QTU Prolongation and Polymorphic Ventricular Tachyarrhythmias Due to Bradycardia-Dependent Early Afterdepolarizations


Polymorphic ventricular tachyarrhythmias occurred spontaneously during bradycardia in dogs given the inotropic polypeptide anthopleurin-A (AP-A). The arrhythmia was investigated in in vitro and in vivo experiments. In in vitro experiments, AP-A (50 μg/l) produced bradycardia-dependent prolongation of action potential duration that was more pronounced in Purkinje than in muscle fibers. Only Purkinje fibers developed early afterdepolarizations (EAD) and triggered activity. These effects could be abolished by rapid pacing, lidocaine (4 mg/l), or tetrodotoxin (1 mg/l). In vivo experiments were conducted in anesthetized healthy dogs with simultaneous recording of surface ECG, monophasic action potentials from the endocardial and epicardial surface of the left ventricle by contact electrode catheter technique, and transmembrane action potentials from the epicardial surface of the left ventricle with a floating microelectrode technique. AP-A in a dose comparable to that used in vitro (4 μg/kg, i.v. bolus) resulted in bradycardia-dependent marked prolongation of both monophasic and transmembrane action potentials. An EAD gradually appeared on both recordings but was more marked in endocardial monophasic action potentials. Eventually, a premature ventricular depolarization arose from or very close to the peak of the EAD. The prolongation of action potentials was associated with similar prolongation of the QTU interval in surface ECG, and in some experiments, the EAD corresponded to a distinct prominent U wave. A ventricular premature depolarization arose from the U or TU complex and initiated polymorphic ventricular tachyarrhythmias that terminated spontaneously or degenerated into ventricular fibrillation. These effects were reversed by rapid pacing or lidocaine (1 mg/kg). The present study provides evidence in support of the hypothesis that AP-A-induced ventricular tachyarrhythmias are due to bradycardia-dependent EAD and triggered activity. (Circulation Research 1988;63:286–305)
Table 1. Effects of Anthopleurin-A (50 μg/l) on Transmembrane Action Potential of Canine Purkinje and Muscle Fibers

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>APA (mV)</th>
<th>MDP (mV)</th>
<th>Vmax (V/sec)</th>
<th>APD90 (msec)</th>
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<td>110±4</td>
<td>83±2</td>
<td>192±13</td>
<td>256±10</td>
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*Statistically significant (Student's t test, p<0.001); †statistically significant (Student's t test, p<0.01).

All fibers were stimulated at a cycle length of 2,000 msec.

APA, action potential amplitude; MDP, maximum diastolic potential; Vmax, maximum rate of depolarization; APD90, duration at 90% repolarization.

Figure 1. Effect of anthopleurin-A (AP-A) (50 μg/l) on action potential duration at 90% repolarization (APD90) as a function of cycle length on canine Purkinje and cardiac muscle fibers. Slopes (±SEM), correlation coefficients (r), and p values are Purkinje (control), 0.03±0.003, 0.88, 0.000; Purkinje (AP-A), 0.176±0.008, 0.98, 0.000; muscle (control) 0.15±0.002, 0.84, 0.000; muscle (AP-A) 0.34±0.002, 0.94, 0.000, respectively. In both Purkinje and muscle fibers, the slopes associated with AP-A were significantly different from control (p<0.000 for Purkinje fibers, p<0.005 for muscle fibers). Increase of APD90 by AP-A showed linear correlation with increase in cycle length (r=0.97 and 0.92 for Purkinje and muscle fibers, respectively). *p<0.05 for paired data (Scheffe's test).

potent positive inotropic agent with a mechanism of action different from catecholamines or digoxin.16-23 AP-A produces significant prolongation of cardiac action potential duration that is more marked at long cardiac cycle lengths. Ventricular fibrillation occurred during in vivo studies of AP-A in dogs.19-20 We hypothesized that the ventricular tachyarrhythmia may be due to bradycardia-dependent EADs and triggered activity induced by AP-A. The present study comprised a series of in vitro and in vivo experiments in dogs that were designed to test this hypothesis. Our study also examines issues that have not been addressed in previous studies: the origin of EAD in the in vivo heart (Purkinje fibers, muscle fibers, or both), the relation between EADs and the U wave in surface ECG, and the mechanism of STU alternans.

Materials and Methods

In Vitro Experiments

Nineteen mongrel dogs weighing 12-18 kg were anesthetized with sodium pentobarbital (30 mg/kg i.v.). A left thoracotomy was performed, and the hearts were rapidly removed and immediately placed in room temperature Tyrode's solution of (mM) Na+ 150.8, K+ 4.0, Ca2+ 2.7, Mg2+ 0.5, Cl- 146.1, HCO3 - 24.0, H2PO4 - 1.8, and dextrose 5.5. Special care was taken to minimize mechanical trauma during the incision and subsequent dissection. Small epicardial or endocardial preparations from 5×10 to 8×16 mm in area and up to 2 mm thick were dissected from the left ventricle, placed in a Plexiglas tissue bath, and superfused with Tyrode's solution equilibrated with 95% O2-5% CO2 and having a constant temperature of 37° C. pH ranged from 7.2 to 7.4, as measured by Model 12 Research pH meter (Corning Scientific Instrument, Corning, New York).

Transmembrane potentials were measured with glass microelectrodes filled with 3 M KCl, which had a resistance of 10-20 MΩ. Each microelectrode was coupled to a W-P Instruments Model M-707 microprobe system with Plexiglas holders and an integral Ag-AgCl electrode. Transmembrane potentials were continuously recorded on a two-channel...
FIGURE 2. Action potential recordings from an endocardial preparation exposed to anthopleurin-A (AP-A) (50 μg/ml). Panel A: Control recording at a drive cycle length of 2 seconds. Panels B–E: Consecutive recordings obtained 18–22 minutes after exposure to AP-A. Preparation was paced at cycle lengths of 2, 4, 6, and 8 seconds in B, C, D, and E, respectively. AP-A significantly prolonged action potential duration, and the changes were more pronounced at long cycle lengths. Panel D: Early afterdepolarization (EAD) developed during late phase 2. Number of EADs increased as the drive cycle length increased in Panel E. Right panel: Recordings obtained 30 minutes after exposure to AP-A from the same endocardial preparation. Preparation became spontaneously active due to slow background automaticity and spontaneous action potentials triggered a series of EADs. A second microelectrode (top) was used to map the preparation (recording was obtained from a site 5 mm away). There was no evidence that EADs were due to electrotonic effects of action potentials occurring at other sites in the preparation.

