An evaluation of automaticity and triggered activity in the canine heart one to four days after myocardial infarction

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ABSTRACT Both abnormal automaticity and triggered activity induced by delayed afterdepolarizations have been proposed as the primary mechanism for ventricular tachycardia (VT) occurring in dogs 24 hr after ligation of the left anterior descending coronary artery. Because of this controversy, we studied the effects of ventricular pacing and therapeutic concentrations of lidocaine and ethmozin on sustained rhythmic activity of isolated subendocardial preparations excised from the infarct, and on VT in conscious dogs. There were differences in the sustained rhythmic activity cycle length of isolated preparations and the VT cycle length that were attributable to the absence of sympathetic input in the former and its presence in the latter. In isolated tissues, pacing for 1 or 10 beats reset the sustained rhythmic activity and pacing for 1 min induced overdrive suppression. Lidocaine (5 μg/ml) had no effect on sustained rhythmic activity but ethmozin (2 μg/ml) suppressed it. Delayed afterdepolarizations occurred but appeared to be induced by pacing or by the hyperpolarization associated with recovery. Although delayed afterdepolarizations were infrequent at 24 hr, their frequency increased with the hyperpolarization of the membrane that occurred at 48 to 96 hr after infarction. Delayed afterdepolarizations also occurred more readily when superfusate temperature was lowered. In conscious dogs, pacing the VT for 1 or 10 beats or 1 min had no effect. Lidocaine (2 to 10 μg/ml) did not affect the VT but ethmozin (2 to 5 μg/ml) increased VT cycle length significantly. Pacing for 1 min in the presence of ethmozin, but not lidocaine, converted VT to sinus rhythm. Our results suggest that although delayed afterdepolarizations occur at 24 hr after infarction in the standard Harris preparation, they are most readily seen as an accompaniment of hyperpolarization, pacing, or lowering of bath temperature. The predominant rhythm at 24 hr appears to be automatic.


PREVIOUS STUDIES1-4 have shown that the origin of the ventricular tachycardia (VT) that occurs 24 hr after ligation of the left anterior descending coronary artery in the dog is in the subendocardial zone of the infarct. These studies attributed the VT to automaticity of depressed subendocardial Purkinje fibers that survived the infarct, although the possibility of reentry was considered.2,5

Triggered activity resulting from delayed afterdepolarizations has also been noted in the 24 hr infarct, and it has been suggested recently that such triggered activity occurring in subendocardial Purkinje fibers is the major mechanism responsible for the VT.5 This conclusion was based on the study of the entire infarct in vitro and of small subendocardial preparations excised from the infarct, and on the identification of delayed afterdepolarizations and triggered activity by microelectrode techniques.5

We had two reasons for undertaking the present study. First, we believed that a model in which abnormal automaticity1-4 and triggered activity5 occurred would be useful for testing whether electrophysiologic and pharmacologic techniques might differentiate between these mechanisms in the intact animal and in isolated tissues. Second, because the 24 hr infarct in the dog was long thought to be a standard model of the abnormal automaticity that occurs at low membrane potentials (e.g., refs. 6 and 7), we believed it would be important to try to resolve the differences between
those studies of infarction that had identified automaticity as the mechanism and that which had identified triggered activity.  

Methods

**General approach.** Recognizing that the response of an arrhythmia to pacing, alone, in the intact animal and in isolated tissues might not be sufficient to aid in the distinction of mechanism, we also used a matrix of drugs that we previously have found useful in differentiating automatic and triggered activity. For the studies of cardiac pacing, we assumed that, given the ability of a paced impulse to gain access to the site of origin of an arrhythmia, any rhythm resulting from a normal automatic mechanism (occurring at high levels of membrane potential) should be readily suppressible by overdrive pacing; any rhythm resulting from abnormal automaticity (occurring at low levels of membrane potential) should respond to overdrive pacing with little or no change in its spontaneous cycle length; and any triggered rhythm should respond to pacing by increasing in its spontaneous rate on the cessation of pacing. For the pharmacologic studies, we used the following matrix to describe the expected responses of the arrhythmogenic mechanisms studied to lidocaine and ethmozin:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Normal automaticity</th>
<th>Abnormal automaticity</th>
<th>Delayed afterdepolarizations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>+ +</td>
<td>-</td>
<td>+ +</td>
</tr>
<tr>
<td>Ethmozin</td>
<td>+ (−)</td>
<td>+ +</td>
<td>+ +</td>
</tr>
</tbody>
</table>

