Electrophysiological Mechanisms in a Canine Model of Erythromycin-Associated Long QT Syndrome

Michael Rubart, MD; Milton L. Pressler, MD; Harald P. Pride, BS; Douglas P. Zipes, MD

Background. Erythromycin is known to prolong ventricular repolarization and has been associated with the occurrence of torsades de pointes. In this study, we have investigated potential mechanisms in vivo and in vitro for induction of an acquired long QT syndrome by erythromycin.

Methods and Results. Ventricular electrograms and endocardial monophasic action potentials were recorded in anesthetized open-chest dogs before and after administration of 40 to 120 mg/kg of erythromycin lactobionate. Conventional microelectrode techniques were used to record transmembrane action potentials in isolated dog Purkinje fibers and papillary muscles. Erythromycin at concentrations >20 mg/L prolonged action potential duration. At higher concentrations (100 to 200 mg/L), erythromycin induced phase 2 and phase 3 early afterdepolarizations (EADs) both in vivo and in vitro. The effects of erythromycin on repolarization were more marked in Purkinje fibers than in papillary muscle. Pretreatment of Purkinje fibers with erythromycin antagonized the effects of dofetilide, a selective delayed-rectifier potassium channel (IK) blocker. Pretreatment with prazosin or tetrodotoxin had no effect on erythromycin-induced changes in action potential duration.

Conclusions. These pharmacological studies suggest that erythromycin prolongs repolarization to a large extent by block of IK. In turn, prolongation of action potential duration resulting from erythromycin's actions on IK may promote the development of EADs. The induction of ventricular arrhythmias observed clinically after exposure to erythromycin may be related to the development of EADs. The rarity of occurrence of ventricular arrhythmias suggests that other predisposing factors contribute to the acquired long QT syndrome associated with erythromycin. (Circulation. 1993;88[part 1]:1832-1844.)

KEY WORDS  • potassium  • depolarizing  • arrhythmia

The long QT syndrome is defined by prolonged ventricular repolarization and the occurrence of a multiformal ventricular tachyarrhythmia called torsades de pointes. In its acquired form, the long QT syndrome has been associated with a variety of interventions and substrates. Administration of the macrolide antibiotic erythromycin has been reported to prolong ventricular repolarization and cause torsades de pointes by an unknown mechanism.

In canine Purkinje fibers in vitro, erythromycin has been shown to lengthen repolarization in a concentration-dependent fashion. Sporadic early afterdepolarizations (EADs) were seen but not recorded. Since increasing evidence implicates EADs and triggered activity as causes of torsades de pointes associated with long QT intervals, we investigated the effects of erythromycin on ventricular repolarization in vivo and in vitro and specifically tested whether it can provoke EADs. In addition, we explored the cellular mechanisms for the effects of erythromycin on the action potential in isolated canine Purkinje fibers and papillary muscles. The results demonstrate that erythromycin markedly prolongs repolarization and acts as a competitive inhibitor of the effects of dofetilide. Erythromycin also provoked EADs similar to those caused by class IA antiarrhythmic agents. Two abstracts of some of these findings have been reported previously.9,10

Methods

In Vivo Experiments

Surgical preparations. Details of the surgical techniques have been described previously.11,12 In brief, 10 mongrel dogs (weight, 12 to 25 kg) were anesthetized with thiopental (25 mg/kg IV) and with chloralose (5 to 10 mg·kg⁻¹·h⁻¹ IV) and ventilated by a constant-volume cycled respirator, and cannulas were placed in the left femoral artery and vein and right femoral vein. After median sternotomy, the ansae subclaviae were transected bilaterally, and both cervical vagi were cut to avoid autonomic reflex responses during experimenta- tion. The sinus node was crushed and the right atrium paced at a constant cycle length of 600 milliseconds using 2-millisecond stimuli. Two pairs of bipolar plunge electrodes were inserted into the right atrium and the left or right ventricular epicardium to record local electrical activity.

Monophasic action potential recordings. Monophasic action potentials (MAPs) were recorded with bipolar contact electrode catheters (EP Technologies Inc, Mountain View, Calif) positioned on the endocardial
right ventricular wall and at the endocardial left ventricular apex (frequency range, 0.04 to 500 Hz). Lead II of the ECG, amplified MAPs, right atrial and left or right ventricular local bipolar electrogams, and arterial blood pressure were displayed on an oscilloscope and digitized through an 8-channel analog-to-digital converter (model PCM-8, Medical Systems Corp., Greenvale, NY) for storage on VCR tape. Analysis of the signals was done off-line with custom software.

Phases 0 to 4 of the MAP were defined as for transmembrane action potentials.13 Data acceptable for MAP analysis followed previously published criteria.11,12 The amplitude of the MAP was defined as the potential difference between phase 2 and the maximal diastolic potential during phase 4.14 Durations of the left and right ventricular monophasic action potentials were determined at 90% repolarization (LV APD90 and RV APD90, respectively).

