD of APD_{90} predicted when reducing I_{Kr} conductance to mimic d-sotalol administration (solid circles) was reduced or nearly abolished when late I_{Na} was inhibited by 1 and 4 μmol/L TTX (solid symbols). D. Representative AP traces obtained when a digital cell was paced at CLs of 500 ms (left) and 2000 ms (right) in control, in presence of I_{Kr} block E-4031 alone, and in presence of simultaneous inhibitions of I_{Kr} and late I_{Na}.

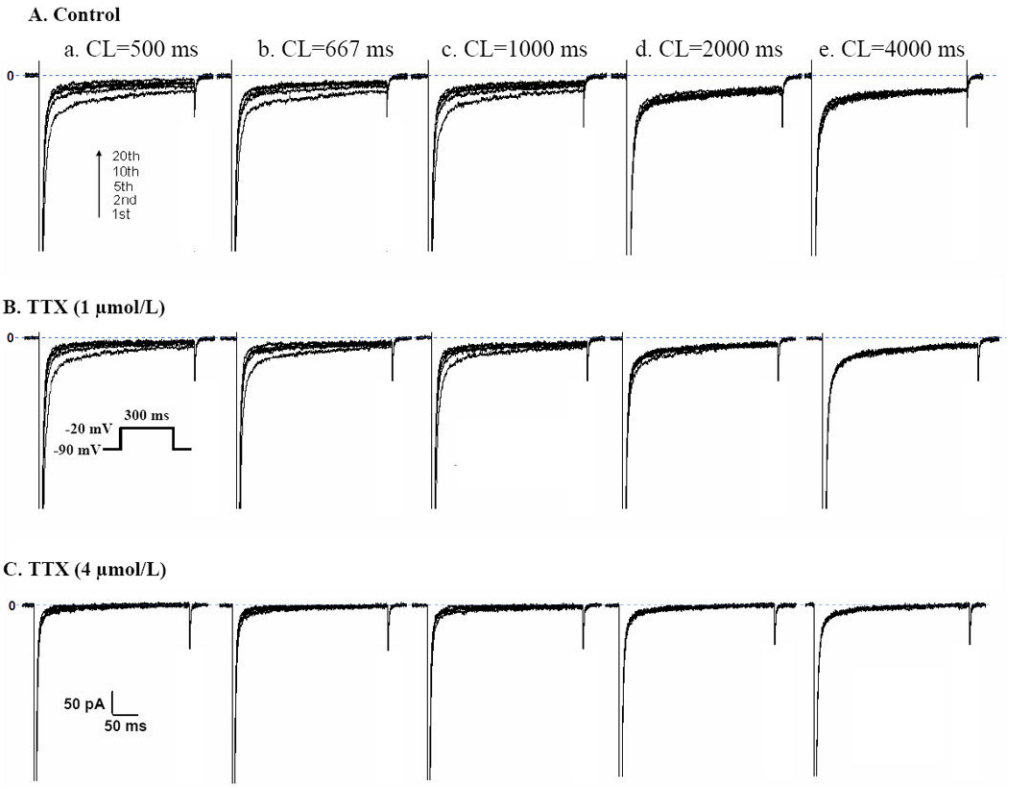

Figure 6.