These observations of drug effects were made in studies of both noninfarcted and infarcted tissues. Moreover, the matrix is dependent on the use of "therapeutic" concentrations of drug, since at higher concentrations (i.e., ≥10 to 15 μg/ml) lidocaine can suppress abnormal automaticity.

On the basis of these considerations, our first goal was to test the effects of lidocaine and of ethmozin on the abnormal automaticity and the delayed afterdepolarizations that occurred in infarcted canine myocardium. Our second goal was to use the information obtained concerning the response to pacing and to drugs in experiments on intact animals with VT 24 hr after infarction. Our third goal was to remove tissues from the hearts of these animals and observe their electrical activity in the tissue bath. This would permit the observation of automatic and triggered activity at the cellular level. Our final goal, based on the results of the first three phases of the study, was to resolve, if possible, any inconsistencies that might be observed between the intact animal and the isolated tissue studies, as well as between our experiments and those previously reported.

**Preparation of the infarct.** Dogs weighing 13 to 20 kg were anesthetized with 30 mg/kg iv sodium pentobarbital and maintained with supplemental doses as required. Under sterile conditions the left anterior descending coronary artery was ligated approximately 1 cm from the tip of the left atrial appendage according to the two-stage Harris procedure. A quadripolar plaque electrode was sewn to the epicardial surface of the left ventricle between the left anterior descending and the circumflex coronary arteries at a site that was close to the border of, but that did not impinge on, the infarct. This was approximately at the level of the tip of the left anterior papillary muscle. Other quadripolar electrodes were sutured on the left atrial appendage and the free wall of the right ventricle. An atrial catheter was inserted through the left atrial appendage. Three electrodes were inserted under the skin to record the electrocardiogram (ECG), and all the electrode wires and the atrial catheter were exteriorized via a subcutaneous tunnel to the intrascapular region. The animal then was allowed to recover from surgery.

Because of the influence that atrial activity can exert on ventricular rhythm, we used formalin to induce complete atrioventricular block in four dogs 3 to 4 weeks before infarction. At the time of surgery to induce infarction, these dogs had a stable, overdrive-suppressible idioventricular rhythm that has been attributed to normal automaticity. Another problem we considered was that the response of the infarct to pacing would be complicated if 1:1 conduction from the stimulation site to the endocardial surface of the infarct were not maintained. For this reason, in four other experiments a single bipolar "hook" electrode was plunged into the center of the infarct. This electrode was verified as being in the correct position by recording a "Purkinje spike" during the experiments, and subsequently on direct inspection at the termination of the experiment. In all four experiments there was delay of conduction from the stimulation site to the subendocardial infarct zone at the pacing cycle lengths used in this study. Nonetheless, 1:1 conduction was maintained 24 hr after infarction.

**Studies of intact animals 1 day after infarction.** Dogs were studied in the conscious state 20 to 24 hr after infarction. They stood in a sling while the ECG and electrograms were recorded with a Gould strip-chart recorder. A programmable stimulator (WPI) was used to deliver rectangular pulses of 2 msec duration and amplitude twice diastolic threshold through the ventricular and atrial electrodes.

We recorded the spontaneous rhythm of the heart and then used the following pacing protocol: (1) We induced single premature stimuli (S2) after every 15 spontaneous beats. These were coupled to the QRS complex of the VT by a Schmitt trigger. The coupling interval for the S2 initially was 95% of the spontaneous cycle length and then was decreased in 20 msec decrements until the effective refractory period was encountered. (2) We induced double extrastimuli (S1S2) in which the coupling interval for the S1 was 250 msec and that for the S2 was reduced in 10 msec decrements until the effective refractory period was attained. (3) We performed sustained pacing starting at a cycle length 5% shorter than that of the spontaneous VT and then reduced the cycle length in 20 to 50 msec decrements until the heart was refractory (at which time the pacing cycle length was in the range of 160 to 200 msec). For each animal two protocols for stimulation were used at each cycle length; these were 10 beats followed by cessation of pacing and 1 min followed by cessation of pacing. With the former protocol, the number of beats at each cycle length was a constant; with the latter, the time at each cycle length was a constant.