Gould strip chart recorder (model 220, Cleveland, Ohio) and monitored on a Tektronix 5111 storage oscilloscope (Beaverton, Oregon). Action potentials were also recorded by Norland Model 3001 digital computerized processing oscilloscope. Action potentials were analyzed for duration at 90% repolarization (APD90), maximal diastolic potential, action potential amplitude, and maximum rate of depolarization (Vmax). Control recordings were obtained after a stabilization period of 30 minutes. The preparation was stimulated by bipolar platinum electrodes (Rhodes Medical Instruments, Woodland Hills, California) and a Bloom DTU-101 MVA programmable stimulator (Bloom Inc., Reading, Pennsylvania). The stimuli consisted of rectangular pulses, 2–5 msec long, at twice threshold voltage. The preparations were stimulated at cycle lengths of 500; 1,000; 2,000; 3,000; 4,000; 6,000; 8,000; and 10,000 msec for 15, 30, 60, and 120 msec. Longer cycle lengths and single stimuli after pauses of up to 90 seconds were also tested in some experiments. After control measurements were obtained, the preparation was superfused with Tyrode’s solution containing AP-A (generously supplied by Dr. T.R. Norton, Pharmacology Department, University of Hawaii). Although we studied AP-A concentrations ranging from 20 to 250 μg/ml, only data at a concentration of 50 μg/ml (10⁻⁸ M) were analyzed. This concentration was reported to exert submaximal positive inotropic effect on ventricular muscles. EADs and triggered activity were defined according to Cranefield and Damiano and Rosen. When AP-A induced EADs, we analyzed the amplitude, coupling interval, and activation voltage of the first EAD in relation to pacing cycle length. These measurements were made as described by Damiano and Rosen. An additional microelectrode was used to map the preparation to determine whether EADs might be the result of electrotonic effects of action potentials occurring at sites other than the primary implanent site. In some experiments, the effects of lidocaine (4 mg/l) or tetrodotoxin (TTX) (1 mg/l) on AP-A–induced changes were also studied.

In Vivo Experiments

Eleven adult mongrel dogs weighing 12–18 kg were anesthetized with sodium pentobarbital (30

FIGURE 3. Effects of cycle length on early afterdepolarization (EAD) amplitude, coupling interval, and activation voltage following exposure to anthopleurin-A (AP-A) (50 μg/ml). EAD amplitude increased as the pacing cycle increased from 2,000 to 4,000 msec and then gradually decreased at longer cycle lengths. EAD coupling interval gradually increased as the pacing cycle length increased, while the EAD activation voltage became less negative. *Significant difference from data obtained at cycle length of 2,000 msec by ANOVA: *p<0.01, **p<0.001.
Figure 4. Action potential recordings from a canine endocardial preparation 20–30 minutes after exposure to anthopleurin-A (50 µg/l) showing the effects of pacing cycle length. Action potential duration was prolonged, but early afterdepolarizations were absent at a cycle length of 2 seconds. Action potential duration showed further lengthening, and a single afterdepolarization developed when the pacing cycle length was increased to 10 seconds. Number of early afterdepolarizations increased as the cycle length was lengthened to 20, 30, and 60 seconds, respectively. Both lengthening of action potential duration and early afterdepolarizations were readily suppressed when the preparation was paced at a cycle length of 1 second (bottom). S, pacing artifact.

mg/kg i.v.) and maintained with supplemental doses as required. Dogs were ventilated with room air through an endotracheal tube with a Harvard positive pressure pump. A jugular vein was cannulated for intravenous administration of drugs, and arterial pressure was monitored through a polyethylene catheter advanced to the ascending aorta through the right common carotid artery. Vagal-induced slowing of the heart was accomplished by insertion of enamel-coated stainless steel wires exposed only at the tip into the right and left cervical vasomotor trunks. Square pulses of 0.05 msec were delivered at 1–20 V at a frequency of 20 Hz. After a left thoracotomy was performed, the epicardium was opened and the heart was exposed. Bipolar pacing leads consisting of two enamel-coated stainless steel wires exposed at the tip were placed in the right atrial appendage and the right ventricular outflow tract. Pacing was performed at twice diastolic threshold with a Bloom DTU-101 MVA programmable stimulator.

Monophasic action potentials (MAPs) were recorded by contact electrode catheter technique. To record endocardial MAPs, a bipolar catheter consisting of a silver–silver chloride distal electrode and a reference lead at a distance of 5 mm was advanced into the left ventricular cavity through the left common carotid artery. Epicardial MAP were obtained by a similar catheter arrangement from the left anterior ventricular surface. The MAP signals were amplified and filtered at a frequency of 0.1–10 kHz. Epicardial transmembrane action potentials (TAPs) were recorded by a modified floating microelectrode technique. Conventional microelectrodes with resistance of 10–20 MΩ were mounted on a specially fabricated holder that coupled the microelectrode to the amplifier. The holder was made from a polyethylene tube approximately 10 cm long, 1 mm i.d., and 1.3 mm e.d. The central portion of the tube was heated under a small flame and stretched to approximately 200 µm in diameter. This stretching rendered the tube flexible at the center and allowed the microelectrode to follow the movement of the heart. The tube was filled with 3 M KCl, and the microelectrode was inserted into one end. The other end was connected to an amplifier (W-P Instruments, M-707 Microprobe System). The holder was placed over the heart with a micromanipulator con-
TABLE 2. Effects of Lidocaine and Tetrodotoxin on Canine Purkinje Fibers Superfused With Anthopleurin-A

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>APA (mV)</th>
<th>MDP (mV)</th>
<th>V_max (V/sec)</th>
<th>APD_90 (msec)</th>
</tr>
</thead>
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<tr>
<td>Control</td>
<td>4</td>
<td>118±8</td>
<td>83±4</td>
<td>440±35</td>
<td>335±30</td>
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<tr>
<td>Anthopleurin-A (50 μg/l)</td>
<td>5</td>
<td>121±10</td>
<td>84±6</td>
<td>444±39</td>
<td>839±83*</td>
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<tr>
<td>Lidocaine (4 mg/l)</td>
<td>4</td>
<td>117±9</td>
<td>84±5</td>
<td>286±22</td>
<td>303±24†</td>
</tr>
</tbody>
</table>

Control                      | 5  | 117±6    | 84±5     | 438±39        | 346±20        |
| Anthopleurin-A (50 μg/l)     | 5  | 120±7    | 85±4     | 441±38        | 878±119*      |
| Tetrodotoxin (1 mg/l)        | 117±8  | 85±5     | 221±29†   | 312±22†       |

Statistical comparisons (one-way analysis of variance and Scheffe’s test).
*p<0.001, anthopleurin-A compared with control group, and †p<0.001, lidocaine and tetrodotoxin compared with anthopleurin-A (one-way analysis of variance and Scheffe’s test).

Fibers were stimulated at a cycle length of 3,000–4,000 msec.

