Induction and termination of triggered activity by pacing in isolated canine Purkinje fibers

JEFFREY P. MOAK, M.D., AND MICHAEL R. ROSEN, M.D.

ABSTRACT The clinical importance of delayed afterdepolarizations and resultant triggered activity as a cause of cardiac arrhythmias is uncertain. We studied the response of ouabain-induced delayed afterdepolarizations and triggered activity to a pacing protocol similar to those used clinically in an effort to quantify the types of responses to pacing that occur as a result of this arrhythmogenic mechanism. Isolated canine Purkinje fibers were superfused with $2 \times 10^{-5}$ M ouabain until delayed afterdepolarizations occurred and attained an amplitude of 5 mV at a paced cycle length of 500 msec. We then studied the induction of triggered activity in these fibers by pacing. We found that: (1) As the pacing cycle length decreased, the coupling interval from the last paced beat to the first triggered beat decreased and 83% of fibers developed triggered activity. (2) The coupling interval of the first triggered beat after single ($S_i$) or double ($S_iS_j$) premature beats was in part dependent on preceding pacing cycle lengths. $S_i$ pacing induced triggered activity in 39% of fibers, and $S_iS_j$ pacing induced triggered activity in 48% of fibers. We then studied the termination of ouabain-induced sustained rhythmic activity by pacing: 89% of sustained rhythmic activity could be terminated by overdrive pacing at a cycle length less than or equal to 300 msec. The coupling interval of the first beat or first delayed afterdepolarization after the termination of overdrive decreased as pacing cycle length decreased. $S_i$ premature beats reset the sustained rhythmic activity and terminated 14% of sustained rhythmic activity. The coupling interval of the first escape beat or delayed afterdepolarization after $S_iS_j$ premature beats decreased as the $S_iS_j$ interval shortened, and $S_iS_j$ terminated 26% of sustained rhythmic activity. Pacing at an $S_iS_j$ cycle length of 400 msec followed by an $S_i$ terminated 50% of sustained rhythmic activity; $S_iS_j$ at a cycle length of 400 msec followed by $S_iS_j$ terminated 85% of sustained rhythmic activity. This quantitative demonstration of the responses of delayed afterdepolarizations, triggered activity, and sustained rhythmic activity to pacing may be useful in differentiating these from other mechanisms for arrhythmias.


TRIGGERED ACTIVITY, defined as impulse initiation induced by afterdepolarizations, has been produced in canine and human Purkinje and ventricular muscle fibers by digitalis,1–5 catecholamines,6–8 and myocardial infarction.9 It also has been produced in canine and human atrial fibers by catecholamines.10,11 and digitalis,12 and in canine coronary sinus by catecholamines.13 In the clinic, a spectrum of arrhythmias that is not explained readily by either reentry or automaticity has been attributed to delayed afterdepolarizations.14–19 However, proof of afterdepolarizations as a cause of arrhythmias in the clinical setting still is lacking.20 Similarly, arrhythmias resulting from myocardial infarction9,21 and digitalis intoxication22–25 in experimental animals have been attributed to delayed afterdepolarizations. Although there is stronger evidence for the role of afterdepolarizations in experimental animals than in the clinical setting, convincing proof still has not been found.

One of the major characteristics of delayed afterdepolarizations that might provide an aid to their identification in intact animals and in the clinic is their response to pacing. It has been noted that as overdrive pacing rate increases, the rate of triggered rhythms induced by afterdepolarizations tends to increase.1,2,26 It also has been found that single premature depolarizations occurring at critical cycle lengths can induce triggered activity or terminate it, a property long thought to be unique to reentry.1,2,27

In the present study, we used pacing techniques that are readily applicable in the clinical setting to deter-
mine the response to pacing of digitalis-induced delayed afterdepolarizations in isolated cardiac Purkinje fibers. We selected digitalis to induce delayed afterdepolarizations because this is the preparation that has been most studied in experimental laboratories and because digitalis-induced arrhythmias remain a clinical problem as well as an experimental problem. The goal of the present study was to identify the characteristics of the response to pacing that might permit guidelines more specific than those already identified \cite{16, 17} to be applied to the study of delayed afterdepolarizations and triggered activity in intact animals; our ultimate goal is to apply these guidelines to the clinical setting as well.

**Methods**

Adult mongrel dogs weighing 10 to 20 kg were anesthetized intravenously with sodium pentobarbital, 30 mg/kg. The heart was quickly removed through a right lateral thoracotomy and was placed in cold Tyrode’s solution. Free running Purkinje fiber bundles from the right and the left ventricle were dissected from the heart and placed in a tissue bath perfused with Tyrode’s solution containing (mmol/l): NaCl, 131; NaHCO$_3$, 18; CaCl$_2$, 2.7; MgCl$_2$, 0.5; NaH$_2$PO$_4$, 1.8; KCl, 4.0; and dextrose, 5.5. The bundles were then bubbled with 95% O$_2$-5% CO$_2$ at 37°C. We paced the Purkinje fibers at a cycle length of 500 msec and allowed them to stabilize for 1 hr. Stimuli were delivered as previously described \cite{28} with bipolar silver wires that were insulated with Teflon. Stimulus pulse width was 2 to 3 msec, and amplitude was 1.5 to 2 times diastolic threshold.