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

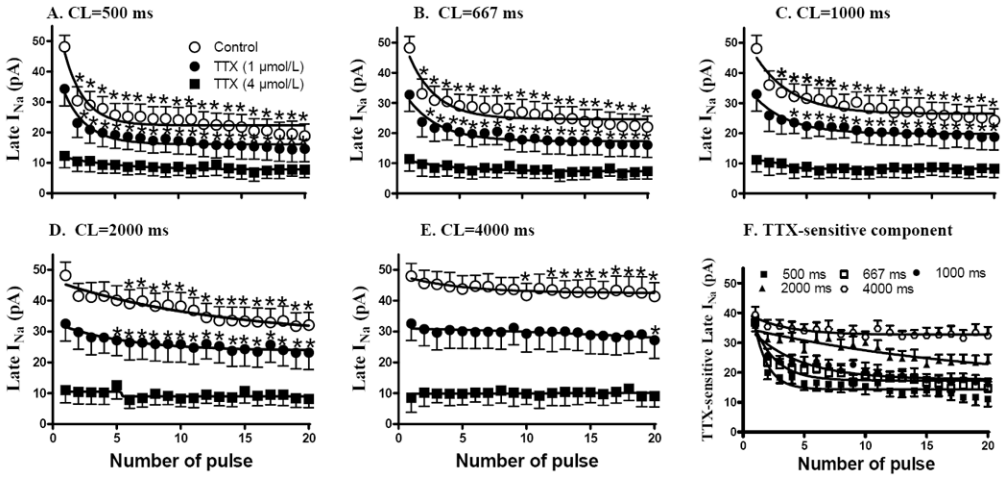

Figure 7.

TTX (1 and 4 μmol/L) reduced the beat-to-beat (from pulse 1 to pulse 20) changes of late sodium current (I_{Na}) at shorter (from 500 to 2000 ms in CL, A-D) but not at longer (4000 ms in CL, E) stimulation cycle length. The TTX-sensitive component of late I_{Na} decreased with a decrease in stimulation CL, as shown in panel F. Each point indicates a mean of measurements from 10 cells. Absolute values of amplitudes of late I_{Na} are shown. *, Compared to pulse #1, $p<0.05$.

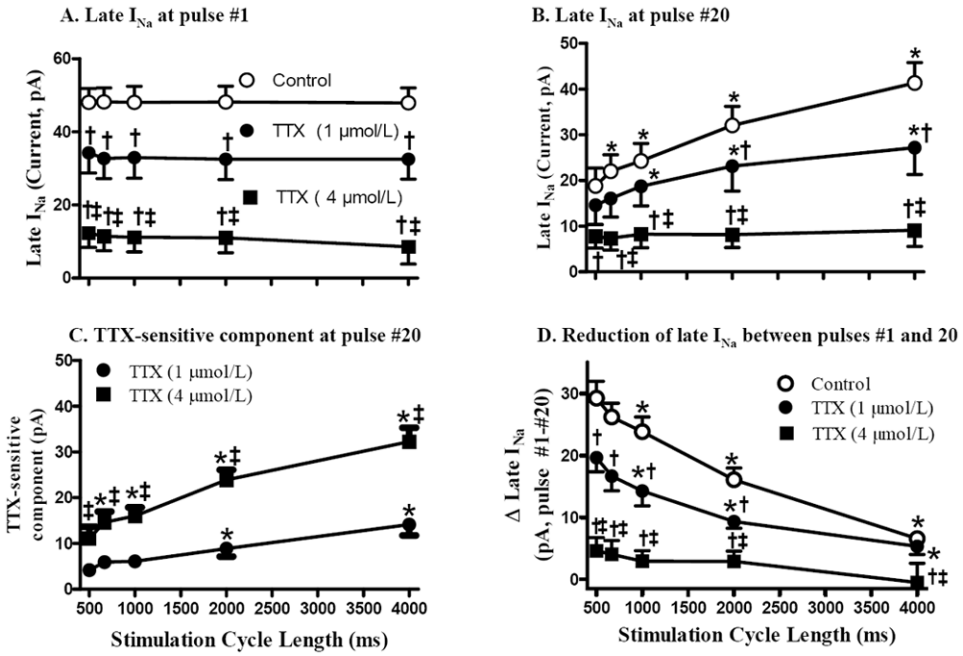

Figure 8.

The rate-dependent decrease of late I_{Na} (measured as TTX-sensitive current). TTX (1 and 4 μmol/L) was used to inhibit late I_{Na}. Late I_{Na} at pulse #1 (A) and #20 (B), the TTX sensitive component (control-TTX) at pulse #20 (C), and the reduction of late I_{Na} between pulses #1 and 20 (D) are plotted. *, Compared with CL of 500 ms; †, compared with control at same rate; ‡, compared to 1 μmol/L TTX at same rate; $p<0.05$. Absolute values of amplitudes of late I_{Na} are shown.