Action Potential Duration Alternans in Dog Purkinje and Ventricular Muscle Fibers

Further Evidence in Support of Two Different Mechanisms

Hirokazu Saitoh, MD, John C. Bailey, MD, and Borys Surawicz, MD

An abrupt shortening of cycle length causes action potential duration (APD) alternation in both canine Purkinje (P) and ventricular (V) muscle fibers. Our recent study suggested that APD alternans is determined by the process controlling APD during electrical restitution in P but not in V fibers. In the latter, alternans was attributed to changes in the availability of intracellular calcium \([\text{Ca}^{2+}]\). We examined this hypothesis further with the following pharmacologic probes known to alter restitution or action of \([\text{Ca}^{2+}]\): tetrabotoxin (0.5–3.0 \(\mu\)M), lidocaine HCl (2.0–12.0 \(\mu\)g/ml), sotalol (10 \(\mu\)M), nicorandil (10–20 \(\mu\)M), 4-amino-pyridine (0.5 \(\mu\)M), ryanodine (10 \(\mu\)M), caffeine (2 mM), and ARL 115 BS (100 \(\mu\)M). Alternans in P fibers persisted under all studied conditions but varied in magnitude depending on the time constant and amplitude of restitution. In V fibers, the magnitude of alternans did not correlate with APD changes during restitution, and APD alternans was associated with the alternans of action potential shape and alternans of developed tension. Alternans in V was suppressed by caffeine at 2.0 mM \([\text{Ca}^{2+}]\), when tension was increased and by ryanodine at 1.0 mM \([\text{Ca}^{2+}]\), when tension was decreased. Alternans in V was not altered by changes in \([\text{Ca}^{2+}]\), within the range of 1.0–4.0 mM; by ARL 115 BS, a compound that increases myofibrillar sensitivity to calcium; or by any other pharmacologic probes. We concluded that in P fibers, APD alternans was determined by the factors controlling APD in the absence of alternans; V fibers possess an independent mechanism of alternans linked to alternans of tension and controlled by \([\text{Ca}^{2+}]\); in V fibers, alternans could be suppressed by both positive and negative inotropic interventions; and calcium released from sarcoplasmic reticulum plays an important role in the V alternans. (Circulation 1989;80:1421–1431)

When Purkinje or ventricular muscle fibers are driven at a regular basic cycle length and the cycle is shortened abruptly, action potential duration (APD) shortens gradually until it reaches a new steady-state value. During the intervening non–steady state, the duration of each action potential is determined by the following three variables: APD at the basic cycle length, the influence of the past history (i.e., the time-dependent decrease of the memory of the preceding APD), and the kinetics of electrical restitution. The memory and the restitution have opposing effects on APD because, during restitution, action potential lengthens with increasing preceding diastolic interval (DI), whereas with declining memory effect, action potential shortens as the preceding DI increases.

Recently, we examined the role of memory and restitution in the process of APD alternans induced by abrupt shortening of cycle in the canine Purkinje and ventricular muscle fibers. We found that the course of memory dissipation was not affected by the presence of alternans in either fiber type. We also found that APD change during alternans was dependent on the preceding DI in the same manner as during restitution in Purkinje but not in ventricular muscle fibers. In ventricular but not Purkinje fibers, alternans was suppressed by the blocker of slow inward current (\(i_{\text{sl}}\)), nisoldipine, and attenuated by the activator of \(i_{\text{sl}}\), Bay K 8644. We postulated that alternans of APD and shape in ventricular muscle fibers resulted from the differences in concentration, handling, or both of intracellular calcium \([\text{Ca}^{2+}]\).

The purpose of our study was to test further the hypothesis that alternans in Purkinje and ventricu-
lar muscle fibers is controlled by two fundamentally different mechanisms. We examined the effects of various changes in restitution with many pharmacologic probes acting on membrane currents other than \(i_{\text{Na}}\), and we investigated the effect of various pharmacologic probes known to affect the concentration, action, or both of \([\text{Ca}^{2+}]\).

Our results show that Purkinje fibers have no independent mechanism of alternans because under all studied circumstances APD alternans in Purkinje fibers could be explained by the mechanisms that control APD during non–steady state in the absence of alternans. However, ventricular muscle fibers possess an independent mechanism of APD alternans that is linked to alternans of developed tension and is controlled by \([\text{Ca}^{2+}]\). Our results suggest that calcium released from sarcoplasmic reticulum plays an important role in this process. Different mechanisms explain the differences in the behavior of alternans in the two fiber types.

**Methods**

Adult mongrel dogs of either sex (weight, 10–20 kg) were anesthetized with sodium secobarbital (30 mg/kg i.v.). Their hearts were removed rapidly through a right thoracotomy and immersed in cool oxygenated Tyrode’s solution. Free-running Purkinje false tendons (5–10 mm long, <1 mm diameter) and attached tips of papillary muscles (3–10 mm wide, 5–10 mm long, 1–2 mm thick) were excised from either ventricle, affixed to the paraffin floor of a 10-ml Lucite muscle chamber, and superfused with Tyrode’s solution gassed with 95% O$_2$-5% CO$_2$.

The composition of Tyrode’s solution (mM) was Na, 148; K, 4.0; Cl, 127; Ca, 2.0; H$_2$PO$_4$, 0.9; Mg, 0.5; and glucose, 5.5. The pH of this solution was 7.3±0.05 after oxygenation. The bath temperature was kept constant at 36±0.2°C.