EADs: Definition and method for measurement. A positive voltage deflection that interrupted the smooth contour of phase 2 or phase 3 repolarization was called an EAD.11,13,14 The amplitudes of EADs were determined as for EADs in transmembrane action potential recordings13 and expressed as a percent of the total MAP amplitude. The QT(U) interval was defined as the time between the first deviation from an isoelectric PR interval until the last deviation from baseline before the isoelectric TP (UP) interval.14,15

Experimental protocol. Changes in MAPs at different cycle lengths were studied 5 to 15 minutes after completion of each dose of erythromycin lactobionate. Pacing cycle length was increased from 600 milliseconds in steps of 200 milliseconds until the intrinsic automaticity interrupted the pacing cycle. Each cycle length was maintained for 2 to 3 minutes to ensure a steady state. After each scan of stimulation frequencies, the pacing interval was returned to 600 milliseconds until the next scan was performed. In 10 dogs, erythromycin lactobionate was dissolved in sterile water and infused intravenously over a period of 10 minutes (single dose, 40 mg/kg); in 6 dogs, the same dose was repeated 60 and 120 minutes later. Measurements of MAP duration at 90% repolarization and the amplitudes of EADs, if present, were determined by the average of three consecutive atrial paced complexes during the control period and at the time of maximum drug effect.

In Vitro Experiments
In vitro experiments were performed according to our published techniques.16 Dogs (15 to 20 kg) were anesthetized with pentobarbital (30 mg/kg IV) and the hearts rapidly excised. Papillary muscles or segments of the Purkinje network were maintained in a 1.5-mL chamber at 36±1°C. Tyrode’s solution was gassed with 95% O2/5% CO2 (pH 7.35) and contained (mmol/L): NaCl 123, KCl 3.5, NaHCO3 21.5, Na2HPO4 0.65, MgCl2 0.5, glucose 5.5, CaCl2 2.0. The superfusion system was made of Teflon to minimize drug carryover and loss of dissolved gases. Preparations were allowed to equilibrate for 1 hour before experiments.

Intracellular recordings. Action potentials were recorded with 3 mol/L KCl-filled microelectrodes (10 to 18 MΩ) connected by Ag/AgCl holders to a unity-gain electrometer (Dagan model 8700). Intracellular signals were digitized and displayed on an oscilloscope (Tektronix 5223) and then entered into a microcomputer. A custom software program provided on-line measurement of action potential parameters: takeoff potential (Vmax); overshoot; action potential amplitude (APA); and action potential duration at 50% and 90% repolarization (APD50, APD90). Maximum upstroke velocity (Vmax) was determined with a derivative circuit that was linear between 50 and 1000 V/s. Voltages were measured with an accuracy of 0.5 mV. Zero drift was corrected by linear interpolation from the zero potential between impalement and withdrawal of the microelectrode.

Measurement of force. Isometric tension was measured in dog Purkinje strands stimulated at 1 Hz and connected via a steel pin to a photoelectric force transducer.17 Studies were done at the peak of the length/tension curve after a 60- to 90-minute period for equilibration. After control measurements, the fibers were superfused for 60 minutes with 100 mg/L erythromycin in Tyrode’s solution. Force recordings were digitized, and peak developed tension was measured on-line with custom software. After each experiment, the transducer was calibrated with known weights. Force was normalized to control values and reported as a percent.

Reagents. Erythromycin, tetrodotoxin (TTX), and prazosin were obtained from Sigma Chemical Co (St Louis, Mo) and added directly to Tyrode’s solution. Dobutamine (UK 68,798; Pfizer Central Research, Sandwich, Kent, UK) was dissolved in 50% ethanol acidified with HCl (=0.05N) to produce a range of stock solutions. The final ethanol concentrations in superfusates did not exceed 0.05%.

In Vitro Protocols
Erythromycin’s effects on transmembrane action potentials were studied in 15 isolated dog Purkinje fibers and 3 papillary muscles that were stimulated continuously at 1 Hz. Measurements were obtained at 600, 800, 1000, 1200, 1500, 2000, 2500, and 3000 milliseconds cycle length during the control period and at 60, 90, and 120 minutes after erythromycin was added to the superfusate. Cycle lengths up to 30 seconds were also tested if permitted by intrinsic automaticity. In studies of Purkinje fibers, only experiments with impalements lasting throughout the experiments were accepted for analysis. In papillary muscles, impalements could not be maintained in the same cell throughout the study. Consequently, the maximal rate (Vmax) of phase 0 depolarization in papillary muscles was too variable for comparison between control and drug-treated states. Because of the cumulative effects of erythromycin, dose-response curves were done in Purkinje fibers by giving the drug in increasing doses (20, 50, 100, and 200 mg/L). Action potentials were recorded before and at 60 minutes after exposure to erythromycin. Three papillary muscles were exposed to 100 and 200 mg/L erythromycin added cumulatively to the superfusate. To further define the mechanism for the action of erythromycin on transmembrane action potentials, the effects of different ion channel antagonists were studied in the absence and presence of erythromycin.

Role of late sodium channels in the effects of erythromycin. Activation of the “late sodium” or “window” current18,19 might be one ionic mechanism by which erythromycin prolongs APD. Coraboeuf et al20 previously found that low doses of TTX shorten APD by
selectively blocking the “late sodium” inward current without affecting the fast inward sodium current. To
explore the role of late sodium channels, five Purkinje fibers were treated with several concentrations of TTX
(6.6x10^-8, 3.3x10^-7, and 6.6x10^-7 mol/L) before and after superfusion with erythromycin. Each dose of TTX
was given for 15 minutes to ensure that changes in APD reached steady state. After determination of the control
dose-response curve, TTX was washed out for 30 minutes before erythromycin (100 mg/L) was added to Tyrode’s solution. After 90 minutes of exposure to erythromycin, the same dose-response curve for TTX, including washout, was repeated in the presence of erythromycin.