APA, action potential amplitude; MDP, maximum diastolic potential; V_max, maximum rate of depolarization; APD_90, duration at 90% repolarization.

trol, and the electrode was lowered onto the surface of the beating heart. Before penetration, the electrode registered an ECG recording. After one or more beats, the electrode penetrated a cell, as indicated by a DC shift and the recording of action potentials. The action potential signal was displayed on a Tektronix 5111 storage oscilloscope. Stable epicardial TAP recordings could be maintained for up to 15 minutes. When a stable impalement was lost, an attempt was made to record close to the original site. No attempt was made to minimize interference from the ECG, which contaminated phase 0 of the action potential in some recordings, or to precisely calculate the maximum rate of depolarization. For the purpose of the present study, it was noted that the only significant effects of AP-A were on phases 2 and 3 of the action potential. Continuous recording of multiple ECG leads (usually

![Figure 5](http://circres.ahajournals.org/)

**Figure 5.** Action potential recordings from two different canine endocardial preparations showing reversal of anthopleurin-A (AP-A) (50 μg/l)-induced action potential prolongation and early afterdepolarizations by lidocaine (LIDO) (4 mg/l) (top) and tetrodotoxin (TTX) (1 mg/l) (bottom).
El-Sherif et al  Ventricular Arrhythmias Due to Early Afterdepolarizations 291

FIGURE 6. Simultaneous recordings of action potentials from a Purkinje fiber (PF) and muscle fiber (MF) in a canine papillary muscle preparation. Sketch of the preparation and the site of recordings is shown on the bottom. Control recordings are shown in the first panel on the left. Recordings in the second to fifth panels were obtained after exposure to anthopleurin-A (AP-A) (50 μg/l) for 18, 24, 26, and 34 minutes, respectively, when the preparation was paced at a constant cycle length of 3 seconds. AP-A resulted in prolongation of action potential duration of both PF and MF, but the changes were more marked in PF. In the third panel, only PF developed an early afterdepolarization. In the fourth panel, the early afterdepolarization triggered an action potential that conducted to MF giving rise to a bigeminal rhythm. In the fifth panel, one microelectrode recorded from the original Purkinje fiber (PF₁), while the second microelectrode was used to map the preparation. A simultaneous recording from a second Purkinje fiber (PF₂) shows the uniform occurrence of early afterdepolarizations and triggered action potential in the Purkinje network. Both fibers show that the triggered action potential arose from the second and not the first early afterdepolarization, as was the case in the fourth panel.

Measurments were made during control and after the administration of AP-A at different cardiac cycle lengths. Phases 0–4 of the MAP were defined in the same way as the phases of a conventional TAP. Action potential amplitude and APD₉₀ of MAP and TAP were determined. When EADs developed, their amplitude (both in millivolts and as a percentage of action potential amplitude), coupling interval, and activation potential (as a percentage of action potential amplitude) were determined at different cycle lengths with similar definitions as in in vitro recordings. The QTU interval in surface ECG leads was measured between the first deviation from an isoelectric PR interval until the last deviation from baseline before the isoelectric UP interval.

**Statistical Analysis**

Student’s t test and one- or two-way analysis of variance (ANOVA) were applied when appropriate.
Simultaneous recordings at an expanded scale of action potentials from a Purkinje fiber (PF₁) and muscle fiber (MF) in the papillary muscle preparation shown in Figure 6. Purkinje recordings in Panels A–E correspond respectively to recordings in the first to fifth panels in Figure 6. Panel B: 24 minutes after exposure to anthopleurin-A (AP-A) (50 μg/l) the PF action potential duration at 90% repolarization increased from a control value of 330 to 580 msec, while the MF action potential duration at 90% repolarization increased from a control value of 180 to 190 msec. Early afterdepolarizations that developed in PF in Panel C gave rise to a triggered action potential in Panel D. Later conducted to MF with a transmission delay of 70 msec (measured from the onset of early afterdepolarizations to the onset of MF action potential). In Panel F, the first early afterdepolarizations failed to trigger an action potential while the second one did. Once again, the triggered action potential in PF conducted to MF with a transmission delay of 80 msec.

When significant difference between groups was detected by ANOVA, the data were also analyzed by Scheffe's test. Data are presented as mean ± SEM. Statistical significance was assumed at p<0.05.

Results

In Vitro Experiments

The effects of AP-A (50 μg/l) on the TAP of canine Purkinje and muscle fibers are summarized in Table 1. The most prominent change was a prolongation of the action potential duration, which was seen 8–15 minutes after superfusion with AP-A. There was no significant change in the maximum diastolic potential, action potential amplitude, or maximal rate of depolarization. The changes in APD were characteristically bradycardia-dependent. Figure 1 shows that prolongation of APD by AP-A was linearly increased as cycle length increased in both Purkinje and muscle fibers. However, the changes in APD were more pronounced in Purkinje fibers than in muscle fibers. Increasing the drive cycle length from 500 to 4,000 msec resulted in a 217% increase in APD of Purkinje fibers compared with 66% in muscle fibers.

Early afterdepolarizations. The effects of drive cycle length on APD were analyzed only in fibers that did not develop EADs. Fifty percent of Purkinje fibers developed EADs at a cycle length of 2,000 msec, and all Purkinje fibers developed EADs at cycle lengths longer than 4,000 msec (Figure 2). EADs occurred in Purkinje fibers at an activation voltage of −20 ± 3 mV (range, −8 to −36 mV) during the terminal portion of phase 2 or the beginning of phase 3. The amplitude, coupling interval, and activation voltage of the first EAD showed characteristic changes in relation to the driving cycle length (Figure 3). The amplitude of the first EAD increased from 8 ± 2 mV to 14 ± 2 mV as the drive cycle increased from 2,000 to 4,000 msec, and then gradually decreased at longer cycle lengths. On the
other hand, the EAD coupling interval gradually increased as the drive cycle length increased reflecting prolongation of phase 2 of the action potential and the consistent occurrence of the first EAD during late phase 2–early phase 3. The activation voltage of the first EAD also showed drive cycle length dependence, becoming less negative as drive cycle length increased. At drive cycle lengths of ≥2 seconds and as the superfusion time increased to 30 minutes, all Purkinje fibers developed repetitive EADs. During repetitive EADs, the amplitude of successive EADs gradually increased as the activation voltage became more negative. Repetitive EADs and triggered responses were more frequent after longer pauses and could last in excess of 20 seconds (Figure 4). The cycle length of repetitive triggered potentials was 480 ± 82 msec (range, 310–630 msec). Triggered activity was terminated by complete repolarization of the last triggered response.

In six experiments, a second microelectrode was used to map the endocardial preparation to determine whether EADs might be the result of electrotonic effects of action potentials occurring at sites other than the primary impalement site. No such action potentials were found in any of the experiments (see Figure 2, right panel). EADs were readily suppressed by many interventions. Resumption of more rapid drive frequency was effective in each experiment (Figure 4). Lidocaine (4 mg/l) and TTX (1 mg/l) were used in four and five experiments, respectively. Each drug completely abolished EADs and the prolongation of APD induced by AP-A (see Table 2 and Figure 5).

Figure 8. Simultaneous recordings of action potentials from canine epicardial (EPI) and endocardial (ENDO) preparations. Two preparations were exposed in the same perfusion chamber to anthopleurin-A (AP-A) (50 µg/l) and were stimulated at the same cycle length. Control (CONT) recordings at a drive cycle length of 3 seconds are shown in the left panel. Recordings in the middle and right panels were obtained 20–22 minutes after exposure to AP-A at a pacing cycle length of 3 and 6 seconds, respectively. AP-A prolonged action potential duration of both muscle and Purkinje fibers, but the effects were more pronounced in Purkinje fibers. At the longer pacing cycle length, only Purkinje fibers showed early afterdepolarizations.