Glass microelectrodes filled with 3 mM KCl (direct current resistance, 10–15 MΩ) were used to record transmembrane action potentials simultaneously from Purkinje and ventricular muscle fibers. Electrical stimuli of 2-msec duration and twice-diastolic threshold strength were delivered through Teflon-coated bipolar silver electrodes placed at ventricular muscle site about 1–2 mm from the insertion of the false tendon. The preparations were driven at a basic cycle length of 1,000 msec for 2–3 hours before the experiments were begun. The basic cycle length was changed abruptly to a series of short cycle lengths. The shortest cycle length corresponded to the effective refractory period, and the subsequent cycle lengths were increased successively by 20 msec until the cycle length was equal to effective refractory period plus 200 msec. Between each change to a new cycle length, the preparation was driven at basic cycle length for a time sufficient to return to control state.

Action potentials were displayed on an oscilloscope and photographed with a Polaroid camera. They also were stored on a magnetic tape and reproduced on a strip-chart recorder at a paper speed of 250 mm/sec. We measured APD at 100% repolarization in both fiber types at the intersection of the tangent line fitted to phase 3 by eye with the horizontal line at the level of maximum diastolic potential. DI was measured from this intersection to the upstroke of the following action potential. APD was also measured at the level of plateau. The plateau duration (D$_p$) in Purkinje fibers was measured at the level of intersection of two tangent lines, one fitted to phase 2 and the other fitted to phase 3. In ventricular muscle fibers, D$_a$ was measured at the level of 30% repolarization. APD alternans was defined arbitrarily as the sequence of at least three action potentials where one in the middle was longer or shorter by 4 or more msec than both the preceding and the following action potential. The ratio of D$_a$ to APD was arbitrarily used as the indicator of the action potential shape in ventricular muscle fibers.

Restitution curves were constructed by plotting the first APD at each cycle length against the preceding DI. The restitution curve was described by:

\[
\text{APD}_t = \text{APD}_0 [1 - A \exp(-t/T)]
\]

where APD$_t$ is the APD preceded by DI of t, APD$_0$ is the APD at a basic cycle length, A is the amplitude (i.e., APD$_0$/APD$_t$ at the shortest DI), and T is the time constant of exponential component. Within the range of DI less than 200 msec, the correlation coefficient between APD, and a single exponential component of the fitted equation was 0.97 or more in Purkinje fiber and 0.94 or more in ventricular muscle fibers. The values of T and A in Purkinje fibers are close to those of the fast component obtained in a two-exponential restitution model.

The values of T and A in ventricular muscle fibers are intermediate values between those of the fast and slow components in a two-exponential restitution model.

The preparation was superfused with solutions containing one of the following substances: tetradotoxin (TTX; Sanky; 0.5–3.0 µM), lidocaine hydrochloride (Astra Pharmaceutical; 2.0–12.0 µg/ml), Sotalol (Mead & Johnson; 10 µM), SG-75 (Chugai; 10–20 µM), 4-aminopyridine (Sigma; 0.5 µM), ryanodine (S.B. Penick & Co; 10 µM), caffeine (Sigma; 2.0 mM), and ARL 115 (Boehringer-Ingelheim, 100 µM). The effects of ryanodine were determined after 60–80 minutes, whereas those of other pharmacologic probes were determined after 20–40 minutes of superfusion.

In a series of experiments using caffeine, ryanodine, and ARL 115, we measured developed isometric tension simultaneously with action potential in isolated trabeculae or papillary muscles from the right ventricle (0.5–1.0 mm in diameter). In these experiments, one end of the preparation was fixed with the rigid pins, and the other end was connected by a silk thread to a rigid rod attached to a Statham
TABLE 1. Effect of Drugs on Restitution and Magnitude of Alternans in Canine Purkinje Fibers

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>BCL (msec)</th>
<th>APDb (msec)</th>
<th>Restitution T (msec)</th>
<th>A</th>
<th>Change of alternans magnitude (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>1,000</td>
<td>329±13</td>
<td>156±10</td>
<td>0.39±0.2</td>
<td>-41.8±3.0</td>
</tr>
<tr>
<td>TTX 1 µM</td>
<td>5</td>
<td>2,720±78</td>
<td>340±13</td>
<td>238±24*</td>
<td>0.37±0.2†</td>
<td>-36.0±3.2</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>1,000</td>
<td>347±13</td>
<td>149±7</td>
<td>0.41±0.1</td>
<td>-29.2±4.7</td>
</tr>
<tr>
<td>Lidocaine 4 µg/ml</td>
<td>5</td>
<td>2,780±74</td>
<td>342±13</td>
<td>226±26*</td>
<td>0.39±0.1†</td>
<td>-30.7±3.2</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>1,000</td>
<td>351 12</td>
<td>135±7</td>
<td>0.37±0.3</td>
<td>+16.4±4.5</td>
</tr>
<tr>
<td>SG-75 10 µM</td>
<td>5</td>
<td>694±20</td>
<td>353±13</td>
<td>94±4*</td>
<td>0.37±0.3</td>
<td>+16.4±4.5</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>1,000</td>
<td>347±30</td>
<td>147±9</td>
<td>0.40±0.2</td>
<td>+36.0±10.1</td>
</tr>
<tr>
<td>4-AP 0.5 µM</td>
<td>5</td>
<td>1,000</td>
<td>347±29*</td>
<td>149±5</td>
<td>0.35±0.2*</td>
<td>+36.0±10.1</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>1,000</td>
<td>341±20</td>
<td>136±4</td>
<td>0.42±0.1</td>
<td>-42.2±3.2</td>
</tr>
<tr>
<td>Caffeine 2 mM</td>
<td>5</td>
<td>802±38</td>
<td>342±21</td>
<td>135±4</td>
<td>0.31±0.2†</td>
<td>-42.2±3.2</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>1,000</td>
<td>349±21</td>
<td>143±4</td>
<td>0.41±0.1</td>
<td>-40.7±3.2</td>
</tr>
<tr>
<td>Ryanodine 10 µM</td>
<td>5</td>
<td>822±29</td>
<td>352±22</td>
<td>138±4</td>
<td>0.35±0.1</td>
<td>-40.7±3.2</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM.
BCL, basic cycle length; APDb, action potential duration at BCL; T, time constant of restitution; A, amplitude of restitution.