Role of delayed rectifier potassium channels in the effects of erythromycin. Block of outward potassium
currents, eg, the delayed rectifier (I_K), was tested as an ionic mechanism by which erythromycin might prolong APD. Voltage-clamp studies in isolated guinea pig ventricular myocytes have shown that dofetilide (UK 68,798) selectively inhibits I_K. To explore the role of I_K, two separate groups of four Purkinje fibers were exposed to increasing concentrations of dofetilide (1, 5, 50, and 200 nmol/L) in the absence and presence of pretreatment with erythromycin (100 mg/L for 90 minutes). Action potential characteristics were measured 45 minutes after each dose of dofetilide to allow the effects to reach steady state.

Role of α_1-adrenergic receptor blockade in the effects of erythromycin. A final mechanism considered for erythromycin-induced prolongation of APD was block of α_1-adrenergic receptors. Rosen et al. have shown that 1 μmol/L prazosin selectively blocks α_1-adrenergic receptors of canine Purkinje fibers without affecting action potential characteristics. In four Purkinje fibers, action potentials were recorded before and 30 minutes after 1 μmol/L prazosin was added to the superfusate. After pretreatment with prazosin, 100 mg/L of erythromycin was added to prazosin/Tyrode’s solution, and steady-state changes in action potential parameters were measured again at 90 minutes.

Data Analysis
Data were expressed as mean±SEM. Student’s paired t test was used to analyze simple pairs of measurements. For three or more conditions, significant trends were identified by repeated-measures ANOVA. When the F value obtained by ANOVA was significant (P<.05), the Fisher PLSD (protected least significant difference) was used to compare pairs of mean values. Two-way ANOVA was used to compare dose-response relations for TTX-, dofetilide-, and prazosin-treated fibers in the absence and presence of erythromycin. Correlations between variables were determined by linear regression.

Results
In Vivo Experiments
Endocardial monophasic action potentials were recorded in 10 animals. In 9, MAPs from both the right and left ventricle were recorded simultaneously; in the remaining dog, only left ventricular MAPs were recorded. Four dogs received a single dose of erythromycin lactobionate (40 mg/kg); the remaining 6 dogs were given a cumulative dose of 120 mg/kg. Fig 1 shows an example of the prolongation of the QT interval and endocardial MAPs after 40 mg/kg IV erythromycin. These effects were highly reproducible, and the prolongation of APD was very similar in the left and right ventricles. Erythromycin (40 mg/kg) significantly (P<.001) lengthened the duration of several parameters (mean±SEM; n=10): QT, 308±5 milliseconds (control) to 341±7 milliseconds (erythromycin); LV APD_90, 265±7 milliseconds (control) to 312±10 milliseconds (erythromycin); RV APD_90, 257±8 milliseconds (control) to 303±7 milliseconds (erythromycin). There was also a significant (P<.05) decrease in mean arterial blood pressure: 98±6 mm Hg (control) versus 78±12 mm Hg (erythromycin). As additional intravenous doses of erythromycin were given, left ventricular endocardial MAP and QT intervals prolonged further up to a cumulative dose of 80 mg/kg. A cumulative dose of
120 mg/kg of erythromycin produced no additional effects on LV APD<sub>90</sub>. The increases in mean QT interval and mean LV APD<sub>90</sub> occurred in parallel and were linearly related (mean±SEM slope, 0.95±0.012; r=.89; n=45). In contrast, erythromycin (40 mg/kg) also prolonged the duration of the MAP of the right ventricular endocardium, but there was no further increase in RV APD<sub>90</sub> until a cumulative dose of 120 mg/kg was given (P<.05). Table 1 summarizes the results from six dogs. Additional doses of erythromycin significantly increased mean QT interval and mean duration of both right and left ventricular MAPs (P<.01) and decreased mean arterial blood pressure (P<.01). There was a significant (P<.05) effect on mean arterial blood pressure at 80 mg/kg with a further decrease at 120 mg/kg. The increases in APD were more pronounced at longer cycle lengths (Fig 2).

Table 2 summarizes the incidence and amplitude of EADs in left and right ventricular MAP recordings. EADs were seen in five MAP recordings from the left ventricular endocardium and in four right ventricular recordings. EADs occurred either at the termination of the plateau (Figs 2 and 3) or during phase 3 (data not shown). EAD amplitudes were more pronounced at longer diastolic intervals resulting from either increases in pacing cycle length (Fig 2) or compensatory pauses following premature ventricular complexes (Fig 3). Repetitive (ie, more than one) EADs were not observed. Neither phase 2 nor phase 3 EADs induced triggered activity in vivo.

EADs in alternate cycles were observed in two experiments. In one experiment, 2:1 alternation of EADs occurred on the terminal portion of the plateau of the left ventricular MAP and late phase 3 of the right ventricular MAP (see Fig 2), coinciding with alternation of QT intervals and a 2:1 occurrence of apparent U waves on the local bipolar electrogram from the left ventricular wall. In another experiment (not shown), 2:1 alternation of phase 3 EADs occurred in both the left and right ventricular MAP recordings, and there was corresponding T wave alternans on the surface ECG and alternations of the QT interval on the local bipolar electrogram from the right ventricular wall. In both experiments, the 2:1 alternation was cycle length dependent. As shown in Fig 2, lengthening of the cardiac cycle resulted in disappearance of the ECG alternans and a 1:1 occurrence of EADs.