We then used the following pacing protocol: (1) We drove the preparations at cycle lengths of 1000 msec through 200 msec (decreasing in 100 msec decrements) for periods of 15, 60, and 180 sec. After each of these drive periods, stimulation was discontinued, which permitted us to observe the spontaneous rhythm. (2) We then drove the preparations for 8 beats at a constant cycle length ($S_1$) followed by a ninth beat ($S_2$) at decreasing coupling intervals until the effective refractory period was reached. This was done at drive cycle lengths ($S_1$) of 1000, 800, 600, and 400 msec. (3) Subsequently, we drove the preparations for 8 beats at a constant cycle length ($S_1$) followed by a ninth ($S_2$) and tenth ($S_3$) beat at decreasing coupling intervals. During this protocol the $S_1 S_2$ interval initially was 60 msec longer than the effective refractory period, and the $S_3 S_4$ interval initially was 300 msec longer than the $S_2 S_3$ interval. Again, we used drive ($S_1 S_2$) cycle lengths of 1000, 800, 600, and 400 msec. The $S_3 S_4$ was held constant as the $S_1 S_2$ interval was decreased in 10 msec decrements until the $S_3 S_4$ encountered the effective refractory period. At this point the $S_3 S_4$ was decreased by 10 msec. This sequence of steps was repeated until the tissue was refractory to the $S_1 S_2$.

After the initial pacing procedure, we superfused the Purkinje fiber bundles with $2 \times 10^{-7}$ M ouabain for 20 to 40 min; the end point for superfusion was the occurrence of delayed afterdepolarizations having an amplitude of 5 mV at a cycle length of 500 msec. At this time the ouabain superfusion was discontinued. We have shown previously that on cessation of ouabain superfusion after attainment of toxicity, the transmembrane potential remains stable for about 1 hr. We, therefore, had 1 hr to repeat the pacing protocol under steady-state conditions of toxicity. In those instances where pacing induced a triggered rhythm, its response to the same pacing protocol (modified depending on the cycle length of the triggered rhythm) was determined.

The Purkinje fiber bundle then was superfused with additional $2 \times 10^{-7}$M ouabain until it either generated sustained rhythmic activity or became inexcitable. For those fibers that exhibited sustained rhythmic activity, the response of this rhythm to the pacing protocol was determined. We have deliberately used the term “sustained rhythmic activity” here for the following reason: As defined by Cranefield \cite{23} this term includes triggered activity and automaticity, as well as repetitive activity induced by reentry. In our experiments the triggered activity induced by pacing preparations that had delayed afterdepolarizations usually was less than 25 beats in duration (see Results). In contrast, the sustained rhythmic activity induced by further superfusion with ouabain lasted for many minutes. Although the latter, when it terminated, ended with a delayed afterdepolarization and presumably was a triggered rhythm occurring in the presence of greater digitalis toxicity, we have retained the term sustained rhythmic activity to emphasize its long duration.

We viewed these protocols as providing us with two types of information analogous to that which one obtains in the clinic: first, those preparations that showed delayed afterdepolarizations but not spontaneous rhythms enabled us to study the inducibility of an arrhythmia in a situation in which the underlying mechanism (i.e., the afterdepolarization) was present, but the arrhythmia was not occurring spontaneously; second, those preparations that showed sustained rhythmic activity enabled us to study the response of a stable arrhythmia to pacing as an intervention. In those instances when the arrhythmia could be terminated, we then could study the requirements for its reinduction.

**Analysis of data.** Delayed afterdepolarizations were measured as described previously \cite{4}; amplitude was measured from the point of maximum hyperpolarization of the membrane before the afterdepolarization to its peak amplitude, and the coupling interval was measured from the phase 0 upstroke of the action potential that induced the afterdepolarization to its peak amplitude. Other characteristics of transmembrane potential measured were maximum diastolic potential, action potential amplitude, maximum upstroke velocity of phase 0 ($V_{max}$), and action potential duration to full repolarization. These were determined with previously described techniques. \cite{3}

Data from each experiment were analyzed as follows: delayed afterdepolarization amplitude, coupling interval, and the cycle length of the first triggered beat induced by a delayed afterdepolarization were plotted as a function of the pacing cycle length that preceded them. This was done for sustained drive periods as well as for premature depolarizations. In some experiments on sustained rhythmic activity there was spontaneous fluctuation in the baseline cycle length of the sustained rhythm. This fluctuation could cause inconsistency in relating the coupling interval of the recovery beat that followed an induced premature beat to the coupling interval of the premature beat. To prevent such inconsistency, we normalized the response by dividing the coupling interval for the recovery beat by the mean cycle length of the 3 beats preceding the premature beat. This was plotted as a function of the coupling interval of the premature beat divided by the mean cycle length.

The statistical analyses used were t tests for paired or grouped data. \cite{30} The results are expressed as mean ± SD.

**Results**

**Induction of triggered activity**

**Effects of sustained drive.** During the control period, sustained pacing at cycle lengths of 1000 to 200 msec for 15 to 180 sec did not induce delayed afterdepolarizations or triggered rhythms. After superfusion of the
TABLE 1
Effect of ouabain $2 \times 10^{-7}$M on the transmembrane potential (mean ± SD)\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>MDP (mV)</th>
<th>Amp (mV)</th>
<th>$V_{\text{max}}$ (V/sec)</th>
<th>APD 100% (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>95 ± 4</td>
<td>134 ± 8</td>
<td>666 ± 48</td>
<td>282 ± 25</td>
</tr>
<tr>
<td>Ouabain</td>
<td>89 ± 7(^b)</td>
<td>117 ± 9(^b)</td>
<td>485 ± 42(^c)</td>
<td>253 ± 34(^c)</td>
</tr>
</tbody>
</table>

\(^a\)Drive cycle length equals 500 msec; n = 17.  
\(^b\)p < .001 of control.  
\(^c\)p < .005 of control.

MDP = maximum diastolic potential; AMP = action potential amplitude; $V_{\text{max}}$ = maximum rate of rise of phase 0; ADP 100\% = action potential duration to full repolarization.

Purkinje fiber bundles with $2 \times 10^{-7}$M ouabain, sustained pacing induced delayed afterdepolarizations alone in five of the 17 preparations and triggered activity as well as delayed afterdepolarizations in the other 12. The characteristics of the transmembrane potentials of the preparations before and after superfusion with ouabain are presented in table 1.