*p<0.01, †p<0.05, ‡p<0.001 compared with control.

transducer (Electronics for Medicine). The resting tension of the preparation was adjusted to approximately 60% of that required to produce maximum tension development as measured at a cycle length of 1,000 msec.

Results are presented as the mean±SEM. Continuous variables such as APD were analyzed by t test. Because ratios are not normally distributed, these were analyzed with the Wilcoxon signed-rank test. The results were considered significant at p less than 0.05.

Results

Purkinje Fibers

Characteristics of action potential at the basic cycle length of 1,000 msec. The mean values of the maximum diastolic potential, action potential amplitude, and maximum depolarization rate of phase 0 (Vmax) were -89.4±0.4 mV, 126.6±0.4 mV, and 616±7 V/sec (n=35). None of these values were changed significantly by any of the pharmacologic probes. APDb averaged 345±8 msec (n=35). Lidocaine shortened APDb by 18.5±3.0% (n=5), TTX by 16.0±2.5% (n=5), and SG-75 by 5.7±1.8% (n=5). Sotalol lengthened APDb by 15.5±2.1% (n=5), 4-AP by 7.5±1.2% (n=5), caffeine by 10.4±0.5% (n=5), and ryanodine by 13.5±1.8% (n=5). All of the above changes were statistically significant.

Effect of pharmacologic probes on restitution. The effects of each pharmacologic probe on the time constant and the amplitude of restitution are summarized in Table 1. In the studies of the pharmacologic probes that either shortened APDb by more than 10% (TTX and lidocaine) or lengthened APDb by more than 10% (sotalol, caffeine, and ryanodine), we adjusted the basic cycle length to obtain an APDb that was nearly equal (within 4 msec) to the control APDb (Table 1). This adjustment resulted in only quantitative changes in the amplitude of alternans (unpublished observations), and the procedure was justifiable because we have shown previously that in Purkinje fibers APDb a difference of about 150 msec had no effect on kinetics of restitution.\(^4\)

Table 1 shows that TTX and lidocaine lengthened significantly the time constant and decreased significantly the amplitude of restitution. Sotalol shortened significantly the time constant without significant change of the amplitude of restitution; 4-AP, caffeine, and ryanodine decreased significantly the amplitude without significant change of the time constant of restitution. SG-75 did not change significantly either the time constant or the amplitude of the restitution.

Effect of pharmacologic probes on the magnitude of APD alternans. As reported previously,\(^3\) APD alternans appeared in each preparation after an abrupt change to a certain critical short cycle. The first APD at the new cycle length was shorter than APDb, the second APD was longer than the first APD, the third APD was shorter than the second APD, and so on. At each cycle length, the magnitude of APD alternans, defined as the difference between two consecutive APDs, decreased progressively with time. APD alternans was not associated with alternans of action potential shape. These characteristics of APD alternans were not changed by any of the pharmacologic probes.

Table 1 shows the changes in the magnitude of alternans induced by the pharmacologic probes. The results represent the average change of the difference between the first and the second APDs at the five shortest cycles in each experiment. The magnitude of alternans was decreased by TTX, lidocaine, SG-75, caffeine, and ryanodine and was
increased by sotalol and 4-AP. Similar changes in the magnitude of alternans occurred between other consecutive APDs during alternans (i.e., difference between the second and the third APD, the third and the fourth APD, and so on).

Relation between the changes in restitution, APD, and magnitude of APD alternans. We compared the DI/APD relation during alternans and during restitution in a previously described manner (i.e., by plotting the differences between two consecutive APDs during alternans against the corresponding differences between two APDs on the restitution curve at common DIs). The plots showed a highly significant correlation between these two values \( r=0.99, p<0.001 \) with the slopes of the regression line being not significantly different from 1. None of the pharmacologic probes changed significantly either the slope or the intercept of the regression lines.

Because each APD, during restitution is determined by the APDb and the T and A values during restitution, we examined the effect of these three variables on the magnitude of APD alternans. For these experiments, we chose lidocaine and TTX because these two probes altered both the APDb and the kinetics of restitution in a concentration-dependent manner. In two preparations, we determined the effect of lidocaine at concentrations of 2, 4, 8, and 12 \( \mu g/ml \); in two other preparations, the effect of TTX at concentrations of 0.5, 1.0, 2.0, and 3.0 \( \mu M \). A representative experiment with lidocaine is shown in Figure 1. To obtain an APDb that was nearly equal (within 4 msec) to the control APDb at the basic cycle length of 500 msec, we adjusted the basic cycle length in the presence of 2, 4, 8, and 12 \( \mu g/ml \) lidocaine concentrations to 700, 1,000, 1,500, and 2,000 msec, respectively. Figure 1 (left panel) shows progressive lengthening of the time constant and progressive decrease of the amplitude of restitution at increasing concentrations of lidocaine. Figure 1 (right panel) shows a concomitant progressive decrease of the magnitude of alternans of the initial six consecutive APDs after the change of cycle length to 300 msec at increasing concentrations of lidocaine.

The inset of Figure 1 shows a plot of the differences between the first and the second APD after the change of cycle length to 300 msec against the time constant of restitution at each concentration of lidocaine. The correlation between these two values is highly significant \( r=0.99, p<0.001 \). Also, the correlation between the magnitude of APD alternans and the amplitude of restitution (not shown in Figure 1) is highly significant \( r=0.97, p<0.01 \). Similar concentration-dependent correlations between the changes in the restitution and the magnitude of alternans were present in the other experiment with lidocaine and in both experiments with TTX. In these four experiments, the time constant of restitution ranged from 61 to 315 msec, the amplitude of restitution from 0.29 to 0.49, and the APDb from 280 to 320 msec.