### In Vitro Experiments

Fig 4 typifies the changes in action potentials recorded from dog Purkinje fibers and papillary muscle before and after exposure to erythromycin. In Purkinje fibers (Fig 4A), erythromycin increased plateau potential and APD at a threshold concentration of 20 mg/L. Increases in erythromycin concentration progressively lengthened APD, but there were no additional changes in plateau potential. Changes in APD began to develop 5 minutes after exposure to erythromycin but were not complete until 60 minutes later. Prolongation of APD was fully reversible after washout for 60 minutes. At 200 mg/L, erythromycin also reduced the V<sub>max</sub> of phase 0 depolarization in Purkinje fibers. Fewer effects on APD were observed in ventricular tissue than in Purkinje fibers (compare Figs 4A and 4B). Table 3 summarizes the effects of erythromycin on action potential characteristics of five Purkinje fibers. Erythromycin produced significant (P<.01) dose-dependent increases in APD<sub>50</sub> and APD<sub>90</sub>; at the highest concentration (200 mg/L), V<sub>max</sub> was significantly depressed. Neither V<sub>max</sub>, overshoot, nor APA was significantly affected.

In three papillary muscles, erythromycin significantly (P<.01) prolonged repolarization from control at both doses tested: APD<sub>50</sub>, 165±13 milliseconds (control), 201±11 milliseconds (100 mg/L), 225±10 milliseconds (200 mg/L); APD<sub>90</sub>, 217±10 milliseconds (control), 258±11 milliseconds (100 mg/L), 288±12 milliseconds (200 mg/L). The maximum lengthening of APD was significantly greater in Purkinje fibers (APD<sub>90</sub>, 67±3%) than in papillary muscles (APD<sub>90</sub>, 33±4%; P<.001). Fig 5 shows that the relation between APD and cycle length was much steeper in Purkinje fibers than papillary muscle.

### Induction of early afterdepolarizations in Purkinje fibers

Since infusion of erythromycin produced EADs in vivo, we investigated the conditions under which EADs might be induced in vitro. Higher concentrations of erythromycin were required. At 100 mg/L, no EADs could be elicited either at extracellular [K<sup>+</sup>] of 3.5 mmol/L (n=12) or 2.7 mmol/L (n=3). However, at 200 mg/L erythromycin, EADs were observed during cycle length–dependent prolongation of APD in four of six
Purkinje fibers (Fig 6). EADs could not be induced in two fibers because spontaneous automaticity precluded pacing at sufficiently long cycle lengths. In one Purkinje fiber, EADs developed at a cycle length of 2 seconds, whereas the other three fibers developed EADs at cycle lengths >5 seconds. EADs occurred during phases 2 and 3 of the action potential at a mean activation voltage (defined as the most negative voltage preceding depolarization) of $-33\pm4$ mV ($n=4$; range, $-40$ to $-21$ mV). The mean amplitude of the EADs from each of the four fibers that developed EADs was $24\pm8$ mV (range, 5 to 45 mV). Triggered activity (ie, a second nonstimulated upstroke of amplitude $>10$ mV arising from an EAD) developed spontaneously in only one of the four fibers. EADs were not observed in any of three papillary muscles exposed to 200 mg/L erythromycin at cycle lengths ranging from 0.6 to 30 seconds.

Mechanism of erythromycin-related changes in repolarization. Fig 7 shows the effects of various concentrations of dofetilide (UK 68,798) on changes in APD$_{90}$ and APD$_{99}$ in the absence and presence of erythromycin. Stepwise increases in dofetilide concentration from a

Table 2. Early Afterdepolarization Amplitude After Erythromycin Administration

<table>
<thead>
<tr>
<th>Erythromycin, mg/kg</th>
<th>40</th>
<th>80</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV</td>
<td>2</td>
<td>4 (3)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>EADA (% MAP)</td>
<td>3-20</td>
<td>8-26</td>
<td>16-23</td>
</tr>
<tr>
<td>LV</td>
<td>3</td>
<td>4 (2)</td>
<td>5 (2)</td>
</tr>
<tr>
<td>EADA (% MAP)</td>
<td>10-69</td>
<td>9-11</td>
<td>10-26</td>
</tr>
</tbody>
</table>

RV indicates right ventricle; LV, left ventricle; EADA, early afterdepolarization amplitude (as a percent of monophasic action potential amplitude). EADA was measured at cycle lengths of 600 to 1400 milliseconds. Values are ranges. $n$ indicates cumulative number of dogs exhibiting EAD; figures in parentheses indicate number of dogs that exhibited EADs at that dose of erythromycin. EADs were not recorded at all doses of erythromycin in some dogs (see text).
threshold concentration of 1 nmol/L progressively lengthened APD\(_{50}\) and APD\(_{90}\). Pretreatment with 100 mg/L erythromycin for 90 minutes significantly (P<.01) prolonged the action potential: APD\(_{50}\), 239±30 milliseconds (control) to 313±24 milliseconds (erythromycin); APD\(_{90}\), 341±36 milliseconds (control) to 453±27 milliseconds (erythromycin). When stepwise additions of dofetilide were made in the presence of erythromycin, the dose-response curves for both APD\(_{50}\) and APD\(_{90}\) were shifted to the right. Higher concentrations of erythromycin and dofetilide could not be tested because the frequent occurrence of EADs prevented analysis of changes in APD.