All measurements were done on strip-chart paper at a speed of 100 mm/sec. Spontaneous VT cycle length was expressed as the mean of 20 successive ventricular beats. During the pacing protocol the recovery cycle length was measured from the last paced QRS complex to the beginning of the first spontaneous QRS complex. To aid in the measurement of ventricular recovery cycle length, we stimulated the atrium and ventricle at the same time in an attempt to overdrive suppress atrial activity. The atrial electrograms and the QRS configuration were analyzed to confirm the occurrence of true ventricular escape vs atrial escape and ventricular capture after overdrive suppression of ventricular activity.

After the control pacing procedure was completed, some dogs were anesthetized with pentobarbital and their hearts were excised for microelectrode studies (see below). Other dogs were studied in the pharmacologic protocol, which was as follows: First, a lidocaine bolus of 4 mg/kg was injected through the atrial line. This was followed by an infusion of 3 mg/kg/hr. The ECG and electrograms were monitored continuously. At 30 min

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a blood sample was drawn for analysis of plasma lidocaine levels (with the EMIT enzyme immunoassay), the cycle length of the VT was measured, and the pacing protocol was repeated. A 7 mg/kg bolus of lidocaine was administered, followed by an infusion of 6 mg/kg/hr. At 30 min the electrophysiologic procedures described above were repeated and another plasma sample was obtained. In preliminary experiments we found that with these protocols there was little difference in the plasma lidocaine levels at 25, 30, and 40 min. Therefore, the results reported here were obtained during the maintenance of stable plasma levels.

A 2 hr recovery period was then permitted, after which we obtained another plasma sample to measure the lidocaine concentration and repeated the pacing procedure. In those instances where the response to pacing was identical to the initial control and the plasma lidocaine level had fallen below 2 µg/ml, we administered a 3 mg/kg bolus of ethmozin. At 30 min we obtained plasma samples for measuring the lidocaine and the ethmozin levels (the latter, kindly performed by Dr. C. C. Whitney of E. I. DuPont de Nemours & Co.). We then repeated the above-mentioned measurements of VT cycle length and repeated the pacing protocol. A second washout was permitted and the experiment was terminated.

To evaluate the contribution of the sympathetic nervous system and circulating catecholamines to the rhythm, after the initial pacing protocol in some experiments we administered the short-acting β-adrenergic blocker ACC 8052 (kindly supplied by Dr. Robert Lee, American Critical Care). This was infused at a rate of 40 µg/kg/min. Twenty-five minutes after the infusion was started, the pacing protocol was repeated. The drug then was discontinued, and a third trial of pacing was performed to confirm washout of its effects.

**Studies of intact animals 2 to 4 days after infarction.**

Animals were prepared as described above and were studied in the conscious state at 48, 72, or 96 hr after infarction. Control measurements of their spontaneous cardiac rhythm and response to pacing were made, after which they were anesthetized with pentobarbital and their hearts were excised for microelectrode studies. No pharmacologic experiments were performed in these animals.

**Studies of isolated cardiac tissues 1 day after infarction.**

All dogs studied here had undergone a pacing study of the heart in situ before anesthesia. Most of the dogs had not been subjected to any pharmacologic intervention during the intact animal study, with the exception of those that had received the short-acting β-adrenergic blocker. These were included after washout of the drug. These animals were used only for the study of the effect of catecholamines on their electrophysiologic characteristics, as described below.