Early afterdepolarizations in Purkinje versus muscle fibers. The development of EADs in Purkinje versus muscle fibers was investigated in three groups of experiments. First, the effects of AP-A (50 µg/l) were studied in four papillary muscle preparations. Simultaneous recordings of Purkinje and muscle fibers were obtained. AP-A resulted in prolongation of APD of both Purkinje and muscle fibers, but the changes were more marked in Purkinje fibers. Only Purkinje fibers developed EADs and triggered action potentials. The latter conducted to endocardial muscle fibers with conduction delays of 40–100 msec (Figures 6 and 7). Multiple recordings were obtained in each experiment and showed that EADs uniformly occurred in the Purkinje network (Figure 6, right panel). However, no attempt was made to map in detail the conduction of triggered action potentials at Purkinje-muscle junctional areas. Second, in four experiments, an endocardial Purkinje fiber preparation and an epicardial muscle fiber preparation were superfused with AP-A (50 mg/l) in the same tissue bath and driven at similar cycle lengths. In each experiment, only Purkinje fibers developed EADs at a slow drive frequency (Figure 8). Third, four epicardial preparations were superfused with a
Figure 9. Action potential recordings from a canine epicardial preparation after exposure to anthopleurin-A (AP-A) (250 μg/l). Control recordings are shown in Panel A. Recordings in Panels B-D were obtained 110–115 minutes after exposure to AP-A during pacing at cycle lengths of 10, 40, and 90 seconds, respectively. Panel C shows marked prolongation of action potential duration to 5.2 seconds and the occurrence of two low-amplitude early afterdepolarizations at the end of the plateau. After a longer pacing cycle in Panel D, the action potential showed further prolongation to 11 seconds. Again, two low-amplitude early afterdepolarizations occurred at the end of the markedly prolonged plateau. No triggered activity was observed in epicardial preparations exposed to AP-A.

In Vivo Experiments

Action potential recordings were obtained in 11 experiments. In nine experiments, endocardial MAPs were simultaneously recorded with either epicardial MAPs (four experiments) or epicardial TAPs (five experiments). In the remaining two experiments, only epicardial TAPs were recorded. Baseline MAPs were very similar to TAPs except for action potential amplitude. The amplitude of epicardial TAPs was 85 ± 12 mV. The amplitudes of epicardial and endocardial MAPs were 33 ± 18 and 22 ± 14 mV, respectively (Table 3).

Effects of AP-A on action potentials. The effects of AP-A were seen 6–12 minutes after drug administration. The only significant effect of AP-A was prolongation of both endocardial and epicardial APD (Figures 10 and 11). The increase in APD was more pronounced at longer cardiac cycle lengths (Figures 11C and 11D). In the nine experiments in which simultaneous endocardial and epicardial action potential recordings were obtained, EAD consistently developed in endocardial MAP but only in seven of nine epicardial recordings (Figures 10 and 11). In endocardial MAPs, EADs occurred at the termination of phase 2 (Figures 10A and 11) or during phase 3 (Figure 10B). In both epicardial MAPs and TAPs, EADs occurred during late phase 3 (Figure 10A). EADs were consistently more pro-
The first ventricular ectopic beat arose from or very close to the peak of the EAD. Ventricular arrhythmias could be induced in every experiment by a critical degree of slowing of the cardiac rate that varied among experiments. The cardiac cycle length associated with ventricular arrhythmias ranged from 480 to 2,400 msec (mean ± SEM, 1,020 ± 340 msec). Ventricular arrhythmias occurred after one or more longer cardiac cycles. However, in 10 of 112 instances, arrhythmias developed when a shorter cycle interrupted a series of long cardiac cycles (Figure 12). The occurrence of ventricular arrhythmias was associated with the development of maximum amplitude of EAD. In eight of 112 instances, there was a beat-to-beat increase of the amplitude of EAD before the occurrence of ventricular arrhythmias (Figure 13). However, in most instances, ventricular arrhythmias occurred with no perceptible change in the amplitude of EAD in preceding cycles. EAD amplitude (as a percentage of endocardial MAP), coupling interval, and activation potential (as a percentage of MAP) showed characteristic changes in relation to the cardiac cycle length (Figures 13 and 14). EAD amplitude and coupling interval gradually increased as the cardiac cycle length increased from 500 to 3,000 msec. Measurements could not be made at longer cycle lengths. Thus, a secondary decrease of EAD amplitude on further lengthening of the cycle length as was observed in vitro could not be demonstrated. In contrast to in vitro observations, the EADs in several in vivo experiments occurred progressively later during phase 3 as the cycle length increased. The cycle length of ventricular tachyarrhythmias ranged from 210 to 390 msec (mean ± SEM, 315 ± 42 msec). The majority of ventricular tachyarrhythmias were polymorphic. Polymorphic ventricular tachyarrhythmias either terminated spontaneously (Figure 15) or degenerated into ventricular fibrillation that required cardioversion (Figure 12). There was no significant difference in the cycle length of the initial few beats of tachyarrhythmias that terminated spontaneously or that degenerated into ventricular fibrillation, and no other characteristic features predicted the behavior of the tachyarrhythmias. During ventricular tachyarrhythmias, action potentials arose from a depolarized level; when the tachyarrhythmia terminated spontaneously, the last action potential was always greater in endocardial compared with epicardial recordings. Because of the smaller amplitude of the action potential in monophasic recordings, the amplitude of EADs was calculated both in millivolts and as a percentage of action potential amplitude. In every experiment, the amplitude of EADs as a percentage of action potential amplitude was always greater in endocardial compared with epicardial recordings. The mean amplitude of EADs in endocardial MAPs was 28 ± 18% the amplitude of the action potential. This was significantly greater (p < 0.0001) than the mean amplitude of EADs in epicardial recordings (10 ± 7% of the amplitude of the action potential).

**Correlation of QTU segment and action potential recordings.** In control recordings, vagal-induced slowing of the heart rate was associated with moderate increase in APD and the QTU segment of the surface ECG. A distinct or prominent U wave was never observed (Figure 11B). However, after AP-A administration, the marked prolongation of action potential and the development of EADs were simultaneously associated with characteristic changes in the QTU segment of the surface ECG. The QTU segment markedly prolonged and "giant" T (or TU) complexes were frequently seen. Excluding the two experiments in which epicardial action potential recordings failed to show EADs, there was a strong correlation between the QTU interval and both endocardial and epicardial APD (r = 0.97, n = 100). In three experiments, a distinct U wave developed after the T wave. The U wave was synchronous with the EADs in the action potential recording (Figures 10A and 11D). In these experiments, the first ventricular ectopic response arose from the peak of the EADs in the action potential recording and the peak of the U wave in the surface ECG (Figure 12).