Increasing concentrations of TTX produced graded shortening of APD\(_{50}\) and APD\(_{90}\) in four fibers (Fig 8). Superfusion with erythromycin (100 mg/L) significantly prolonged APD whether TTX was present or not. Erythromycin significantly (P<.01) increased mean APD\(_{50}\) from 186±5 to 265±12 milliseconds and mean APD\(_{90}\) from 288±9 to 401±15 milliseconds. No significant difference occurred in the percent decrease in APD\(_{50}\) and APD\(_{90}\) at each concentration of TTX whether erythromycin was present (hatched bars in Fig 8) or absent (solid bars), suggesting that erythromycin has little competition with TTX for binding to the late sodium channels.

\(\alpha_1\)-Adrenergic receptor stimulation has been shown to promote induction of EADs.\(^{23,24}\) We investigated whether erythromycin might prolong APD via \(\alpha_1\)-adrenergic receptor stimulation by pretreating four dog Purkinje fibers with 1 \(\mu\)mol/L prazosin. Prazosin (1 \(\mu\)mol/L) had no significant effect on action potential characteristics: control, \(V_m\) -94±2 mV, APA 133±3 mV, APD\(_{50}\) 229±14 milliseconds, APD\(_{90}\) 324±11 milliseconds, \(V_{max}\) 705±10 V/s versus prazosin, -93±2 mV, 134±2 mV, 234±16 milliseconds, 339±16 milliseconds, and 705±14 V/s, respectively. In the continued presence of 1 \(\mu\)mol/L prazosin, 100 mg/L erythromycin prolonged APD\(_{50}\) and APD\(_{90}\) to a similar degree as in the absence of prazosin (Fig 9). None of the effects of erythromycin on repolarization were antagonized by \(\alpha_1\)-adrenergic receptor blockade. The increase in plateau potential and APD associated with erythromycin (see Fig 4) might result from an increase in inward calcium current (I\(_{Ca}\)). If so, developed tension might be expected to increase. Consequently, we measured changes in developed tension in three dog Purkinje fibers after exposure to erythromycin. Fig 10 shows a summary of the mean changes in developed tension (as a percent of control force) during a 60-minute exposure to 100 mg/L erythromycin. The
FIG 4. Tracings showing effects of erythromycin on transmembrane action potentials of (A) dog Purkinje fiber and (B) dog papillary muscle. Each panel shows plots of digitized transmembrane action potentials and the first derivative of the action potential upstroke (insets). Superimposed are records before (control, cd) and 60 minutes after superfusion of various concentrations of erythromycin (numbers labeling signals; in mg/L). Erythromycin increased the length of the plateau and prolonged action potential duration. The effects were much greater in Purkinje fibers than in papillary muscle. The papillary muscle recordings before and after 100 mg/L erythromycin were from different impalements. Time, voltage, and volts per second scales as marked; pacing cycle length, 1000 milliseconds. $V_m$, takeoff potential.

FIG 5. Plot showing effects of erythromycin (200 mg/L) on action potential duration at 90% repolarization (APD$_{90}$) in dog Purkinje fibers ($\circ$) and papillary muscles ($\triangle$) at different cycle lengths (CL). Values are mean±SEM; number of experiments shown in parentheses. The magnitude of erythromycin-induced prolongation of APD$_{90}$ was proportional to CL. The effects of erythromycin were more steeply dependent on CL in Purkinje fibers than in papillary muscles. Fibers that developed early afterdepolarizations were excluded.

Discussion

Major Findings

The new findings in this study were that erythromycin prolonged APD in both Purkinje fibers and papillary muscles, elevated the action potential plateau of Purkinje fibers without changing $V_m$, APA, or overshoot, and in vivo prolonged the QT(U) interval of the surface ECG and the APD of the monophasic action potentials. Erythromycin induced phase 2 and phase 3 EADs in vivo and in Purkinje fibers but not ventricular muscle in vitro; triggered activity arising from EADs was observed exposure to 100 mg/L erythromycin ($P<$0.01). Although this decrease in force was statistically significant, the change was only slightly greater than the intrinsic error (5% to 10%) and may have been affected by “run-down” of the fibers over time. Because changes in developed tension during erythromycin exposure were minimal, we did not pursue additional studies of the role of $I_{Ca}$.

TABLE 3. Effects of Erythromycin on Action Potential Characteristics in Canine Purkinje Fibers

<table>
<thead>
<tr>
<th>Erythromycin</th>
<th>$V_m$, mV</th>
<th>OVS, mV</th>
<th>APA, mV</th>
<th>APD$_{50}$, ms</th>
<th>APD$_{90}$, ms</th>
<th>$V_{max}$, V/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$-90\pm2$</td>
<td>$37\pm1$</td>
<td>$127\pm1$</td>
<td>$280\pm21$</td>
<td>$370\pm22$</td>
<td>$690\pm31$</td>
</tr>
<tr>
<td>20 mg/L</td>
<td>$-91\pm1$</td>
<td>$39\pm1$</td>
<td>$129\pm1$</td>
<td>$309\pm27$</td>
<td>$407\pm32^*$</td>
<td>$709\pm38$</td>
</tr>
<tr>
<td>50 mg/L</td>
<td>$-91\pm2$</td>
<td>$39\pm1$</td>
<td>$130\pm1$</td>
<td>$338\pm30^*$</td>
<td>$457\pm35^+$</td>
<td>$710\pm37$</td>
</tr>
<tr>
<td>100 mg/L</td>
<td>$-91\pm2$</td>
<td>$39\pm2$</td>
<td>$130\pm2$</td>
<td>$381\pm35^+\ddagger$</td>
<td>$528\pm38^*\ddagger$</td>
<td>$701\pm39$</td>
</tr>
<tr>
<td>200 mg/L</td>
<td>$-90\pm1$</td>
<td>$38\pm1$</td>
<td>$128\pm2$</td>
<td>$412\pm40^*\ddagger$</td>
<td>$620\pm39^*\ddagger$</td>
<td>$640\pm44^*\ddagger$</td>
</tr>
</tbody>
</table>