The results of the above four experiments were pooled and subjected to multiple regression analysis with the magnitude of alternans as the dependent variable and the time constant of restitution, the amplitude of restitution, and the APDb as the independent or predictor variables. The following equation was obtained (MINIT Statistical Software, University of California Press, 1983):

\[
d-\text{APD} = -102.5 - 0.18t + 2.0A + 0.33\text{APD}_b
\]

where \( d-\text{APD} \) is the difference between the first and the second APD after changing cycle length to 300 msec, \( t \) is the time constant of restitution, and \( A \) is the amplitude of restitution. All the terms are highly significant \( p<0.001 \), and the overall significance of the regression equation has a multiple \( r \) value of 0.986 and a \( p \) value of less than 0.001. These results indicate that each of the three variables contributes independently to the magnitude of alternans as follows: the magnitude of alternans is decreased by the higher value of time constant of restitution and

![Figure 1. Plots of the effect of four different lidocaine concentrations on restitution (left) and action potential duration (APD) alternans (right) of the first six action potentials after change to cycle length of 300 msec. With increasing lidocaine concentration, the time constant of restitution (T) increases progressively from 64 in control to 122, 227, 277, and 312 msec; the amplitude of restitution decreases from 0.49 in control to 0.46, 0.44, 0.41, and 0.37; and the magnitude of alternans decreases. In the inset, difference between the second and the first APD is plotted against the T of restitution. Solid line in the inset is fitted to the data by linear regression \( r=0.99, p<0.001 \).](image-url)
TABLE 2. Effect of Drugs on Restitution and Magnitude of Alternans in Canine Ventricular Muscle Fibers (Basic Cycle Length, 1,000 msec)

<table>
<thead>
<tr>
<th>Drugs</th>
<th>n</th>
<th>APD&lt;sub&gt;b&lt;/sub&gt; (msec)</th>
<th>D&lt;sub&gt;pl&lt;/sub&gt; to APD (%)</th>
<th>Restitution</th>
<th>Alternans magnitude* (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>234±8</td>
<td>60.9±2.3</td>
<td>T (msec)</td>
<td>A</td>
</tr>
<tr>
<td>TTX 1 μM</td>
<td>6</td>
<td>222±9*</td>
<td>63.1±3.2</td>
<td>159±23</td>
<td>0.17±0.1</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>221±4</td>
<td>65.3±1.8</td>
<td>157±24</td>
<td>0.20±0.2</td>
</tr>
<tr>
<td>Lidocaine 4 μg/ml</td>
<td>6</td>
<td>218±3</td>
<td>65.3±1.8</td>
<td>157±25</td>
<td>0.20±0.4</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>233±7</td>
<td>66.3±1.3</td>
<td>153±16</td>
<td>0.20±0.3</td>
</tr>
<tr>
<td>SG-75 20 μM</td>
<td>5</td>
<td>223±5†</td>
<td>64.0±2.6</td>
<td>152±16</td>
<td>0.19±0.3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>219±5</td>
<td>65.7±1.1</td>
<td>139±7</td>
<td>0.17±0.3</td>
</tr>
<tr>
<td>Sotalol 10 μM</td>
<td>6</td>
<td>237±4‡</td>
<td>65.5±1.6</td>
<td>152±12</td>
<td>0.15±0.3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>218±8</td>
<td>61.5±1.3</td>
<td>161±19</td>
<td>0.15±0.2</td>
</tr>
<tr>
<td>4-AP 0.5 μM</td>
<td>5</td>
<td>231±7†</td>
<td>63.8±2.2</td>
<td>165±24</td>
<td>0.17±0.3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>217±9</td>
<td>56.7±2.4</td>
<td>165±10</td>
<td>0.18±0.2</td>
</tr>
<tr>
<td>Caffeine 2 mM</td>
<td>6</td>
<td>228±5</td>
<td>60.6±2.5*</td>
<td>124±4†</td>
<td>0.26±0.1*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>224±6</td>
<td>61.4±2.4</td>
<td>158±8</td>
<td>0.19±0.2</td>
</tr>
<tr>
<td>Ryanodine 10 μM</td>
<td>6</td>
<td>248±5*</td>
<td>70.7±1.9*</td>
<td>106±4*</td>
<td>0.30±0.1*</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>214±5</td>
<td>62.0±3.1</td>
<td>141±6</td>
<td>0.17±0.2</td>
</tr>
<tr>
<td>ARL 115 100 μM</td>
<td>7</td>
<td>206±4*</td>
<td>63.4±3.1</td>
<td>146±10</td>
<td>0.23±0.1†</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM.

* Difference of action potential duration between the seventh and the eighth action potential.

APD<sub>b</sub>, action potential duration at basic cycle length; D<sub>pl</sub>, plateau duration; T, time constant of restitution; A, amplitude of restitution.

*p<0.01, †p<0.05, ‡p<0.001 compared with control.

increased by the higher value of the amplitude of restitution and of the APD<sub>b</sub>.

The results in Table 1 are consistent with these findings. The attenuation of alternans by SG-75 can be attributed to shortening of APD<sub>b</sub>, the attenuation of alternans by caffeine and ryanodine to reduced amplitude of restitution, the enhancement of alternans by sotalol to shortened time constant of restitution, and the enhancement of alternans by 4-aminoypyridine to lengthening of APD<sub>b</sub>.

Ventricular Muscle Fibers

Characteristics of action potential at the basic cycle length of 1,000 msec. The mean values of resting membrane potential, action potential amplitude, and V<sub>max</sub> were -83.1±0.2 mV, 112±0.2 mV, and 199±2 V/sec, respectively. These values were not changed significantly by any pharmacologic probes except for a significant increase of the action potential amplitude by caffeine from 110±3 to 114±3 mV and by ryanodine from 113±2 to 116±2 mV. As shown in Table 2, APD<sub>b</sub> was significantly shortened by TTX, SG-75, and ARL 115; significantly lengthened by sotalol, 4-AP, and ryanodine; and not significantly changed by lidocaine or caffeine. The ratio of D<sub>pl</sub> to APD was increased significantly by caffeine and ryanodine and not changed significantly by the remaining probes.