Dogs were anesthetized and the hearts were quickly excised and placed in cold oxygenated Tyrode’s solution of the following composition (mM): NaCl 131, NaHCO3 18, CaCl2 2.7, MgCl2 0.5, NaH2PO4 1.8, KCl 4.0, dextrose 5.5. All of the infarcted endocardium and a border zone of normal tissue were excised carefully to avoid trauma to the infarcted area. These tissues were trimmed to a thickness of approximately 3 mm and pinned to the waxed bottom of a Lucite tissue bath. This held a volume of 25 ml and was perfused at a rate of 30 ml/min with Tyrode’s solution that was maintained at 37.5°C and bubbled with 95% O2 and 5% CO2.

The tissues were stimulated with bipolar silver electrodes placed on noninfarcted endocardium near the tip of the anterior papillary muscle at its junction with the anterior division of the left bundle branch. Standard techniques were used to deliver rectangular pulses 2 msec in duration and twice diastolic threshold to the preparation.20, 21 Previously described techniques were used to calibrate the system, to record transmembrane potential, and to calibrate, differentiate, and display the maximum upstroke velocity of phase 0.20, 21

The preparations were not paced when they initially were placed in the tissue bath. Rather, they were “mapped” with three microelectrodes until the site of earliest origin of their spontaneous rhythm was located. Then the tissues were stimulated, according to a protocol identical to that described above for the intact animals. Thereafter, two different methods were used. In some experiments the intact preparation was studied as described below, while maintaining one microelectrode at the site of earliest impulse initiation. However, in most experiments, a piece of tissue about 1.5 to 2 cm2 was excised, incorporating the site of earliest impulse initiation at its center. This was studied further with microelectrode and pacing techniques. The response to pacing and drugs was comparable in both preparations, although the actual site of earliest origin of the impulse tended to vary markedly in the intact preparation and little in the smaller preparation.

We paced both the large and the small preparations to permit observation of the responses of the rhythms to single premature stimuli (S2), to two premature stimuli (S2S3), and to sustained pacing at fixed cycle lengths for either 10 beats or 1 min. For all preparations we recorded the maximum diastolic potential, the activation voltage at which phase 0 of the action potential was initiated, and the overshoot and duration of the action potential measured to full repolarization. Standard methods were used to make these measurements20, 21 as well as to measure the coupling interval and amplitude of delayed afterdepolarizations.9 After the cessation of pacing, the recovery cycle length for the first spontaneous impulse that occurred was measured from the midpoint of phase 0 of the last paced beat to the midpoint of phase 0 of the spontaneous impulse.

The following terminology was used, as suggested by Cranefield:22

**Delayed afterdepolarization:** a depolarizing afterpotential that occurs after full repolarization of the action potential and is dependent on the action potential for its initiation. It cannot occur de novo. Its amplitude tends to increase and its coupling interval to the action potential that induces it tends to decrease as the drive rate is increased.8

**Triggered activity:** rhythmic activity that results from the attainment of threshold by a delayed afterdepolarization. The rate of the triggered rhythm tends to increase as the drive rate for any preparation increases.23

**Automaticity:** impulse initiation that can occur de novo and that results from phase 4 depolarization. When occurring in Purkinje fibers with high levels of membrane potential, it is readily suppressed by overdrive pacing.24 However, as membrane potential is decreased, this response to overdrive pacing is blunted and ultimately does not occur.11

**Sustained rhythmic activity:** a sustained rhythm, irrespective of its mechanism. This may be automatic, triggered, or reentrant.

After completing the control pacing study we used the following pharmacologic protocols: One set of tissues was superfused with Tyrode’s solution containing 5 µg/ml lidocaine. Thirty minutes after the onset of superfusion, we repeated the pacing protocol. Lidocaine then was washed out for 30 min and a second, control pacing study was done. If results returned to the initial control value, we then superfused the preparation with 2 µg/ml ethmozin. At 30 min pacing again was performed.

A second set of tissues received no lidocaine. Rather, after the control pacing study we superfused it with ethmozin only and studied it as described above. A third set of tissues received no drug at all. We did this because previous investigators have shown that infarcted preparations will hyperpolarize and show
improved action potential characteristics with time after they are placed in the tissue bath.\textsuperscript{1-3} This group of tissues, then, served as a control.