**Relation of ventricular arrhythmias to early afterdepolarizations and cardiac cycle length.** In all experiments, spontaneous ventricular ectopic rhythms developed in the form of isolated beats, couplets, or runs of ventricular tachyarrhythmia. One hundred twelve instances of spontaneous ventricular ectopic rhythms were available for analysis. In every case, the first ventricular ectopic beat arose from or very close to the peak of the EAD. Ventricular arrhythmias could be induced in every experiment by a critical degree of slowing of the cardiac rate that varied among experiments. The cardiac cycle length associated with ventricular arrhythmias ranged from 480 to 2,400 msec (mean ± SEM, 1,020 ± 340 msec). Ventricular arrhythmias occurred after one or more longer cardiac cycles. However, in 10 of 112 instances, arrhythmias developed when a shorter cycle interrupted a series of long cardiac cycles (Figure 12). The occurrence of ventricular arrhythmias was associated with the development of maximum amplitude of EAD. In eight of 112 instances, there was a beat-to-beat increase of the amplitude of EAD before the occurrence of ventricular arrhythmias (Figure 13). However, in most instances, ventricular arrhythmias occurred with no perceptible change in the amplitude of EAD in preceding cycles. EAD amplitude (as a percentage of endocardial MAP), coupling interval, and activation potential (as a percentage of MAP) showed characteristic changes in relation to the cardiac cycle length (Figures 13 and 14). EAD amplitude and coupling interval gradually increased as the cardiac cycle length increased from 500 to 3,000 msec. Measurements could not be made at longer cycle lengths. Thus, a secondary decrease of EAD amplitude on further lengthening of the cycle length as was observed in vitro could not be demonstrated. In contrast to in vitro observations, the EADs in several in vivo experiments occurred progressively later during phase 3 as the cycle length increased. The cycle length of ventricular tachyarrhythmias ranged from 210 to 390 msec (mean ± SEM, 315 ± 42 msec). The majority of ventricular tachyarrhythmias were polymorphic. Polymorphic ventricular tachyarrhythmias either terminated spontaneously (Figure 15) or degenerated into ventricular fibrillation that required cardioversion (Figure 12). There was no significant difference in the cycle length of the initial few beats of tachyarrhythmias that terminated spontaneously or that degenerated into ventricular fibrillation, and no other characteristic features predicted the behavior of the tachyarrhythmias. During ventricular tachyarrhythmias, action potentials arose from a depolarized level; when the tachyarrhythmia terminated spontaneously, the last action potential was always greater in endocardial compared with epicardial recordings. Because of the smaller amplitude of the action potential in monophasic recordings, the amplitude of EADs was calculated both in millivolts and as a percentage of action potential amplitude. In every experiment, the amplitude of EADs as a percentage of action potential amplitude was always greater in endocardial compared with epicardial recordings. The mean amplitude of EADs in endocardial MAPs was 28 ± 18% the amplitude of the action potential. This was significantly greater (p < 0.0001) than the mean amplitude of EADs in epicardial recordings (10 ± 7% of the amplitude of the action potential).

**Correlation of QTU segment and action potential recordings.** In control recordings, vagal-induced slowing of the heart rate was associated with moderate increase in APD and the QTU segment of the surface ECG. A distinct or prominent U wave was never observed (Figure 11B). However, after AP-A administration, the marked prolongation of action potential and the development of EADs were simultaneously associated with characteristic changes in the QTU segment of the surface ECG. The QTU segment markedly prolonged and "giant" T (or TU) complexes were frequently seen. Excluding the two experiments in which epicardial action potential recordings failed to show EADs, there was a strong correlation between the QTU interval and both endocardial and epicardial APD (r = 0.97, n = 100). In three experiments, a distinct U wave developed after the T wave. The U wave was synchronous with the EADs in the action potential recording (Figures 10A and 11D). In these experiments, the first ventricular ectopic response arose from the peak of the EADs in the action potential recording and the peak of the U wave in the surface ECG (Figure 12).

**Relation of ventricular arrhythmias to early afterdepolarizations and cardiac cycle length.** In all experiments, spontaneous ventricular ectopic rhythms developed in the form of isolated beats, couplets, or runs of ventricular tachyarrhythmia. One hundred twelve instances of spontaneous ventricular ectopic rhythms were available for analysis. In every case, the first ventricular ectopic beat arose from or very close to the peak of the EAD. Ventricular arrhythmias could be induced in every experiment by a critical degree of slowing of the cardiac rate that varied among experiments. The cardiac cycle length associated with ventricular arrhythmias ranged from 480 to 2,400 msec (mean ± SEM, 1,020 ± 340 msec). Ventricular arrhythmias occurred after one or more longer cardiac cycles. However, in 10 of 112 instances, arrhythmias developed when a shorter cycle interrupted a series of long cardiac cycles (Figure 12). The occurrence of ventricular arrhythmias was associated with the development of maximum amplitude of EAD. In eight of 112 instances, there was a beat-to-beat increase of the amplitude of EAD before the occurrence of ventricular arrhythmias (Figure 13). However, in most instances, ventricular arrhythmias occurred with no perceptible change in the amplitude of EAD in preceding cycles. EAD amplitude (as a percentage of endocardial MAP), coupling interval, and activation potential (as a percentage of MAP) showed characteristic changes in relation to the cardiac cycle length (Figures 13 and 14). EAD amplitude and coupling interval gradually increased as the cardiac cycle length increased from 500 to 3,000 msec. Measurements could not be made at longer cycle lengths. Thus, a secondary decrease of EAD amplitude on further lengthening of the cycle length as was observed in vitro could not be demonstrated. In contrast to in vitro observations, the EADs in several in vivo experiments occurred progressively later during phase 3 as the cycle length increased. The cycle length of ventricular tachyarrhythmias ranged from 210 to 390 msec (mean ± SEM, 315 ± 42 msec). The majority of ventricular tachyarrhythmias were polymorphic. Polymorphic ventricular tachyarrhythmias either terminated spontaneously (Figure 15) or degenerated into ventricular fibrillation that required cardioversion (Figure 12). There was no significant difference in the cycle length of the initial few beats of tachyarrhythmias that terminated spontaneously or that degenerated into ventricular fibrillation, and no other characteristic features predicted the behavior of the tachyarrhythmias. During ventricular tachyarrhythmias, action potentials arose from a depolarized level; when the tachyarrhythmia terminated spontaneously, the last action

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**Table 3. Action Potential and Early Afterdepolarization Amplitude After Anthopleurin-A Administration in Eleven Dogs**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>APA (mV)</th>
<th>EADA (% of APA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>END MAP</td>
<td>9</td>
<td>12-37 (22 ± 14)</td>
<td>7-65 (28 ± 18)</td>
</tr>
<tr>
<td>EPI MAP</td>
<td>4</td>
<td>20-54 (33 ± 18)</td>
<td>1-8 (4 ± 3)</td>
</tr>
<tr>
<td>EPI TAP</td>
<td>7</td>
<td>68-98 (83 ± 12)</td>
<td>2-14 (8 ± 6)</td>
</tr>
</tbody>
</table>