$V_m$, transmembrane potential; OVS, overshoot; APA, action potential amplitude; APD$_{50}$ and APD$_{90}$, action potential duration at 50% and 90% repolarization, respectively; $V_{max}$, maximum upstroke velocity (dV/dt) of action potential. Values are mean±SEM; n=five fibers; stimulation frequency, 1 Hz.

$^*P<.01$ vs control.

$^\dagger P<.05$, $^\ddagger P<.01$ vs 50 mg/L.

$^\ddagger P<.01$ vs 20 mg/L.

$^\ddagger\ddagger P<.01$ vs 100 mg/L.
FIG 6. Tracings showing erythromycin-induced early after-depolarizations (EADs) in an isolated dog Purkinje fiber. Shown are action potentials 60 minutes after exposure to 200 mg/L erythromycin. Pacing cycle length (CL) as marked. A, At a CL of 3 seconds, action potential duration was prolonged, but no EADs were observed. B, At a CL of 5 seconds, action potential duration lengthened further, and EADs occurred near the end of phase 2. C, Middle record from panel B at an expanded time scale. Some of the late phase 2 EADs had sufficient amplitude to reach threshold. Time and voltage scales marked.

in one Purkinje fiber at extremely long pacing intervals. The effects of erythromycin on APD were much more pronounced in Purkinje fibers than in papillary muscle. Erythromycin antagonized the lengthening of APD observed with dofetilide, an I\textsubscript{K} blocker. Pretreatment of the Purkinje fibers with prazosin (1 \mu mol/L) or low doses of TTX (up to 6.6 \times 10\textsuperscript{-7} mol/L) did not alter the prolongation of the action potential by erythromycin. Erythromycin did not result in substantive changes in twitch tension even at doses that greatly prolonged APD.

The observations in vitro and in vivo probably represent identical electrophysiological phenomena. However, higher concentrations of erythromycin were required to induce EADs in Purkinje fibers in vitro, and the cycle lengths at which EADs occurred were significantly longer than in the intact heart. The difference might possibly be a result of circulating catecholamines released from the adrenal medulla. \alpha- and \beta-adrenergic receptor agonists have been shown to cause EADs or facilitate their induction both in vivo\textsuperscript{23,25} and in vitro\textsuperscript{24,26}. Circulating catecholamines may have shifted the cycle length–EAD relation to shorter cycle lengths under in vivo conditions, thereby increasing the propensity of the heart to develop EADs. A second possibility is that intravenous administration of erythromycin may have led to a more rapid initial tissue uptake and a higher intracellular concentration of the drug than when it was superfused in vitro. The observation that the maximum drug effect in vivo occurred 10 to 20 minutes after intravenous infusion of erythromycin, whereas its effects on transmembrane action potential characteristics did not reach steady state until 60 minutes of drug exposure, supports this mechanism. Finally, since low [K\textsuperscript{+}]o is known to facilitate EAD induction\textsuperscript{27} [K\textsuperscript{+}], may have been lower in vivo relative to in vitro.

FIG 7. Plots showing erythromycin (eryth)-related shift in dofetilide (dof) dose-response curve. Ordinate, mean \pm SEM change from baseline action potential duration at 50\% (APD\textsubscript{50}) and 90\% repolarization (APD\textsubscript{90}); abscissa, logarithm of dofetilide concentration ([dof]) in nanomoles per liter (nM). Open symbols, changes in APD from control at various concentrations of dofetilide; filled symbols, changes in APD with dofetilide in the presence of 100 mg/L erythromycin. Baseline APD\textsubscript{50} and APD\textsubscript{90} were recorded 90 minutes after exposure to erythromycin. The two dose-response curves were from separate sets of four dog Purkinje fibers.

Origin of EADs and Production of Ventricular Arrhythmias In Vivo

EADs may result from spatial inhomogeneities of repolarization that cause electrotonic interactions between differently polarized regions or from oscillations of membrane currents.\textsuperscript{26-31} At first glance, our findings seem to support the former mechanism. Erythromycin was shown to increase Purkinje fiber APD much more than that of papillary muscle. Differences in repolarization between Purkinje fibers and contiguous muscle can simulate phase 3 EADs\textsuperscript{1,32} and the observed EADs could have resulted from electrotonic interactions or reentrant activity within the tissue. However, the similarity of the phenomena produced by erythromycin to those induced by quinidine,\textsuperscript{27,33-35} anthopleurin A (AP-A),\textsuperscript{15} cesium,\textsuperscript{11,12,36} clofilium,\textsuperscript{37} and BAY K 8644\textsuperscript{20} in vivo in dogs,\textsuperscript{38} in vitro in Purkinje fibers only 1 to 2 mm long, and in isolated myocytes\textsuperscript{38} supports the theory that EADs originate from events within the cellular membrane. Intracellular recordings from isolated Purkinje cells will be necessary to unequivocally establish the cellular basis of these phenomena.