A fourth group was used for the study of catecholamine effects. We used tissues from the hearts of dogs that had received the short-acting β-adrenergic blocker ACC 8052 during the intact animal study. The catecholamine used was epinephrine (1 × 10^{-8}M). To retard its oxidation, we added EDTA (5 × 10^{-3}M) to the Tyrode’s solution (for these experiments only). We have used this concentration previously; it has no demonstrable effect on the transmembrane potential or on automatic or triggered rhythms.\textsuperscript{25}

In some experiments we studied the effects of temperature changes on the spontaneous rhythms and on the response to pacing. We did this because of the prior demonstration of the marked temperature sensitivity of autonomic rhythms.\textsuperscript{26} Here we used animals that had not been subjected to pharmacologic study. The protocol was as follows: In some experiments, the preparations were stabilized for 10 to 25 min at a temperature of 38.5\degreeC to 39\degreeC, and in other experiments the preparations were initially superfused with Tyrode’s solution\textsuperscript{27} at 36\degreeC. A fiber in the pacemaker region was impaled, and the preparation was overdriven for 15 sec intervals at cycle lengths between the spontaneous cycle length and the maximum following frequency of the preparation. In some experiments, 3 to 5 sec periods of “burst pacing” at cycle lengths shorter than the maximum following frequency were tested. Then the temperature was changed to 36\degreeC (if the preparation had been superfused initially at 38.5\degreeC to 39\degreeC) or to 39\degreeC (if the preparation had been superfused initially at 36\degreeC), and the overdrive protocol was repeated. Finally, the temperature was adjusted to its initial value, and the overdrive pacing was repeated.

\textbf{Studies of isolated cardiac tissues 2 to 4 days after infarction.} We obtained these tissues from the same animals that were studied in the intact, conscious state at 48, 72, and 96 hr after infarction. We used the same methods described above to isolate the infarcts, to record their transmembrane potentials, and to observe their response to pacing. No pharmacologic experiments were performed here.

\textbf{Data analysis.} Data for transmembrane potentials are presented only when an impalement was maintained throughout an experiment. Results are expressed as mean ± SE. Although the paired t test was appropriate for the analysis of certain of our data, the test used to analyze the response to pacing and to compare curves before and after various interventions was the nested analysis of variance.\textsuperscript{28} When the ANOVA permitted us to do so, further comparison of individual data points was done with Scheffe’s test.\textsuperscript{28}

\section*{Results}

\textbf{Isolated tissues from the 24 hr infarct.} Subendocardial tissues from the infarct zone were obtained from 38 dogs. Their transmembrane potential characteristics were recorded within 20 min of placement in the tissue bath. The values for the transmembrane potentials at the site of earliest onset of electrical activity were: maximum diastolic potential, -64.8 ± 1.1 mV; activation voltage, -49.7 ± 2.5 mV; overshoot, 11.2 ± 0.9 mV; action potential duration, 415 ± 13 msec; and spontaneous cycle length, 668 ± 191 msec. The pacing protocol was performed and 19 preparations were then subjected to pharmacologic interventions (see below). The other 19 preparations were not superfused with drugs, and their transmembrane potentials were observed for the next 60 min. This provided a time control for the pharmacologic studies that took into account any electrophysiologic changes that might occur while the tissues remained in the superfusate. (These values are used in table 2 [see below] as the concurrent controls for the drug studies.)

We noted the following general characteristics of the preparations when they initially were placed in the tissue bath: First, all had sustained rhythmic activity and this was regular in its rhythm in all but seven. However, within 10 to 20 min of their placement in the tissue bath, the rhythm of these seven had become regular as well. Second, as previously described, there was a general tendency for the preparations to show changes in transmembrane potential characteristics over the course of several hours. Eight of the preparations were observed for 4 hr. Two of these gradually hyperpolarized and became quiescent. After 4 hr these did not initiate impulses spontaneously but did respond to extrinsic stimulation with delayed afterdepolarizations and triggered activity. Records from one of these are presented in figure 1. As shown here, during stimulation at long cycle lengths, only a delayed afterdepolarization was evoked (upper left panel and filled circles on graph). However, as drive cycle length was decreased, triggered activity supervened (upper right panel and unfilled circles on graph). The characteristic shortening of both the delayed afterdepolarization and triggered action potential coupling intervals occurred with reduction of the drive cycle length.