For those fibers that exhibited only delayed afterdepolarizations, a distinctive and previously described relationship\(^1,2\) was observed between pacing cycle length and the coupling interval of the delayed afterdepolarization (figure 1A). That is, as the pacing cycle length was decreased, the coupling interval of the delayed afterdepolarization shortened, and at cycle lengths of 500 msec or less, second and sometimes third delayed afterdepolarizations were detected. These also showed a decrease in coupling interval as drive cycle length decreased. Increasing the duration of pacing from 15 to 180 sec did not modify the coupling interval of the delayed afterdepolarization.

Amplitude of delayed afterdepolarizations also varied with the pacing cycle length (figure 1B). The first delayed afterdepolarization increased in amplitude as pacing cycle length decreased from 1000 msec, peaking at cycle lengths of 600 to 700 msec. At pacing cycle lengths less than 600 msec, the first delayed afterdepolarization began to decrease in amplitude. The response of the second delayed afterdepolarization to pacing was qualitatively and quantitatively different from that of the first delayed afterdepolarization. The magnitude of the increase in amplitude as cycle length decreased was much greater than that for the first delayed afterdepolarization; the second delayed afterdepolarization continued to increase in amplitude as cycle length was further decreased. Increasing the duration of pacing from 15 to 180 sec did not further influence the amplitude of the delayed afterdepolarizations. This should not be construed as implying that there was no pacing influence at all on the amplitude of delayed afterdepolarizations. As has been demonstrated previously\(^3,4\) and as will be shown below, when the number of paced beats is increased from 1 to 10 or 15 there is an increase in afterdepolarization amplitude and, with this, an increase in the likelihood of their attaining threshold and inducing a triggered rhythm. However, as shown here, at any given cycle length once pacing has occurred for more than 15 sec there is no further effect on amplitude.

For those fibers in which triggered activity was induced by pacing, the relationship between the coupling

![Figure 1A](http://circ.ahajournals.org/)

**FIGURE 1A.** Effect of pacing at different basic cycle lengths for 15 sec on coupling interval of the delayed afterdepolarization (DAD). The filled circles and the unfilled circles are the first and second delayed afterdepolarization, respectively.
interval of the first triggered beat and the pacing cycle length (figure 2) was nearly superimposable on the relationship between the coupling interval of the delayed afterdepolarizations and pacing cycle length (figure 1A). As the pacing cycle length was decreased, the percentage of fibers that triggered at each cycle length increased from 5% at 800 msec to 83% at 200 msec. The duration of pacing (15 to 180 sec) did not have a major influence on the percentage of fibers that developed triggered activity after termination of pacing at any specific cycle length. For example, during pacing at a cycle length of 500 msec, three of 13 fibers triggered when pacing duration was 15 sec, and two of 13 fibers triggered when pacing duration was 180 sec. The duration of triggered activity tended to be brief; in fact, a triggered rhythm of more than 25 beats occurred

FIGURE 1B. Effect of pacing at different cycle lengths for 15 sec on delayed afterdepolarization amplitude (DAD Amp). The filled circles and the unfilled circles are the first and second delayed afterdepolarization, respectively.

FIGURE 2. Effect of pacing at different cycle lengths for 15 sec on the coupling interval of the first triggered beat that followed the termination of pacing. The filled circles represent those beats that were triggered by the first delayed afterdepolarization (DAD), and the unfilled circles display those beats that were triggered by the second delayed afterdepolarization.
in only four of the 17 fiber bundles. When triggered rhythms occurred, their rate tended to increase as the preceding drive cycle length decreased.

In eight experiments we repeated the pacing protocol a second time to determine whether triggering occurred reproducibly at the same cycle length on two successive occasions. Although triggering occurred during the second run, the cycle length at which it was initiated was reproducible in only three of 8 fibers.

Single premature beats ($S_2$). We paced the preparations for 8 beats at each of the four primary cycle lengths ($S_1S_1$ equal to 1000, 800, 600, and 400 msec), and then for each cycle length we introduced a ninth beat ($S_2$), decreasing the coupling interval until the effective refractory period was encountered. This protocol elicited a cycle length–dependent response in the coupling interval of the delayed afterdepolarization and in the percentage of fibers that triggered.

At each of the four primary cycle lengths no fibers showed triggered activity during $S_1S_1$ pacing for 8 beats. However, at each primary cycle length, the percentage of fibers that triggered increased as the $S_1S_2$ interval decreased. The following patterns of coupling intervals were seen after the $S_2$: At $S_1S_1$ drive cycle lengths of 1000 and 800 msec, those triggered rhythms that were induced by the $S_2$ showed a longer coupling interval when $S_1S_2$ was short than when $S_1S_2$ was long. For example, at a cycle length of 1000 msec, the range of $S_1S_2$ intervals studied was 800 to 360 msec, and three of 18 fibers showed a triggered rhythm. At $S_1S_2$ equal to 800 msec the cycle length of the initial triggered action potential was $930 \pm 70$ msec, and at $S_1S_2$ equal to 360 msec the cycle length was $1133 \pm 58$ msec ($p < .005$). This resulted from the fact that at long $S_1S_2$ intervals it was the first delayed afterdepolarization in the sequence that initiated the triggered activity, whereas at short $S_1S_2$ intervals it was the second delayed afterdepolarization.

At $S_1S_2$ equal to 800 msec, six of 18 fiber bundles showed triggered activity in the presence of $S_2$. The range of $S_1S_2$ intervals studied was 700 to 340 msec. At $S_1S_2$ equal to 700 msec the cycle length for the initial triggered action potential was $838 \pm 80$ msec, and at $S_1S_2$ equal to 340 msec the cycle length was $911 \pm 95$ msec ($p < .05$).