END MAP, endocardial monophasic action potential; EPI MAP, epicardial monophasic action potential; EPI TAP, epicardial transmembrane action potential; APA, action potential amplitude; EADA, early afterdepolarization amplitude (in millivolts and as a percentage of APA). EADA was measured at cycle length of 1,000 ± 100 msec. *EADs were not recorded in EPI MAP in one dog and in EPI TAP in one dog.
FIGURE 10. Simultaneous recordings of endocardial monophasic action potential (MAP), epicardial MAP, or transmembrane action potential (TAP) and surface ECG from two experiments 12–20 minutes after administration of anthopleurin-A (AP-A) (4 μg/kg). Recordings were obtained at cardiac cycle lengths of 1,900 msec in Panel A and 1,750 msec in Panel B. Panel A: AP-A resulted in similar prolongation of endocardial MAP, epicardial MAP, and the QTU segment. A very prominent early afterdepolarization (EAD) occurred from the terminal part of the plateau in the endocardial MAP. A smaller EAD occurred very late on phase 3 of the epicardial MAP, simulating a delayed afterdepolarization. However, the peak of the epicardial EAD corresponded to the peak of the endocardial EAD and to a distinct U wave in surface ECG (marked by arrows). Panel B: AP-A resulted in prolongation of endocardial MAP and a prominent EAD arising from phase 3 (marked by arrows). Epicardial TAP failed to show an EAD but revealed an early afterhyperpolarization that corresponded to the EAD in the endocardial MAP. Duration of the epicardial TAP was 120 msec shorter than the endocardial MAP. QTU segment in lead VI showed an initial negative deflection followed by a terminal positive deflection that corresponded to the endocardial EAD. However, there was no distinct U wave as there was in Panel A.

potential returned to full repolarization and failed to show EADs (Figure 15).

STU alternans. STU alternans was seen in four experiments. In two experiments, simultaneous endocardial and epicardial recordings were obtained. In these experiments, the STU alternans corresponded to 2:1 alternation of EADs occurring on phase 3 of the action potential on both endocardial and epicardial recordings but was more pronounced in the endocardial MAPs (Figure 16). In the two other experiments, only epicardial TAPs were obtained. In one experiment (Figure 17), the STU alternans was again due to 2:1 alternation of an EAD that occurred late on phase 3. In the second experiment, STU alternans was associated with alternation of the duration of the epicardial TAPs. EADs in successive action potentials seemed to arise from alternately high and low levels of phase 3 (Figure 15). In all four experiments, the STU alternans and the 2:1 alternation of EADs was tachycardia-dependent.
A. Control B. Panel A. Duration of control endocardial and epicardial MAP was the same (220 msec) and was equal to the QTU interval. Phase 2 of the MAP was more prominent in the endocardial recording, which also showed slow phase 4 depolarization. Panel B. Lengthening of the cardiac cycle length resulted in slight increase of the duration of both endocardial and epicardial MAP and the QTU interval. Recordings in Panels C and D were obtained 10–12 minutes after administration of AP-A. In Panel C, the drug resulted in marked prolongation of phase 2 of the endocardial MAP and the development of an early afterdepolarization (EAD) at the end of phase 2. Epicardial MAP was also prolonged, but its duration was 60 msec shorter than the endocardial MAP and it failed to show EAD. QTU interval was also prolonged and, when measured in lead V1, had the same duration as the endocardial MAP. T wave was markedly inverted in lead II. Recordings in Panel D were obtained after the recordings in Panel C when the cardiac cycle length was increased from 710 to 1930 msec and the slower rate was maintained for 1 minute. There was further prolongation of endocardial MAP and an increase in the amplitude of EAD. Epicardial MAP also showed further prolongation, but its duration was shorter than the endocardial MAP by 80 msec and it still failed to show EAD. On the other hand, the QTU segment showed the development of a distinct U wave, the onset of which was synchronous with the onset of EAD in endocardial MAP.

Lengthening of the cardiac cycle length resulted in disappearance of STU alternans and a 1:1 occurrence of EADs (Figures 16C and 17). The longer cycle length was also associated with characteristic increase in the duration of the action potential and the QTU segment (Figures 16C and 17) as well as with increase in EAD amplitude and the occurrence of ventricular arrhythmia (Figure 16C).

Effects of lidocaine. In each experiment, AP-A-induced prolongation of APD, EADs, and ventricular arrhythmias could be reversed at shorter cycle lengths (Figure 13). These effects were also reversed by lidocaine (1 mg/kg i.v. bolus) in four experiments (Figure 18). The effects of lidocaine were observed while the cardiac cycle length was kept constant.

Discussion

The present study was designed to test the hypothesis that ventricular tachyarrhythmias induced by AP-A are due to bradycardia-dependent EADs and triggered activity. In vitro experiments showed that AP-A-induced action potential prolongation was associated with the development of EADs at low levels of membrane potential similar to those induced by aconitine,12 quinidine,3 N-acetyl procainamide,13 sotalol,14 and clofilium.15 On the other hand, although cesium resulted in EAD at both low and high membrane potential, only those at high membrane potential were capable of inducing triggered activity.4 Mapping the endocardial preparation provided evidence that AP-A–induced EADs represented oscilla-
Figure 12. Recordings from the same experiment shown in Figure 11 obtained 1 minute later and shows the spontaneous development of a polymorphic ventricular tachyarrhythmia that degenerated into ventricular fibrillation requiring cardioversion. First ectopic action potential arose very close to the peak of the early afterdepolarization in the endocardial monophasic action potential (MAP), while the ectopic QRS in the surface ECG occurred from the summit of the U wave. On the other hand, the first ectopic action potential occurred after complete repolarization of the epicardial MAP. Ventricular tachyarrhythmia occurred when a shorter cycle interrupted a series of long cardiac cycles; this was uncommon. Characteristically, ventricular arrhythmias developed after one or more longer cardiac cycles.

tions of membrane potential and not electrotonic effects occurring in nearby fibers. In in vivo experiments, AP-A in a dose comparable to that studied in vitro also resulted in prolongation of endocardial MAPs and both epicardial MAPs and TAPs and in the development of EADs. Epicardial TAP recordings provide the first direct evidence of the occurrence of EADs in vivo. Epicardial MAPs were very similar to the direct intracellular recordings, except for amplitude. The time course of repolarization of MAPs has been shown to correlate closely with that of simultaneous TAPs under a variety of conditions.

Evidence of the similarities between observations in endocardial preparations in vitro and endocardial MAPs in vivo include 1) a comparable time course of the changes in vitro and in vivo. However, inferences based on the similarities in the time courses of in vitro and in vivo observations must necessarily be limited because these were nonsteady state experiments, particularly the in vivo ones. 2) Similar changes in APD, EAD amplitude, and coupling interval in relation to cardiac cycle length and 3) the response to lidocaine also provided evidence. Some differences between in vitro and in vivo observations were also noted. The cycle length of EADs in vitro (480 ± 82 msec) was longer than the cycle length of ventricular tachyarrhythmias in vivo (350 ± 42 msec). The cycle length difference could be explained by the intact sympathetic system in vivo. In endocardial MAPs, single rather than multiple EADs were observed before the development of an ectopic action potential from the peak of the EAD. On the other hand, in endocardial Purkinje fibers, one or more subthreshold EADs frequently occurred before the development of triggered action potentials. This may be related to the difference in coupling within the Purkinje network and between it and myocardium in vivo, which may influence the amplitude and the propagation of the first EAD. In contrast to in vitro observations, EADs occurred progressively late on phase 3 of the endocardial MAPs on lengthening of the cardiac cycle length in some in vivo experiments (Figure 16). This difference may be related to the nature of the endocardial MAP, which reflects the potential changes from a relatively large endomyocardial region compared with intracellular recordings.