The in vitro data provide evidence that erythromycin-induced EADs in vivo may have originated from the endocardial Purkinje network. One inconsistency is that Purkinje fibers do not have sufficient mass to explain the ECG changes of QT interval prolongation, U waves,
and ST-T alternans. Recent studies demonstrate a subpopulation of ventricular cells (M cells) within the deep subepicardium and midmyocardium of the free ventricular wall with electrophysiological properties more akin to Purkinje fibers. M cells have a steeper APD/rate relation than endocardial or epicardial fibers. M cells have also been found in the deep subendocardium of the ventricular septum, papillary muscles, and trabeculae. M cells seem to be more susceptible to the development of EADs and EAD-induced triggered activity when exposed to quinidine, ceflofilium, cesium, 4-AP, and amiloride and may be more sensitive to erythromycin as well.

Intravenous administration of erythromycin lactobionate failed to induce ventricular arrhythmias in vivo, and only one Purkinje fiber preparation exhibited triggered activity at an extremely long cycle length arising from a phase 3 EAD. This may have occurred because we did not attempt to reduce [K⁺]₀ or [Mg²⁺]₀ in our in vivo experiments, and both ions may have been too plentiful to initiate triggered activity from EADs. It is also possible that the EAD amplitude was too low to attain threshold or that locally generated EADs blocked at the Purkinje/muscle junction. Circulating catecholamines also have been shown to play a significant role for EAD transmission in the intact heart, and these too may have contributed to the lack of ventricular arrhythmias in the sympathetically denervated dogs of the present study.

**T Wave Alternans**

T wave alternans has been demonstrated in a variety of experimental and clinical settings and may be a marker of electrical instability of the ventricle.

Electrical alternans has been induced by ischemia, abrupt changes in heart rate, hypocalcemia, low pH, and stimulation of the left stellate ganglion. The mechanisms for beat-to-beat changes in repolarization may involve alternation in the extent to which potassium outward currents (especially Iₚ and Iₜ) decay, alternation in recovery from inactivation of late sodium channels, rate-related 2:1 conduction block to portions of the heart, and/or beat-to-beat variation of the dome portion of the epicardial action potentials.

In the present study, T wave changes on the surface ECG (Fig 3) and QTU alternans on the local bipolar electrogram (Fig 2) were related to 2:1 alternation of phase 2 EADs and were associated with an abrupt change in heart rate. Tachycardia-dependent QTU alternans caused by 2:1 alternation of EADs has also been demonstrated in dog hearts in vivo after AP-A administration. Such periodicity in the occurrence of EADs was not observed in Purkinje fibers in vitro, possibly...
because T wave alternans results from 2:1 alternation of the propagation of locally generated EADs rather than from slowing the kinetics of repolarizing ion currents such as I_K.

Ionic Basis for the Effects of Erythromycin on the Action Potential

The present study provides insights into the role of several ionic currents in the effects of erythromycin on the action potential. However, voltage-clamp studies of single dog Purkinje cells will be necessary to precisely define the ionic basis of the effects of erythromycin. Concentrations of erythromycin up to 100 mg/L markedly prolonged APD yet did not change V_max or APA, which are determined primarily by the fast inward sodium current (I_Na). At 200 mg/L erythromycin (and presumably higher concentrations), it is likely that I_Na was reduced because V_max decreased without changes in V_m. The results suggest little involvement of I_Na in the effects of erythromycin at low doses (≤100 mg/L/L) but do not exclude a potential contribution of a decrease in I_Na to the generation of events like EADs that were observed at 200 mg/L erythromycin.

We explored the possible contribution of the late sodium current to the effects of erythromycin on APD. Prolongation of APD and elevation of the plateau could be explained by activation of this TTX-sensitive current.20 If so, erythromycin would be expected to antagonize, at least in part, the action potential shortening produced by TTX and shift the relation between TTX concentration and change in APD. The results illustrated in Fig 8 do not support this hypothesis; therefore, we conclude that erythromycin probably does not prolong APD by increasing this slowly inactivating sodium current. However, this conclusion is based on the evidence that TTX selectively binds to sodium channels19,59-61 and that such binding is not voltage or use dependent. Studies by Coraboeuf et al20 in intact Purkinje fibers and Wasserstrom and Salata62 in isolated dog cardiomyocytes have shown that TTX causes tonic block of late sodium channels and that the effects of TTX on APD are independent of cycle length. What is known about the specificity of the action of TTX tends to uphold our interpretation of the data.

Erythromycin could also prolong APD by blockade of potassium outward currents such as the transient outward current (I_o) and/or delayed rectifier potassium current (I_K). The lack of effect of erythromycin on the early "notch" of repolarization in Purkinje fibers suggests that block of I_o is unlikely. The data in Fig 7 suggest that blockade of I_K is far more likely. Dofetilide (UK 68,798) selectively blocks the rapid component of the delayed rectifier potassium outward current (I_Kr) in cardiac cells21,63-65 prolonging APD without affecting V_max or APA.21,65 Erythromycin significantly attenuated the action of 1 to 200 nmol/L dofetilide to prolong APD, shifting the dose-response curve to the right (Fig 7). This result would be consistent with competitive interaction of erythromycin and dofetilide at a common binding site of the I_K channel. The validity of this conclusion depends on the specificity of dofetilide for the I_K channel and the absence of a confounding effect, perhaps related to the known reverse use dependence of dofetilide.64,65 Pharmacological studies have limitations because few drugs are perfectly specific. However, there have been other examples of competitive inhibi-

![Graph showing mean ± SEM changes in peak isometric force after exposure to 100 mg/L erythromycin in dog Purkinje fibers.](http://circ.ahajournals.org/).
tion of the I\(\text{K}\) channel. A recent report\(^{60}\) found competitive displacement of \([\text{H}]\)dofetilide by several drugs (eg, clofilium, quinidine, sotalol, and selmidil) that are thought to block I\(\text{K}\). The results in Fig 7 suggest that erythromycin also should be included as one of those agents that competitively inhibit I\(\text{K}\).