At shorter $S_1S_1$ drive cycle lengths (600 and 400 msec) there were two patterns for the cycle length of the first triggered action potential. One of these showed an increasing cycle length for the first triggered action potential as $S_1S_2$ decreased (similar to that described above); the other pattern was a shorter cycle length as $S_1S_2$ decreased. To illustrate, at $S_1S_2$ equal to 600 msec, nine of 17 fibers showed triggered activity at $S_1S_2$ equal to 500 to 340 msec (figure 3). For four of the nine fibers at $S_1S_2$ equal to 500 msec, the cycle length of the first triggered action potential was $760 \pm 41$ msec, and at $S_1S_2$ equal to 340 msec, it was $845 \pm 64$ msec ($p < .05$) (figure 3, A). For five of the nine fiber bundles at $S_1S_2$ equal to 500 msec, the cycle length of the first triggered action potential was $760 \pm 41$ msec, and at $S_1S_2$ equal to 340 msec, it was $661 \pm 112$ msec ($p < .01$) (figure 3, B).

The same pattern was seen at a $S_1S_2$ drive cycle length equal to 400 msec. Here, nine of 14 fiber bundles showed triggered activity at $S_1S_2$ equal to 380 to 240 msec. For two of the nine, the mean cycle length of the first triggered beat was $483 \pm 3$ msec at $S_1S_2$ equal to 380 msec, it was $590 \pm 4$ msec at $S_1S_2$ equal to 240 msec. For seven of the nine fiber bundles at $S_1S_2$ equal to 380 msec, the cycle length of the first triggered action potential was $777 \pm 202$ msec; at $S_1S_2$ equal to 260 msec, the cycle length was $643 \pm 138$ msec ($p < .02$).

At all drive cycle lengths, amplitude of delayed afterdepolarizations increased as the $S_1S_2$ interval decreased. There was no correlation between the $S_1S_2$ coupling interval and the number of triggered beats elicited at any basic drive cycle length. Triggering for more than 25 beats occurred infrequently (in only three of 14 preparations). We tested the reproducibility of triggering at critical cycle lengths in seven preparations. Reproducibility was seen in four of these.

Two premature beats ($S_2S_2$). As above, we used $S_1S_1$ cycle lengths of 1000, 800, 600, and 400 msec for eight cycles. The $S_1S_1$ interval (the $S_1$ being the ninth beat in the sequence) initially was 60 msec longer than the effective refractory period, and the $S_1S_2$ was 300 msec longer than the $S_1S_2$ interval. While holding the $S_1S_2$ constant, we reduced the $S_1S_2$ in 10 msec decrements until the effective refractory period was encountered. We then decreased the $S_1S_2$ interval by 10 msec and repeated the $S_1S_2$ testing. This sequence was repeated until refactoriness to the $S_1S_2$ was encountered.

Whereas single premature beats ($S_2S_2$) induced 39% of all fiber bundles studied to show triggered activity, this increased to 48% after dual premature beats ($S_2S_2$). At a $S_1S_2$ drive cycle length of 1000 msec, the coupling interval of the initial triggered beat that followed the $S_1S_2$ increased as the $S_1S_2$ coupling interval decreased. For example, at a cycle length equal to 1000 msec, the $S_1S_2$ induced triggered activity in three of 13 fibers. At an $S_1S_2$ coupling interval equal to 300 msec the coupling interval of the first triggered action potential was $900 \pm 86$ msec, whereas at an $S_1S_2$ coupling interval equal to 200 msec the coupling interval of the first
We tested the reproducibility of triggered action potentials. The pattern described above is comparable to that for single premature beats (S₂), in that at long basic cycle lengths (1000 and 800 msec) decreasing the S₂ coupling interval prolonged the recovery cycle. At drive cycle lengths of 600 and 400 msec, S₂S₃ stimulation either prolonged or had no effect on the cycle length of the first triggered action potential as the S₂S₃ interval of eight of the 13 fibers studied. In the other five fibers, as S₂S₃ interval decreased so did the cycle length of the first triggered action potential. There was no relationship between the S₂S₃ coupling interval and the mean number of triggered beats. Triggered activity for more than 25 beats was infrequent (five of 18 fibers). We tested the reproducibility of triggering on 10 preparations. Reproducibility at the same critical cycle length was seen in five.

**Termination of sustained rhythmic activity.** As in triggered activity, the sustained rhythmic activity induced by digitalis was initiated by an action potential and was terminated with delayed afterdepolarizations. However, those sustained rhythms that did not terminate might have been induced by an automatic mechanism, since delayed afterdepolarizations were never clearly identified in these. The mean cycle length of the sustained rhythmic activity in all preparations was 669 ± 295 msec (range of 285 to 1250 msec). Analysis of the stability of cycle length over the course of 5 to 10 min in 34 sustained rhythms (measurement commencing at least 2 min after they started) revealed a mean fluctuation in cycle length of ±5%.

**Effect of overdrive pacing for 15 and 60 sec.** Sixteen experiments were performed. The relationship between drive cycle length and the first escape beat after the cessation of pacing (in instances when the arrhythmia persisted) or the delayed afterdepolarization (in instances when the arrhythmia was suppressed) is shown in figure 4. This curve is virtually superimposable on that in figure 1A. We then determined whether overdrive pacing abruptly terminated triggered activity, resulting in a delayed afterdepolarization only, or whether it gradually terminated triggered activity (in which case triggered activity persisted for 1 to 10 beats followed by quiescence), or whether pacing was followed by continuation of triggered activity for 25 beats or more. In all instances, we determined whether the re-
response to pacing was associated with any consistent change in membrane potential. For those rhythms that terminated after overdrive pacing, 26% ceased abruptly and 74% showed continued triggered activity for 1 to 10 beats before terminating. Pacing at short drive cycle lengths invariably caused a greater percentage of fibers to terminate their sustained rhythmic activity than that which occurred at long cycle lengths (figure 5). For example, 40% of triggered rhythms were terminated at a paced cycle length of 600 msec, whereas 89% were terminated at a paced cycle length of 300 msec. Of those rhythms that terminated in 1 to 10 beats, 61% slowed before termination and 29% accelerated. In 12 preparations we tested whether termination was reproducible during a second run of pacing. It was reproducible in 11 of the 12.