In in vivo experiments, ectopic action potentials that developed from or very close to the peak of EAD corresponded to early coupled ventricular premature beats in surface ECC showing the R-on-T or R-on-TU phenomenon. This suggests that the first ectopic beat is triggered by an EAD. Because tridimensional mapping of the heart in vivo was not available, the mechanism of subsequent beats of ventricular tachyarrhythmias is less clear. Repetitive triggered activity secondary to EADs were common in vitro. There was also some evidence in vivo that the second and subsequent ectopic ventricular responses were triggered by EADs (Figure 16). However, the possibility that subsequent ectopic beats during polymorphic ventricular tachyarrhythmias, and particularly the degeneration into ventricular fibrillation, are due to reentry caused by inhomogeneity of refractoriness cannot be excluded.
Similar to in vitro observations, the occurrence of EAD and ventricular arrhythmias in vivo were characteristically bradycardia-dependent. The cardiac cycle length associated with ventricular arrhythmias ranged from 480 to 2,400 msec (mean ± SEM, 1,020 ± 340 msec). Thus, in contrast to ventricular arrhythmias induced by cesium chloride, marked bradycardic rates were not always necessary for the occurrence of ventricular arrhythmias induced by AP-A.

AP-A is one of several neurotoxins that have become essential tools for the analysis of molecular aspects of nerve conduction and transmission. Although the mechanism of AP-A–induced prolongation of APD is not precisely defined, it is likely to be qualitatively different from most antiarrhythmic drugs. Most of the studies on the mechanism of action of AP-A and the closely related polypeptide ATXII derived from the sea anemone Anemonia sulcata have been in nerve cells. These studies suggest that sea anemone toxins markedly slow the sodium-inactivation process without changing the kinetics of sodium or potassium activation. Of interest was the finding that binding of the sea anemone toxins to the membrane site that controls closing of the sodium channel could only occur when the channel was open for sodium. When TTX caused the channel to be closed to sodium entry, the binding of the sea anemone toxins did not occur. In a study on canine ventricular muscle, AP-A–induced prolongation of action potential was accompanied by a decrease in net outward current that was attributed to an increase of sodium current during the plateau because the slow inward current and the delayed potassium current were not changed. In a preliminary study from this laboratory, we analyzed whole cell currents and potentials...
**FIGURE 14. Effects of cycle length on endocardial early afterdepolarization (EAD) amplitude (as percentage of monophasic action potential [MAP]), coupling interval, and activation potential (as percentage of MAP). EAD amplitude and coupling interval increased as the cycle length increased from 500 to 3,000 msec. *Significant difference from data obtained at cycle length of 500 msec by ANOVA: *p<0.01. On the other hand, there was no consistent change in EAD activation potential on lengthening of the cardiac cycle length. Individual results from five different experiments are shown. In three experiments, the EAD occurred progressively late during phase 3 as the cycle length increased. In the two other experiments, the EAD occurred at a lower membrane potential as the cycle length increased to 1,000 msec; then the activation potential remained essentially constant at longer cycle lengths.**

in myocytes isolated from neonatal rat ventricles with gigaohm seal electrodes before and after exposure to AP-A.38 Our findings suggest that marked prolongation of action potential by AP-A can be explained by slowed sodium inactivation. EADs were seen that corresponded in timing and magnitude to spontaneous inward currents recorded during clamp steps near the plateau level.

**Origin of Early Afterdepolarizations In Vivo**

Drug-induced EADs commonly have been demonstrated in endocardial preparations.3-5,13-15 When arrhythmias in vivo have been attributed to EADs, there was no discussion as to whether EADs originated from the Purkinje network, the myocardium, or both. In the present study, there was evidence that AP-A–induced EAD in vivo may have originated from the endocardial Purkinje network. First, in vitro studies of papillary muscle preparations exposed to a concentration of AP-A equivalent to that in vivo showed that only Purkinje fibers developed marked bradycardia-dependent prolongation of action potential, EADs, and triggered activity with a time course similar to in vivo observations. The same was true when endocardial Purkinje fibers and epicardial muscle fiber preparations were exposed to AP-A in the same tissue bath. It was extremely difficult to induce EADs in epicardial preparations, and triggered activity was never demonstrated. It is possible, however, that AP-A administration in vivo may have resulted in a more rapid initial tissue uptake and a higher concentration of the drug in myocardial cells compared with in vitro superfusion. Second, in nine experiments in which endocardial MAP was simultaneously recorded with either epicardial MAP or TAP, EADs were demonstrated in the endocardial recording in all experiments but failed to be recorded from the epicardium in two experiments. Because the entire epicardial surface was not explored, it is possible that recording from other epicardial sites may have demonstrated EADs in these two experiments. Third, the amplitude of EADs (as percentage of action potential amplitude) in endocardial recordings was significantly greater than the amplitude of EADs in epicardial recordings. However, it is important to note that the endocardial MAP probably reflects the electrical activity of Purkinje as well as subendocardial myocardial fibers.26

The possibility that the administration of AP-A in vivo results in prompt prolongation of APD, EAD, and triggered activity in Purkinje fibers and that these abnormalities are transmitted within the Purkinje network and to the myocardium is intriguing. However, the nature of the transmission of repolarization abnormalities from Purkinje network to overlying myocardium in vivo is not precisely clear. Previous studies in canine false tendon10-39 and squid axon40 have shown that repolarization abnormalities could be transmitted to a varying degree. Electrotonic transmission, actual propagation, or both have been invoked. Our observations in Purkinje-muscle preparations showed that triggered action potentials generated in Purkinje fibers conducted to endocardial muscle fibers with transmission delays of up to 100 msec. We have recently studied the transmission of repolarization abnormalities induced by clofilium in long, thin canine endocardial strips that contained 24-hour-old ischemic regions and contiguous normal endocardium.15 Clofilium selectively induced marked prolongation of the plateau and EADs in ischemic endocardium. Repetitive action potentials, but not the prolonged plateau, were transmitted to the normal region.

**Origin of U Wave in Surface Electrocardiogram**

The precise electrophysiological mechanism(s) of normal and abnormal U waves in surface ECG is
FIGURE 15. Epicardial transmembrane action potential (TAP) and surface ECG from a dog 15 minutes after administration of anthopleurin-A (4 µg/kg) showing QTU alternans and a short run of polymorphic ventricular tachycardia. QTU alternans was associated with alternation of the duration of the epicardial TAP. Early afterdepolarizations (EAD) (marked with arrows) were present in successive action potentials but arose alternately from a low and high level of phase 3 with the shorter action potential associated with EAD occurring higher on phase 3. Arrhythmia followed the beat with shorter action potential duration. First ectopic action potential seemed to arise from an EAD occurring early on phase 3, while the first ectopic QRS showed the R-on-T phenomenon. Successive ectopic action potentials arose from a depolarized level of membrane potential. Arrhythmia terminated by complete repolarization of the last ectopic action potential, which failed to show an EAD. Sinus beats after termination of the arrhythmia were again associated with EAD in the epicardial TAP, but the QTU alternans became much less prominent.