Effect of pharmacologic probes on restitution. The effects of each pharmacologic probe on the kinetics of restitution are summarized in Table 2. The time constant of restitution was shortened significantly by caffeine and ryanodine and not changed significantly by the remaining probes. The amplitude of restitution was decreased significantly by TTX; increased significantly by caffeine, ryanodine, and ARL 115; and not changed significantly by lidocaine, SG-75, sotalol, and 4-AP.

Effect of pharmacologic probes on APD alternans. Similar to Purkinje fibers, APD alternans occurred after an abrupt change to a certain critical short cycle length. However, APD alternans was always associated with alternans of action potential shape (i.e., alternans of the ratio of D<sub>pl</sub> to APD). The first action potential after the change in cycle length was squarelike with a longer plateau and a steeper repolarization course, whereas the second action potential was more triangular with a shorter plateau and a slower course of terminal repolarization. The third action potential was again squarelike; the fourth, triangularike, and so on. Beginning with the third action potential, alternans of action potential shape was accompanied by an alternans of APD with the triangular action potentials being longer than both the preceding and the following square action potentials.

Table 2 shows the effects of each pharmacologic probe on the magnitude of APD alternans between the seventh and the eighth APD after the change of cycle length to 240 msec. In seven of 35 experiments in which a one-to-one response to stimulation could not be maintained at a cycle length of 240 msec (three with sotalol, two with 4-AP, and one each with SG-75 and ryanodine), alternans was examined at a cycle length of 260 msec. The magnitude of APD alternans was not changed signifi-
significantly by lidocaine, TTX, SG-75, sotalol, 4-AP, or ARL 115. APD alternans was abolished by caffeine and markedly attenuated by ryanodine.

Relation between the changes in restitution, APD<sub>b</sub>, and magnitude of APD alternans. The magnitude of APD alternans in ventricular muscle fibers had no consistent relation to the three variables that controlled the magnitude of alternans in Purkinje fibers (i.e., the time constant of restitution, the amplitude of restitution, and the APD<sub>b</sub>). Figure 2 shows a representative experiment in which TTX decreased the amplitude of restitution, prolonged the time constant of restitution, and shortened APD<sub>b</sub> from 210 to 200 msec. Each of these three effects would have decreased the magnitude of alternans in Purkinje fibers. However, TTX did not change the magnitude of APD alternans in this experiment and in other experiments (Table 2).

Figure 3 shows a representative experiment in which caffeine increased the amplitude and shortened the time constant of restitution. These effects would have increased the magnitude of alternans in Purkinje fibers, yet caffeine abolished APD alternans in this and in five other preparations at each cycle length.

Ryanodine increased the amplitude of restitution, shortened its time constant, and lengthened APD<sub>b</sub>. Each of these effects would have increased the magnitude of alternans in Purkinje, but in ventricular muscle alternans was attenuated by ryanodine. SG-75, sotalol, and 4-AP did not change the magnitude of APD alternans despite significant changes in APD<sub>b</sub>.

**Alternans of Action Potential Shape and Developed Tension in Ventricular Muscle Fibers**

The results of our previous study<sup>3</sup> using nisoldipine and Bay K 8644 suggested that in ventricular muscle fibers APD alternans was linked to factors controlling action potential shape. We postulated that alternans of APD and shape resulted from differences in the concentration, handling, or both of [Ca<sup>2+</sup>]. To examine further this hypothesis, we studied the effects of several procedures known to alter the concentration, action, or both of [Ca<sup>2+</sup>] on the alternans of action potential shape and the developed tension.

**Effect of caffeine.** Figure 4 shows an example of simultaneous recording of tension and ventricular action potential in an experiment in which cycle length was changed from 1,000 to 240 msec before and during superfusion with 2 mM caffeine. In control, there is continuous alternans of tension beginning with the first beat after change in cycle length. During alternans, the weaker tension is associated with a square and the stronger tension with a triangular action potential. This relation was present during alternans at each cycle length in each experiment.

In agreement with previously reported findings in dog ventricular muscle,<sup>6,7</sup> maximum increase of developed tension after administration of 2 mM caffeine occurred within 5 minutes. Subsequently, tension decreased gradually but remained above the
control level for more than 2 hours. In six preparations, the average maximum tension was 29.6±6.9% more than control. The average tension at the time of the change to shorter cycles was 14.9±9.3% more than control. Figure 4B shows that the tension of the first beat after change in cycle length is weaker than that of the basic beat. Subsequently, tension increases gradually without any alternation. Similarly, there is no alternans of the action potential shape.

In each of the six preparations, 2 mM caffeine completely suppressed alternans of both tension and the ratio of $D_p$ to APD at each cycle length. These results are summarized in Figure 5, which shows the average values of tension and the ratio of $D_p$ to APD after change of cycle length from 1,000 msec to 240 msec. In control (top), the stronger tension is accompanied by the smaller and the weaker tension by the larger ratio of $D_p$ to APD. During superfusion with 2 mM caffeine (bottom), both tension and the ratio of $D_p$ to APD change gradually without alternans.

**Effect of ARL 115 BS.** Superfusion with 100 M ARL 115 BS increased tension at a basic cycle length of 1,000 msec by 64.2±8.2% in seven preparations (Figure 6). After change of cycle length to 240 msec, tension in the presence of ARL 115 BS was also more than in control. However, ARL 115 BS did not change significantly the percent difference between two consecutive magnitudes of tension and of action potential shape alternans.