For those preparations with sustained rhythmic activity that persisted on cessation of overdrive pacing, we analyzed the cycle lengths of the first 3 to 5 beats as well as the cycle length once the rhythm stabilized. Sixty-six percent of the rhythms accelerated during the first 3 to 5 beats at a drive cycle length of 300 msec, whereas only 30% accelerated at a cycle length of 600 msec. Seventy-five percent of the rhythms resumed their initial rate within 10 beats, whereas the other 25% either slowed (46% of these) or accelerated (54%).

We measured the maximum diastolic potentials at the start and cessation of pacing for those preparations in which sustained rhythmic activity was terminated and for those in which it was not. There was no significant difference identified here. For the former group, on termination of pacing, maximum diastolic potential was $-82.7 \pm 7.5$ mV; for the latter, it was $-83.8 \pm 6.9$ mV.

Single premature beats ($S_2$). To analyze the response of

**FIGURE 4.** Influence of overdrive pacing for 15 sec on sustained rhythmic activity. The paced cycle lengths are displayed on the abscissa. The ordinate displays the coupling interval of the first escape beat after cessation of pacing (when the sustained rhythmic activity continued) or of the delayed afterdepolarization (when sustained rhythmic activity terminated).

**FIGURE 5.** Effect of overdrive pacing cycle length on the percentage termination of all sustained rhythmic activity at each basic cycle length. The filled circles represent pacing for 15 sec, and the unfilled circles represent pacing for 60 sec. Because there is no significant difference between the two curves, the bars indicating SD have been eliminated. Results are the means of 16 experiments.
sustained rhythmic activity to single premature beats, we scanned phase 4 of the transmembrane potential by triggering the S2 from the upstroke of the preceding action potential. At least 10 cycles elapsed between test stimuli. We grouped the sustained rhythms based on their spontaneous cycle lengths (i.e., 300 to 500 msec, 500 to 700 msec, 700 to 900 msec). The response to single premature beats was similar in all groups. As the interval between the spontaneous beat and the S2 decreased, the normalized coupling interval (see Methods) was unchanged (p > .05); that is, the sustained rhythm showed reset (figure 6). Single premature beats rarely terminated the sustained rhythmic activity (in only three of 22 rhythms) or accelerated its rate (two of 22 rhythms). The termination of sustained rhythmic activity by single premature beats was reproducible in only one of three preparations in which this was tested. In the others, termination occurred over a variable range of premature coupling intervals.

Two premature beats (S2S3). The response of sustained rhythmic activity to two premature beats was analyzed by grouping the sustained rhythms together by cycle length (i.e., 300 to 500 msec, 500 to 700 msec, and 700 to 900 msec) (figure 7). The response to two premature beats was similar in the first two groups of cycle lengths. Of the 15 fibers studied here, the rhythm was terminated by S2S3 in four. In these four fibers in which the sustained rhythmic activity was terminated, it usually was followed by 1 triggered beat (12 attempts at termination were made in these four fibers; in nine of these attempts, termination occurred and a single triggered beat followed the S2S3). Reproducibility of termination during successive drive protocols occurred in one of three preparations where this was studied. For the 11 fibers in which the rhythm did not terminate, as the S2S3 interval decreased from 300 to 210 msec, the interval from the S2 to the first triggered beat was constant; however, as the S2S3 interval was decreased from 210 to 160 msec, there was a significant decrease in the coupling interval of the first escape beat (p < .01).

The response of the two preparations whose cycle lengths were between 700 to 900 msec was different from that described above. Here, as the S2S3 coupling interval was shortened from 300 to 180 msec, the sustained rhythmic activity did not cease, and the escape interval was unchanged.

Single premature beats (S2) after 8 paced beats at a constant cycle length (S1). The S1S1 cycle length selected was determined by the cycle length of the sustained rhythmic activity and was either 800, 600, or 400 msec. Three preparations were studied at a S1S1 cycle length of 800 msec. Decreasing the S1S2 interval from 700 to 320 msec had no consistent effect on the escape interval but did terminate one of the three rhythms. At S1S2 equal to 600 msec, initiation of an S1 terminated three of seven rhythms studied. For the other four, decreasing the S1S2 interval from 500 to 300 msec had no effect on the escape interval; i.e., the escape interval was 701 ± 58 msec at S1S2 equal to 500 msec and 680 ± 31 msec at S1S2 equal to 300 msec (p > .05). A different result was seen at S1S2 equal to 400 msec (figure 8). In nine of the 13 rhythms studied, as S1S2 was decreased from 380 to 210 msec, the escape interval decreased from 613 ± 63 msec to 440 ± 46 msec (p < .001). In four of the 13 rhythms, as the S1S2 interval was decreased from 380 msec to 240 msec, the escape interval increased from 563 ± 127 msec to 611 ± 120 msec (p < .02). Seven of 13 rhythms could be terminated by initiating S1 at a S1S1 equal to 400 msec. Termination was reproducible at a critical cycle length during successive runs in only one of four preparations in which this was studied.