still unknown. Hoffman and Cranefield suggested that normal U wave could correspond to terminal repolarization of Purkinje fibers because their APD is approximately 30% longer than that of ventricular muscle. The mechanism of prominent U waves associated with hypokalemia was frequently studied experimentally. Watanabe produced hypokalemia in intact dogs with hemodialysis and recorded surface ECG. The changes in surface ECG were compared with corresponding changes in canine Purkinje and muscle action potentials exposed to low K+ in vitro. The study concluded that the U wave corresponds to prolonged repolarization of Purkinje fibers. In experiments on isolated rabbit heart perfused with K+-free Krebs-Henseleit solution, Surawicz et al observed U wave–like deflections in epicardial electrograms that corresponded to slowed terminal repolarization of ventricular action potentials in suction electrograms (negative afterpotential or EAD). On the other hand, Tai Fu et al observed positive U wave in local epicardial electrograms by regional intracoronary infusion of K+-free Tyrode's solution. The U waves corresponded to early afterhyperpolarization of MAP recorded from the same epicardial region. In the present study, a distinct U wave was demonstrated in three experiments synchronous with the EAD in action potential recordings (Figures 10 and 11). In three other experiments, although a distinct U wave was not seen, a very prominent terminal deflection in the QTU segment corresponding to the EAD was recorded in one or more surface ECG leads (Figure 16). These observations suggest that a significantly large EAD can give rise to a prominent and distinct U wave in surface ECG.

STU Alternans

In the experimental setting, ST alternans is a frequent phenomenon in the very early stages of acute myocardial ischemia. It has also been induced by electrical stimulation of the left stellate ganglion, hypocalcemia, hypoxia, low pH, and abrupt rate changes. The electrophysiological mechanism(s) of STU alternans is not well established. Two basic postulates have been suggested. First, repolarization alternans is secondary to alternation of depolarization, which may be caused by rate-related 2:1 conduction block in some portion of the heart. It is not uncommon for rate-related 2:1 conduction block to develop in severely ischemic regions because of abnormal prolongation of refractoriness. STU alternans in surface recordings in this situation may not be associated with discernible alternation of the QRS because of the
Figure 16. Recordings of epicardial (EPI) transmembrane action potential (TAP), endocardial (END) monophasic action potential (MAP), and surface ECG showing QTU alternans. Panel A: Control recordings. Panel B: Recordings obtained 14 minutes after the administration of anthopleurin-A (AP-A) (4 µg/kg) showing QTU alternans due to 2:1 alternation of an EAD (marked by arrows) that was more prominent in the endocardial MAP. Cardiac cycle during the alternans was 450 msec. Both epicardial and endocardial recordings were repostioned in Panel B. Panel C: Cardiac cycle length was increased to 700–750 msec by vagal stimulation. Epicardial TAP could not be maintained. There was further prolongation of the endocardial MAP and QTU segment with disappearance of the STU alternans. Every action potential was followed by an early afterdepolarization (EAD). Amplitude of the EAD significantly increased, and the deflection occurred during late phase 3 to simulate a delayed afterdepolarization. A short run of monomorphic ventricular tachycardia occurred and was initiated by an ectopic beat with different QRS configuration. First ectopic action potential arose from the peak of the EAD and was followed by five action potentials with relatively monomorphic configuration. Arrhythmia terminated by full repolarization of the last action potential, which did not show an EAD. Second sinus beat after termination of the arrhythmia was again followed by an ectopic beat with QRS configuration similar to the ectopic beat that ushered the tachycardia run (both beats are marked by asterisks). Ectopic action potential arose from the peak of the preceding EAD, but it failed to initiate subsequent action potentials when it completely repolarized. A small EAD occurred at late phase 2–early phase 3 of the action potential and at exactly the same level from which the second ectopic action potential arose during the tachycardia. This suggests that the second and, probably subsequent, action potentials during the tachycardia were triggered by EAD. QTU interval was markedly prolonged, and the QTU segment showed a terminal prominent deflection synchronous with the EAD in the endocardial MAP. However, there were no distinct features as is shown in Figure 11.
FIGURE 17. Epicardial (EPI) transmembrane action potential (TAP) and surface ECG leads recorded from another dog 12 minutes after the administration of anthopleurin-A (4 μg/kg) showing rate dependence of the QTU alternans. Alternans was due to 2:1 alternation of an early afterdepolarization. Increasing the cardiac cycle length (arrows) resulted in disappearance of QTU alternans and 1:1 occurrence of early afterdepolarizations as well as the characteristic prolongation of action potential and QTU segment associated with longer cycle lengths.

FIGURE 18. Recordings from the same experiment shown in Figure 17 illustrating the effects of lidocaine (1 mg/kg i.v. bolus). Panel A: At 15 minutes after anthopleurin-A administration (4μg/kg), marked prolongation of epicardial (EPI) transmembrane action potentials (TAP) and a bigeminal ventricular rhythm developed. Ectopic action potentials arose alternately early and late on phase 3. Last ectopic beat arising from early phase 3 initiated polymorphic ventricular tachyarrhythmia. Panels B-D: Recordings obtained 1, 2, and 3 minutes after lidocaine administration showing suppression of ventricular arrhythmias and gradual reversal of anthopleurin-A–induced action potential prolongation. This was associated with normalization of the QTU segment to control configuration before anthopleurin-A administration.
minimal contribution of slowed and delayed depolarization of an ischemic region to the overall QRS complex. Second, alternans develops at the level of single cardiac cells because of alternation in the rate and extent of electrolyte transfers across the cardiac membrane. Alternation of APD on increase of stimulus frequency can be attributed to alternation in the extent to which the outward current $i_o$ decays or to alternation in the degree of recovery from inactivation of the slow inward current. $^{51-53}$ It has been suggested that alternation of slow inward current may be involved in STU alternans during acute ischemia. $^{54}$ There is no reason to assume that a single mechanism is responsible for all cases of STU alternans. In the present study, STU alternans after AP-A administration was due to 2:1 alternation of an EAD and may have specifically represented U wave alternans. Similar to most other reported examples of STU alternans, the phenomenon was characteristically tachycardia-dependent. A 2:1 alternation of EAD was not observed in in vitro Purkinje fibers exposed to AP-A. This suggests that the in vivo observation may not be due to alternation of the ionic current(s) involved in the generation of EAD at the Purkinje fiber level but is rather a manifestation of 2:1 alternation of the propagation of locally generated EAD.

**Clinical Implication**

The present study is relevant to clinical examples of drug-induced QTU prolongation and polymorphic ventricular tachyarrhythmias. Some reports have already described EAD-like deflections in MAP recordings in patients with long QTU and torsades de pointes. $^{55,56}$ Future recordings of endocardial MAP in patients with idiopathic or drug-induced QTU prolongation may provide further evidence in support of the hypothesis that bradycardia-dependent EAD and triggered activity is the underlying electrophysiological mechanism for the arrhythmia in these cases.

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**Key Words** • polymorphic ventricular tachyarrhythmias • early afterdepolarizations • long QTU • U wave • TU alternans
QTU prolongation and polymorphic ventricular tachyarrhythmias due to bradycardia-dependent early afterdepolarizations. Afterdepolarizations and ventricular arrhythmias.

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