**Effect of ryanodine.** In six preparations, 10 μM ryanodine reduced tension at a basic cycle length of 1,000 msec, by 83.7±2.8%, and attenuated APD alternans. The longest cycle at which alternans appeared decreased from 320±9 to 251±6 msec ($p<0.001$), and the range of cycle lengths at which alternans occurred decreased from 93±10 to 11±3 msec ($p<0.001$). Figure 7 shows a representative experiment in which cycle length was changed from 1,000 to 240 msec. In control, alternans of tension and of the ratio of $D_p$ to APD is present after the first beat after the change in cycle length. During superfusion with ryanodine, tension alternans begins with the eighth beat and the action potential shape alternans with the seventh action potential after the change in cycle length. The delayed appearance of alternans of both tension and action potential shape in the presence of ryanodine occurred in each of the six preparations (Figure 8).

We postulated that the small amount of $[\text{Ca}^{2+}]$, available for contraction in the presence of ryanodine was not sufficient to produce an early appearance of alternans and that the delayed appearance of alternans was caused by an increase in $[\text{Ca}^{2+}]$. 

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**FIGURE 4.** Simultaneous record of developed tension (top) and action potential (bottom) after change of cycle length from 1,000 (last action potential shown) to 240 msec in a ventricular muscle fiber before (A) and during superfusion with 2 mM caffeine (B). Note that in the presence of caffeine the basic tension is slightly more than in control and that action potential duration alternans does not occur.

**FIGURE 5.** Plots of developed tension and the ratio of plateau duration ($D_p$) to action potential duration (APD) in control (open symbols) and in the presence of 2 mM caffeine (solid symbols) after change of cycle length from 1,000 (last action potential shown) to 240 msec (first 16 action potentials) in six ventricular muscle fibers. Symbols and vertical bars show means and SEM, respectively.

**FIGURE 6.** Plot of developed tension after change of cycle length from 1,000 (last beat shown) to 240 msec before and during superfusion with ARL 115 in seven ventricular muscle fibers. Symbols and vertical bars show means and SEM, respectively.
resulting from influx during action potential. We tested this postulate in five additional experiments in which we compared the effects of ryanodine at 1.0, 2.0, and 4.0 mM [Ca\(^{2+}\)]\(_{o}\). In the absence of ryanodine, action potential shape alternans was present at each of these concentrations, and there was no significant difference between the magnitudes of alternans at these three [Ca\(^{2+}\)]\(_{o}\).

Figure 9 shows the effects of 10 \(\mu\)M ryanodine on the ratio of D\(_{pl}\) to APD of initial 20 action potentials after change to cycle length of 240 msec at calcium concentration of 1.0 (upper panel) and 2.0 mM (lower panel) in a representative experiment. In this experiment, cycle length of 240 msec was the shortest cycle at which a one-to-one response to stimulation was maintained in the presence of ryanodine at 1.0 mM [Ca\(^{2+}\)]\(_{o}\). Ryanodine prevented the appearance of delayed alternans of action potential shape at 1.0 mM [Ca\(^{2+}\)]\(_{o}\), but not at 2.0 mM [Ca\(^{2+}\)]\(_{o}\). At 4.0 mM [Ca\(^{2+}\)]\(_{o}\), the effect of ryanodine was similar to that at 2.0 mM [Ca\(^{2+}\)]\(_{o}\) (not shown).

Table 3 summarizes the results of five experiments. At increasing [Ca\(^{2+}\)]\(_{o}\), both APD\(_{b}\) and the ratio of D\(_{pl}\) to APD decreased progressively both in control and in the presence of ryanodine. Ryanodine prolonged APD and increased the ratio of D\(_{pl}\) to APD significantly at each [Ca\(^{2+}\)]\(_{o}\] concentration. In control, the ranges of cycle lengths at which alternans appeared at three different [Ca\(^{2+}\)]\(_{o}\] were not significantly different from each other. During superfusion with ryanodine at [Ca\(^{2+}\)]\(_{o}\] of 1.0 mM, alternans did not occur at any cycle length; at [Ca\(^{2+}\)]\(_{o}\] of 2.0 and 4.0 mM, alternans was present but within a significantly shorter range of cycle lengths than in control.

The magnitude of action potential shape (i.e., ratio of D\(_{pl}\) to APD alternans) was examined as the difference between the seventh and the eighth and also as the difference between the 19th and the 20th action potentials after change in cycle length. Both differences were examined at the shortest cycle length at which a one-to-one response to stimulation was maintained. At 2.0 and 4.0 mM [Ca\(^{2+}\)]\(_{o}\], ryanodine decreased significantly the D\(_{pl}\)-APD difference between the seventh and the eighth action potentials but not the D\(_{pl}\)-APD difference between the 19th and the 20th action potentials. The effects of ryanodine on the magnitude of action potential

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**Figure 7.** Simultaneous record of developed tension (top) and action potential (bottom) after change of cycle length from 1,000 (last action potential shown) to 240 msec in a ventricular muscle fiber before (A) and during superfusion with 10 \(\mu\)M ryanodine (B). Note that ryanodine decreases the tension and delays the appearance of an attenuated alternans.

**Figure 8.** Plot of developed cycle and the ratio of plateau duration (D\(_{pl}\)) to action potential duration (APD) in the presence of 10 \(\mu\)M ryanodine after change of cycle length from 1,000 to 240 msec in five ventricular muscle fibers and to 260 msec in one ventricular muscle fiber (first 16 action potentials). Bars show means and SEM, respectively.

**Figure 9.** Plot of ratio of plateau duration (D\(_{pl}\)) to action potential duration (APD) after change of cycle length from 1,000 to 240 msec in a ventricular muscle fiber in control and during superfusion with 10 \(\mu\)M ryanodine in the presence of 1.0 mM [Ca\(^{2+}\)]\(_{o}\) (top) and 2.0 mM [Ca\(^{2+}\)]\(_{o}\) (bottom) (see text).
shape alternans at $[\text{Ca}^{2+}]_o$ of 2.0 and 4.0 mM were not significantly different from each other.