Two premature beats (S2S3) after 8 beats at a constant cycle length (S1). The S1S1 cycle lengths studied were 600 and 400 msec. At S1S2 equal to 600 msec, as the S1S2 coupling interval was decreased from 300 to 190 msec, the coupling interval of the first escape beat did not change significantly (661 ± 40 to 575 ± 62 msec, p > .05). Three of five rhythms studied at S1S2 equal to 600 msec were terminated at short S1S2 intervals. At a S1S2 cycle length equal to 400 msec, as S1S2 was decreased from 300 to 190 msec, the escape interval decreased
from 519 ± 40 to 444 ± 36 msec in seven fibers studied (p < .005). Six of the seven rhythms were terminated. Termination of these rhythms was preceded by 1 or 2 triggered beats in three of the six fibers. Termination of triggered activity was reproducible at a critical cycle length during successive runs in only one of three preparations in which reproducibility was studied.

Discussion

In this study, we used digitalis as a means to induce delayed afterdepolarizations and sustained rhythmic activity, and then used a pacing protocol to identify the responses to pacing that were characteristic of the mechanisms studied. In so doing, we made two assumptions: first, that sustained rhythmic activity studied in the tissue bath was analogous to digitalis-induced tachycardias in the heart in situ; second, that the initiation of triggered activity in preparations having delayed afterdepolarizations but no spontaneous arrhythmias was analogous to induction of an arrhythmia in the heart in situ. It would be helpful if we could assume that all sustained rhythmic activity induced by digitalis would be triggered by delayed afterdepolarizations. Although there is evidence to support this (i.e., the sustained rhythmic activity was preceded by delayed afterdepolarizations and, when it terminated, was followed by one or more delayed afterdepolarizations), there remains the possibility — for those rhythms that never terminated — that they might have been the result of an abnormal automatic mechanism. It also should be stressed that although one might attempt to apply the responses to pacing seen in this study to all rhythms induced by delayed afterdepolarizations, we can state only that they are characteristic of digitalis-induced rhythms. Whether triggered activity induced by catecholamines, infarction, and other interventions is the same or different from that induced by digitalis remains to be seen. That some differences exist is suggested by the observation that whereas delayed afterdepolarizations induced by catecholamines in simian mitral valve show an increase in amplitude as drive cycle length is decreased, their coupling interval

**FIGURE 7.** The influence of two programmed premature beats (S₂S₃) on the escape interval of the sustained rhythmic activity (on vertical axis, represented as coupling interval) after the second programmed premature beat. A. Four sustained rhythms whose basic cycle length was between 300 to 500 msec. B. Five sustained rhythms for which basic cycle length was between 500 to 700 msec. C. Two sustained rhythms whose cycle length was between 700 to 900 msec. The S₂S₃ coupling interval (horizontal axis) is the coupling interval between the first and second programmed premature beats.
does not decrease.\textsuperscript{31} It is difficult to compare these different types of delayed afterdepolarizations further because there has been no quantification of the consistency of response of nondigitalis-induced delayed afterdepolarizations to pacing and to premature depolarizations. Finally, in relating our results to those that might occur in the heart in situ, we have assumed that a straightforward relationship can be found between these cellular electrophysiologic events and those that occur in the intact heart and that the relationship is not necessarily encumbered by complicating factors such as exit or entry block. Although this assumption might seem an oversimplification, the recent reports by Gorgets et al.\textsuperscript{32} and by us\textsuperscript{33} suggest that the response to pacing of rhythms caused by digitalis toxicity in the intact animal is not dissimilar from that which we report in isolated tissues. There are some differences, however, between our results with premature stimuli and those of Gorgets et al.,\textsuperscript{32} which might be explained by the differences between the isolated tissue and models of the heart in situ.

In the remainder of this discussion we will compare the responses to pacing of digitalis-induced delayed afterdepolarizations and sustained rhythmic activity with those of two other arrhythmogenic mechanisms: automaticity and reentry. To aid in the discussion, we have summarized the results of our pacing studies in tables 2A and 2B. In considering automaticity we shall further subdivide this into two categories: that which occurs at high membrane potentials (often referred to as "normal automaticity") and that which occurs at low membrane potentials (referred to as "abnormal automaticity").\textsuperscript{34} That automaticity that occurs at high membrane potentials in the Purkinje system is suppressed readily by overdrive.\textsuperscript{35,36} This is in sharp contrast to automaticity in Purkinje fibers having membrane potentials of $-40$ to $-60$ mV. Overdrive pacing of this automatic activity either fails to suppress it, or may actually slightly increase the rate at which the automatic focus fires (especially at more positive membrane potentials).\textsuperscript{37} Nevertheless, such automatic activity may be suppressed by long periods (i.e., several minutes) of overdrive.\textsuperscript{37} Recent studies of automaticity at low membrane potentials have suggested that the failure to suppress, by overdrive, this type of impulse initiation is a result of the lesser Na\textsuperscript{+} entry that occurs at low rather than at high membrane potentials.\textsuperscript{38} Finally, it should be mentioned that there is not a sharp demarcation between the overdrive-suppressible automaticity that occurs at high membrane potentials and that which either is not suppressed or actually increases in rate at low membrane potentials. Rather, there is a gradation of responses to overdrive, depending on the membrane potential of the automatic focus.\textsuperscript{38} As a result, overdrive may induce variable degrees of suppression or no suppression at all of automatic foci. This characteristic of automaticity should be kept in mind when attempting to differentiate the responses of automatic and triggered rhythms to pacing.