We did not evaluate the role of the inward rectifier potassium current (I\(\text{Kr}\)), which contributes to repolarization during late phase 3. I\(\text{Kr}\) channels are a major determinant of the membrane potassium conductance at high negative potentials and thereby regulate the resting potential. We think it unlikely that erythromycin has substantial effects on I\(\text{Kr}\) because there were no significant changes in \(V_m\) even when APD was lengthened by >100 milliseconds. Likewise, the minimal effect of erythromycin on developed tension argues against a substantive contribution of the sarcolemmal I\(\text{ca}\) and/or calcium-release channels of the sarcoplasmic reticulum to the changes in APD. Agents that raise the plateau of the action potential by enhancing I\(\text{ca}\), eg, isoproterenol, also significantly shorten APD, an effect opposite to that observed with erythromycin. We have no explanation for the small transient increase in developed force occurring \(\pm\)10 minutes after exposure to erythromycin other than to state that it was not much larger than the error of the measurements. Furthermore, we cannot exclude an effect of erythromycin to block calcium-activated potassium channels. The potential role of erythromycin to stimulate sodium/calcium exchange or to inhibit the sodium/potassium pump current also remains within the realm of possibility.

We did consider whether erythromycin acts as an agonist for \(\alpha_1\)-adrenergic receptors in Purkinje cells. In rat ventricular myocytes, \(\alpha_1\)-adrenergic receptor stimulation decreases I\(\text{Kr}\), thereby prolonging APD.\(^{67,68}\) We discount this possibility, however, because pretreatment with the \(\alpha_1\)-adrenergic receptor antagonist prazosin did not affect erythromycin-induced lengthening of APD (Fig 9).

**Clinical Implications**

Five cases of torsades de pointes have been reported after administration of erythromycin, four after intravenous doses\(^5-8\) and one after oral intake of 1.5 g/d.\(^4\) The erythromycin concentrations used in our in vitro and in vivo studies were high compared with plasma levels in patients. In healthy volunteers, peak serum erythromycin concentrations reach an average of 30 mg/L after intravenous administration of 900 mg erythromycin.\(^69\) This is above the threshold concentration for significant prolongation of APD in vitro (see Fig 4). Thus, significant changes in repolarization could result from a single 1-g intravenous dose of erythromycin. The consequences of these changes in APD would depend on coexisting factors. Patients with undiagnosed congenital long QT syndrome might be "unmasked" by erythromycin. Multiform ventricular arrhythmias after oral erythromycin have been reported,\(^70\) even though 500 mg of oral erythromycin results in plasma levels of only 2 to 4 mg/L. One explanation may be that the fact that tissue concentrations of erythromycin exceed those in plasma, and it accumulates in the heart muscle.\(^71\) Other factors in the extracellular milieu may predispose to ventricular tachyarrhythmia. Of the five reported patients with erythromycin-associated torsades de pointes, four had experienced myocardial ischemia or been given other class III antiarrhythmic agents that affect repolarization. Our in vivo and in vitro experiments suggest that EAD-induced ventricular arrhythmias may occur after erythromycin administration even in normal animals and that bradycardia promoted these events.

In conclusion, erythromycin at doses exceeding 20 mg/L prolonged action potential duration both in vitro and in vivo. The changes in repolarization were much more pronounced in Purkinje fibers than in ventricular muscle. Pharmacological studies suggest that blockade of delayed rectifier potassium channels may underlie much of the effects of erythromycin on APD. The relative rarity of EADs suggests that other predisposing factors contribute to the acquired long QT syndrome associated with erythromycin administration. For example, erythromycin recently was shown to alter the metabolism of terfenadine, resulting in greater accumulation of the parent compound.\(^72\) Terfenadine can cause torsades de pointes in susceptible patients, and coadministration of erythromycin may potentiate its effects on the QT interval. One can only speculate that other drugs that lengthen APD might also become arrhythmogenic in the presence of erythromycin.

**Acknowledgments**

This study was supported in part by grant HL-42370 from the National Institutes of Health, grant 91015190 from the American Heart Association, Dallas, Tex, by the Veterans Administration, Washington, DC, and by the Herman C. Krannert Fund. M.R. was a recipient of a postdoctoral fellowship grant from the Max-Kade Foundation, New York, NY. The authors wish to thank Dr Gregory Ayres for his development of software for analysis of in vivo recordings, Dr Naomi Fineberg for assistance with statistical analysis, and Pfizer Central Research, Kent, UK, for their generous gift of dofetilide.

**References**

11. Ben-David J, Zipes DP. Differential response to right and left anse subclavia stimulation of early afterdepolarizations and ven-


Electrophysiological mechanisms in a canine model of erythromycin-associated long QT syndrome.
M Rubart, M L Pressler, H P Pride and D P Zipes

Circulation. 1993;88:1832-1844
doi: 10.1161/01.CIR.88.4.1832
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/88/4/1832

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/