**Discussion**

**Action of Pharmacologic Probes on Action Potential**

Our selection of pharmacologic probes was aimed at producing diverse effects on membrane currents affecting the shape and duration of action potential. The shortening of action potential by TTX and lidocaine is attributed to the depression of TTX-sensitive window current$^8,9$ and the shortening of action potential by SG-75 to increased $g_K$. 10 The lengthening of action potential by sotalol is attributed to the reduction of time-dependent outward potassium current$^{11}$ and the lengthening of action potential by 4-aminoptyridine to the suppression of transient outward current ($i_{to}$).$^{12-14}$ Both caffeine and ryanodine prolonged the action potential plateau in both fiber types. Lengthening of plateau by caffeine is probably due to depression of $i_{to}$. $^{7,15}$ The mechanism of ryanodine effect on plateau is probably similar to that postulated for caffeine because ryanodine inhibits the component of $i_{to}$ that is insensitive to 4-aminoptyridine$^{16}$ and slows the inactivation course of $i_{to}$. $^{17}$ ARL 115 BS shifted the plateau to more positive potential and shortened APD. To our knowledge, there is no information about the mechanism of this action.

**Action of Pharmacologic Probes on Restitution**

Tables 1 and 2 show that the pharmacologic probes produced diverse effects on the kinetics of restitution in both fiber types. The effects of lidocaine, TTX, and SG-75 in Purkinje fibers and those of lidocaine in ventricular muscle fibers are in agreement with earlier findings.$^2,18,19$ In our previous studies, we attributed the slowing of restitution by TTX and lidocaine to the altered kinetics of the reavailability of the sodium channel$^2$ and the increased amplitude of the restitution curve to a more triangular action potential shape (T. Nakaya, A. Varro, V. Elharrar, and B. Surawicz unpublished observations). The effects of the remaining probes on the restitution in the two fiber types have not been studied previously. We found that the compounds that lengthened APD (i.e., 4-aminoptyridine, caffeine, and ryanodine) tended to increase the amplitude of restitution in Purkinje fibers and decrease the amplitude of restitution in the ventricular muscle fibers. Similar differences between the premature APD in Purkinje and ventricular muscle fibers were found in the presence of several antiarrhythmic drugs.$^{18,19}$

**Relation Between APD During Alternans and Restitution**

We found no exceptions to an excellent correlation between APD difference during alternans and restitution in Purkinje fibers under conditions resulting in diverse alterations of action potential shape and duration. These results confirm our previous findings with nisoldipine and Bay K 8064.$^3$ The experiments with lidocaine and TTX revealed that the magnitude of alternans was controlled by three independent variables (i.e., the APD, the time constant, and the amplitude of restitution). The effects of other probes were consistent with these findings.

We have concluded that Purkinje fibers have no independent mechanisms responsible for the appearance of alternans induced by sudden shortening of the cycle and that alternans occurs only because of the alternans of the preceding DI. These findings support the hypothesis that APD alternans in Purkinje fibers depends on the differences in the recovery of membrane currents generated by the preceding action potential.$^{10}$ We cannot rule out the possibility that the same factor influences the APD in ventricular muscle, but we have shown that it cannot be a dominant mechanism because in these fibers the magnitude of APD alternans correlated poorly with the DI-dependent APD changes during restitution, and alternans of action potential shape occurred even when the DI was constant.

**Alternans of Action Potential Shape and Developed Tension**

Concomitant occurrence of action potential shape and tension alternans at a constant preceding DI

### Table 3. Effect of 10 μM Ryanodine at Three Different $[\text{Ca}^{2+}]_o$ on Alternans of Action Potential Shape in Five Canine Ventricular Muscle Fibers

<table>
<thead>
<tr>
<th>$[\text{Ca}^{2+}]_o$ (mM)</th>
<th>drug</th>
<th>APD$_o$ (msec)</th>
<th>$D_p$ to APD ratio (%)</th>
<th>CL range (msec)</th>
<th>$D_p$ to APD alternans magnitude at CL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>Control</td>
<td>235±8</td>
<td>68.5±2.1</td>
<td>104±10</td>
<td>7.1±1.2, 5.9±0.9, 264±8</td>
</tr>
<tr>
<td></td>
<td>Ryanodine</td>
<td>261±7*</td>
<td>72.7±1.1*</td>
<td>(−)</td>
<td>0, 0</td>
</tr>
<tr>
<td>2.0</td>
<td>Control</td>
<td>213±2</td>
<td>61.9±1.8</td>
<td>94±5</td>
<td>6.3±1.0, 5.6±0.5, 242±4</td>
</tr>
<tr>
<td></td>
<td>Ryanodine</td>
<td>244±5†</td>
<td>71.1±1.0*</td>
<td>16±8†</td>
<td>2.0±1.4*, 5.2±0.9</td>
</tr>
<tr>
<td>4.0</td>
<td>Control</td>
<td>208±7</td>
<td>59.2±1.7</td>
<td>88±13</td>
<td>5.2±0.6, 4.6±0.4, 216±7</td>
</tr>
<tr>
<td></td>
<td>Ryanodine</td>
<td>218±7*</td>
<td>66.2±1.7*</td>
<td>16±5†</td>
<td>3.1±0.6*, 6.6±1.2</td>
</tr>
</tbody>
</table>

*Values are given as mean±SEM.

APD$_o$, action potential duration at basic cycle length; $D_p$, plateau duration; CL range, range of cycle length at which alternans could be elicited.