**FIGURE 8.** The influence of $S_1S_2$ pacing during sustained rhythmic activity on the escape interval (vertical axis) after $S_2$. A, The response of those fibers in which the $S_1S_2$ coupling interval was shortened, so did the $S_2$ (coupling interval of the first escape beat). B, The response of those fibers in which as the $S_1S_2$ coupling interval was shortened, the $S_2$ (coupling interval of the first escape beat) lengthened. For both panels, $S_1S_1$ equals 400 msec.
TABLE 2A
Induction of triggered activity

<table>
<thead>
<tr>
<th>By sustained drive</th>
<th>By S₂</th>
<th>By S₂S₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>As pacing CL ↓ escape</td>
<td>No triggered activity</td>
<td>No triggered activity</td>
</tr>
<tr>
<td>CL of 1st triggered beat ↓</td>
<td>after 8 S₁ paced beats</td>
<td>after 8 paced S₁ beats</td>
</tr>
<tr>
<td>At BCL 800 msec, 5% of fibers trigger</td>
<td>As S₁S₂ ↓ in presence of S₂, percent fibers</td>
<td>As S₂S₃ ↓ in presence of S₂S₃, percent fibers</td>
</tr>
<tr>
<td>At BCL 200 msec, 83% of fibers trigger</td>
<td>triggering ↑</td>
<td>triggering ↑</td>
</tr>
<tr>
<td>As duration of pacing ↑ from 15 to 180 sec, no effect on percent of fibers triggering</td>
<td>triggering ↑</td>
<td>triggering ↑</td>
</tr>
<tr>
<td>Triggering for &gt; 25 beats infrequent (23% of fibers)</td>
<td>Triggering for &gt; 25 beats infrequent (21% of fibers)</td>
<td>Triggering for &gt; 25 beats infrequent (28% of fibers)</td>
</tr>
<tr>
<td>At long S₂S₃ (1000 to 800 msec) triggering CL ↑ as S₁S₂ ↓</td>
<td>At long S₂S₃ (1000 to 800 msec) triggering CL ↑ as S₂S₃ ↓</td>
<td></td>
</tr>
<tr>
<td>At short S₂S₃ (600 to 400 msec) triggering CL ↑ or ↓ as S₁S₂ ↓</td>
<td>At short S₂S₃ (600 to 400 msec) triggering CL ↑ or ↓ as S₂S₃ ↓</td>
<td></td>
</tr>
</tbody>
</table>

Induction reproducible in 38%

CL = cycle length; BCL = basic cycle length.

TABLE 2B
Pacing of sustained rhythmic activity

<table>
<thead>
<tr>
<th>Sustained drive</th>
<th>S₂</th>
<th>S₂S₃</th>
<th>S₁S₂</th>
<th>S₁S₂S₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>As pacing CL ↓ CL of 1st escape beat ↓</td>
<td>76% show reset</td>
<td>At SRA CL = 700 to 900 msec, no consistent response</td>
<td>At S₁S₂ = 600 msec no consistent response</td>
<td></td>
</tr>
<tr>
<td>At BCL = 600 msec, 40% terminate; at BCL = 300 msec, 89% terminate</td>
<td>14% terminate</td>
<td>At SRA CL = 300 to 700 msec 27% terminate</td>
<td>At S₁S₂ = 400 msec 54% terminated by S₂</td>
<td></td>
</tr>
<tr>
<td>Pacing for 15 or 60 sec does not change percent terminating</td>
<td>10% accelerate</td>
<td>Termination reproducible at critical coupling interval in 33%</td>
<td>Termination reproducible at critical coupling interval in 25%</td>
<td></td>
</tr>
<tr>
<td>Termination reproducible at critical CL in 92%</td>
<td>Termination reproducible at critical coupling interval in 33%</td>
<td>Termination reproducible at critical coupling interval in 33%</td>
<td>Termination reproducible at critical coupling interval in 33%</td>
<td></td>
</tr>
<tr>
<td>For those terminating 26% abrupt</td>
<td>For those terminating, 80% show afterbeats</td>
<td>For those terminating, 86% show afterbeats</td>
<td>For those terminating, 50% show afterbeats</td>
<td></td>
</tr>
<tr>
<td>74% show 1 to 10 afterbeats, 61% slow, 29% accelerate</td>
<td>For those not terminating as S₂S₃ ↓ from 210 to 160 msec, CL of 1st triggered beat ↓</td>
<td>For those not terminating 69% show ↓ CL of 1st triggered beat as S₂S₃ ↓</td>
<td>For those not terminating 100% show ↓ CL of 1st triggered beat as S₂S₃ ↓</td>
<td></td>
</tr>
<tr>
<td>For those not terminating at CL = 600 msec, 30% accelerate for 3 to 5 beats</td>
<td>For those not terminating as S₂S₃ ↓ from 210 to 160 msec, CL of 1st triggered beat ↓</td>
<td>Remainder show ↑ CL as S₁S₂ ↓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At CL = 300 msec, 60% accelerate for 3 to 5 beats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75% resume initial CL in &lt; 10 beats</td>
<td>25% ↑ or ↓ in rate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CL = cycle length; BCL = basic cycle length; SRA = sustained rhythmic activity.
on membrane potential, automaticity may or may not be suppressed by overdrive and, when suppressed, it then recurs.38, 39

Reentry, like digitalis-induced sustained rhythmic activity, can be terminated by pacing.40, 42 This may occur abruptly on cessation of pacing,41, 42 or there may be several “afterbeats” following the cessation of pacing.40, 42 It would be useful to obtain precise information about the frequency of afterbeats in reentrant rhythms, since this might help in discriminating between triggered and reentrant rhythms.

Single programmed premature beats usually reset digitalis-induced sustained rhythmic activity, although in the present study they did terminate 14% of such rhythms (usually at short S2S3 coupling intervals). We must stress, however, that such termination was not consistent and reproducible in any one preparation. This response differs quantitatively from that of reentrant rhythms, which reportedly are terminated abruptly by S1, 80% of the time in experimental animals39, 43-46 and 50% to 60% in human subjects.41, 42 Moreover, there is another important difference: that is, in reentry, termination by single premature beats at critical cycle lengths usually is consistent and reproducible; in the present experiments on triggered activity, consistency and reproducibility were not high. While dissimilar to reentry, the response of sustained rhythmic activity to S1 is similar to the responses of automatic rhythms, which can be reset by single premature beats.38

The use of double premature beats (S1S2) without prior paced beats terminated 27% of digitalis-induced sustained rhythmic activity. In the remaining 73%, the recovery cycle length of the arrhythmia decreased with the prematurity of the S1S2. The percentage of rhythms terminated by S1S2 increased to 86% when a brief period of S1S1 pacing preceded the S2S3. Approximately 75% of reentrant rhythms in human subjects are terminated by S1S2.42 In contrast, automatic rhythms usually are reset.

Summing up these observations concerning the termination of arrhythmias we find: automatic rhythms are best distinguished from the other mechanisms discussed by the fact that they cannot be terminated and are overdrive suppressed to a variable degree by pacing, and they can be reset (although their behavior may be complicated by phase shifting and annihilation phenomena).47, 48 Both digitalis-induced sustained rhythmic activity and reentry can be terminated by sustained pacing, but there appears to be a far higher likelihood of termination with single and double premature beats for reentry than for triggered activity. More important, termination by single or double premature beats is not consistent and reproducible during the sustained rhythmic activity (see table 2B), but is so in reentry. The only means by which a reproducible response was obtained in the presence of triggered activity occurred as a result of sustained pacing. Another possible differentiator of mechanism that requires further exploration is the effect of a single premature beat on the escape interval of an arrhythmia that is not terminated by that premature beat. Whereas for digitalis-induced sustained rhythmic activity, the escape interval often decreases as does the premature cycle length, for reentrant rhythms there is often an inverse relationship: as premature beats are induced earlier, the reentrant coupling interval becomes longer.16

Initiation of arrhythmias. In considering those situations in which an arrhythmia is not occurring and its induction is attempted, 83% of the fibers superfused with digitalis that we studied developed triggered activity in response to rapid pacing. This is qualitatively similar to the effect of rapid pacing in inducing reentry in animals43, 45, 46 and in human subjects41, 42 (where approximately 80% and 38%, respectively, will show induction of the arrhythmia). Normal and abnormal automatic mechanisms reportedly are not initiated by rapid pacing.38 The initiation of triggered activity in digitalis-toxic fibers occurs more readily at short cycle lengths than at long cycle lengths. In contrast, for reentrant rhythms, cycle length may be long or short.41 In the case of triggered rhythms as drive cycle length decreases, the coupling interval of the first beat of the induced arrhythmia usually decreases and the rate of the arrhythmia tends to increase. This does not hold for reentry. Finally, although triggered activity can be reinduced consistently, the reproducibility at a specific cycle length is low (38%, table 2A).

Premature stimulation induced triggered activity in 48% of preparations where S1S2S were used and 39% where S2S were alone were used. Similarly, in reentry there is a critical dependence of the arrhythmia on the premature cycle length, with double rather than single premature beats being more effective in induction.42, 43 However, at critical cycle lengths the reproducibility of induction is reportedly very high in reentry,41, 43 whereas for triggered activity there is a tendency for induction at short cycle lengths rather than at long cycle lengths; however, reproducibility at any one cycle length is not consistent (50%, table 2A). Moreover, in triggered rhythms at long S1S1 cycle lengths (i.e., 1000 and 800 msec), the coupling interval of the triggered action potential is prolonged as the S1 is induced earlier in diastole, whereas at shorter S1S1 cycle
lengths (600 and 400 msec), an increasing percentage of the fibers show a decrease in the coupling interval of the triggered action potential as the S1S2 decreases. This relationship also occurs in studies that use S1S3.

With respect to automatic rhythms, it has been assumed for some time that they could not be induced by premature stimulation. However, Janse and Van Cappelle have suggested that under appropriate circumstances in the ischemic heart an automatic focus may be triggered by a premature impulse. This observation still awaits direct experimental verification.

Once a reentrant or a triggered arrhythmia has been induced by pacing or premature stimulation, it tends to be stable. For both mechanisms there may be instability for a few beats (and either acceleration or deceleration), but thereafter variability is small (less than 5% for triggered rhythms). The vast majority of the triggered rhythms that were induced were not sustained (less than or equal to 28% persisting for more than 25 beats). One might interpret these results as indicating that most triggered rhythms, by definition, are not sustained. One cannot accept this conclusion, however, when one considers that the majority of the sustained rhythm activity that occurs with digitalis toxicity appears to be the result of triggered activity. This suggests that triggering can be as sustained an arrhythmia as reentry.

We express our gratitude to the following individuals: Dr. Irina Golyakovsky for assisting in certain experiments; Dr. Pedro Brugada for his helpful and incisive comments; and to Andrea Diano for her careful attention to the preparation of the manuscript.

References
7. Carmiliet E, Vereecke J: Adrenaline and the plateau phase of the cardiac action potential. Phleugers Arch 313: 300, 1969
8. Yeh BK, Lazzara R: Genesis of triggered and spontaneous automaticity in mammalian ventricular muscle fibers at high resting potentials. Experentia (Basel) 35: 500, 1979
39. Davis J, Glassman R, Wit AL: Method for evaluating the effects of antiarrhythmic drugs on ventricular tachycardias with different electrophysiologic characteristics and different mechanisms in the infarcted canine heart. Am J Cardiol 49: 1176, 1982
44. Fisher JD, Mehra R, Furman S: Termination of ventricular tachycardia with bursts of rapid ventricular pacing. Am J Cardiol 41: 94, 1978
Induction and termination of triggered activity by pacing in isolated canine Purkinje fibers.
J P Moak and M R Rosen

Circulation. 1984;69:149-162
doi: 10.1161/01.CIR.69.1.149

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/69/1/149

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

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

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