$p<0.05$, $\dagger p<0.1$, $\ddagger p<0.001$ compared with control.
can result from one of the following processes: 1) action potential shape determines the developed tension, 2) the developed tension determines the action potential shape, and 3) both action potential shape and tension change independently, perhaps owing to a common factor. The first two possibilities appear unlikely. If the currents generated during repolarization contributed to a positive inotropic effect, the developed tension should be greater in association with a longer plateau and a more positive plateau potential. However, this was not the case. Similarly, if alternans of action potential shape was caused by passive mechanoelectrical coupling,\textsuperscript{21} we would expect a positive correlation between the developed tension and the amplitude of alternans. However, this was not the case.

It is reasonable to attribute the tension alternans to the difference in the concentration, handling, or both of $[\text{Ca}^{2+}]$.\textsuperscript{2,22} We postulated earlier that the lower $[\text{Ca}^{2+}]$, concentration associated with weaker tension may contribute to the positive shift and shortening of plateau by increasing the driving force for $\text{Ca}^{2+}$, slowing the inactivation of the $\text{Ca}^{2+}$ current, and decreasing the conductance of one or more outward currents. As a result, $[\text{Ca}^{2+}]$, concentration increases, and the subsequent tension is stronger. Conversely, the higher $[\text{Ca}^{2+}]$, concentration associated with stronger tension may contribute to shortening of the plateau by opposite actions (i.e., decreasing the driving force for $\text{Ca}^{2+}$, accelerating the inactivation of $\text{Ca}^{2+}$ current, and increasing the conductance of one or more outward currents.

We found that alternans in the ventricular muscle fibers was not appreciably altered by either doubling or decreasing in half the $[\text{Ca}^{2+}]$, and by increasing the sensitivity of contractile proteins to calcium.\textsuperscript{17,31} However, alternans was either suppressed or markedly attenuated by caffeine and ryanodine. Both caffeine (at higher concentrations than that used in this study) and ryanodine inhibit the function of the sarcoplasmic reticulum in cardiac muscle,\textsuperscript{24-26} but they do so via different mechanisms.\textsuperscript{27} Both caffeine and ryanodine abolish the oscillations of $[\text{Ca}^{2+}]$, and of tension in calcium-overloaded ferret ventricular muscle.\textsuperscript{28}

Consistent with the earlier findings in mammalian ventricular muscle,\textsuperscript{29,30} ryanodine decreased the developed tension. Caffeine, however, increased the developed tension, presumably by increasing sensitivity of the contractile proteins to calcium. The effects of caffeine and ryanodine suggest that $[\text{Ca}^{2+}]$, responsible for the maintenance of alternans is released from the sarcoplasmic reticulum. Consistent with this hypothesis is the recent report that the tension alternans in ferret papillary muscle was associated with altered intensity of the intracellular aequorin signal,\textsuperscript{32} a finding attributed to the delayed $\text{Ca}^{2+}$ transport from the uptake compartment to the release compartment of the sarcoplasmic reticulum.

**Suppression of Alternans**

The suppression of alternans by caffeine and ryanodine may be due to lesser fluctuations of the amounts of calcium released by sarcoplasmic reticulum. However, this may not be the only mechanism of alternans suppression because we have shown earlier\textsuperscript{3} that alternans is attenuated or suppressed by Bay K 8644 and nisoldipine, presumably as a result of changing amounts of calcium entering through the sarcolemmal calcium channel.

Neither the maintenance nor the suppression of alternans was dependent on any particular level of developed tension or plateau duration. Alternans was suppressed or attenuated when the duration of plateau was decreased (nisoldipine) or increased (Bay K 8644, caffeine, and ryanodine). Also, alternans was suppressed or attenuated in the presence of both increased tension (Bay K 8644 and caffeine) and decreased tension (nisoldipine and ryanodine).

**Similarities and Differences Between Two Types of Alternans**

Both types of alternans require a critical short cycle for induction and, in both the magnitude and the duration of alternans, increase with shortening of the new cycle length.\textsuperscript{3} However, the differences in mechanism account for certain differences in the behavior of alternans. In Purkinje fibers, the APD during alternans is controlled by preceding DI. Because of declining memory effect and increasing DI between action potentials, the magnitude of alternans in Purkinje fibers may decrease progressively, and the alternans must disappear before the APD reaches the new steady state. Also, in Purkinje fibers, the alternans can always be interrupted at any time by interpolating an interval in which the sum of preceding DI and following APD equals the cycle length.\textsuperscript{3}

In the ventricular muscle fibers, the magnitude of APD alternans also tends to decrease progressively because of declining memory effect. However, at very rapid rates, alternans of action potential shape may continue indefinitely without change in DI. In ventricular muscle fibers, alternans of APD also can be interrupted by interpolating a single cycle, but the sum of the preceding DI and the following APD of the cycle interrupting alternans does not need to equal the cycle length.\textsuperscript{3}

**Limitations of the Study**

The reported findings and conclusions are applicable only to the type of alternans induced by sudden decrease in cycle length and are not necessarily pertinent to types of electrical or mechanical alternans elicited by other interventions. Also, the results obtained in dog cardiac fibers may not be applicable to other species that may have different mechanisms controlling $[\text{Ca}^{2+}]$. For instance, the effects of ryanodine on action potential and tension differ in different species. Thus, ryanodine did not
change APD and tension in guinea pig papillary muscle but increased APD and reduced tension in rat ventricular muscle. Implications

A more complete understanding of the mechanism of alternans in ventricular muscle must await further advances in the understanding of the excitation-contraction coupling in cardiac muscle. In the meantime, the suggested working hypothesis is that alternans is caused by an imbalance between the available calcium and the calcium required to maintain a steady cycle length–dependent tension level. Therefore, cycle length–dependent alternans can be eliminated by either an increased availability or a decreased availability of calcium. The efficacy of an inotropic intervention may depend, in part, on the behavior of alternans induced at rapid heart rates. This suggests that the characteristics of alternans should be considered in the evaluation of pharmacologic agents.

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