The other six preparations also hyperpolarized over the course of 4 hr, but showed no loss of sustained rhythmic activity. Their characteristics are presented in table 1. Note that significant increases in maximum diastolic potential, activation voltage, overshoot, and cycle length occurred with time. Nonetheless, all these

\begin{table}
\centering
\caption{Changes in transmembrane potential characteristics with time (n = 6)}
\begin{tabular}{cccc}
\hline
 & MDP (mV) & AV (mV) & OS (mV) & SRA CL (msec) \\
\hline
Control & -64.5 ± 1.3 & -51.6 ± 2.6 & 11.3 ± 2.8 & 737 ± 96 \\
4 hr later & -75.8 ± 1.2 & -65.6 ± 4.7b & 28.7 ± 4.7b & 972 ± 44a \\
\hline
\end{tabular}
\end{table}

\textsuperscript{b}p < .01; \textsuperscript{c}p < .001.

\textsuperscript{a}
preparations remained automatic and showed no delayed afterdepolarizations.

An example of the response to pacing of one automatic rhythm is shown in figure 2. This preparation had a low maximum diastolic potential and was not suppressed by overdrive pacing at a cycle length of 600 msec for 1 min. However, pacing at a cycle length of 400 msec was accompanied by hyperpolarization and, with this, modest overdrive suppression.

A comparison of the effects of pacing for 1 beat, for 10 beats, and for 1 min on the recovery cycle length of the infarcted preparations is demonstrated in figure 3. Note that over a wide range of pacing cycle lengths the use of single premature stimuli or trains of 10 stimuli was insufficient to modify the recovery cycle length. However, overdrive for 1 min at cycle lengths of 400 msec or less was associated with significant overdrive suppression. In all those preparations that were suppressed by overdrive pacing, the membrane hyperpolarized during the pacing procedure (e.g., figure 2). When the maximum diastolic potential attained a range of $-65$ to $-75$ mV, delayed afterdepolarizations usually were seen. We must stress, however, that although present, they did not appear to contribute to the sustained rhythmic activity. An example is shown in figure 4. Here, pacing at a cycle length of 400 msec induced hyperpolarization and overdrive suppression (panel B). The last paced action potential was followed by a delayed afterdepolarization. This, in turn, was superimposed on phase 4 depolarization, which led to reemergence of the automatic rhythm. It is important to note that as the preparation proceeded to depolarize (i.e., to show a reduced maximum diastolic potential), the delayed afterdepolarization became smaller in amplitude, finally disappearing as the preparation attained its previous membrane potential and automatic rate.

Response of the isolated tissues to drugs. We studied the response to lidocaine followed by ethmozin in 12 preparations, and to ethmozin alone in seven preparations. In table 2, A, the first comparison is between lidocaine and a concurrent control. Although there were significant increases in maximum diastolic potential and overshoot in the presence of lidocaine, these changes did not reverse on washout. Even in the presence of these changes in membrane potential, lidocaine did not exert a significant effect on spontaneous rate.

Because of the loss of three impalements, only nine of the 12 preparations were superfused subsequently with ethmozin (table 2, A). In all nine the sustained rhythmic activity ceased. No data for washout are presented here because, as described previously, the effects of ethmozin are difficult to reverse in isolated tissue studies.\(^\text{13}\)

A shortcoming of the experimental design in table 2, A, is that the comparisons of lidocaine and ethmozin are sequential. One cannot be entirely certain that over this period, cessation of electrical activity did not occur spontaneously (although the data in table 1 argue against this). For this reason we performed the experiments in table 2, B. The period of time involved was the same as that for the lidocaine experiment in table 2, A. Note that significant hyperpolarization again occurred. In six of the seven fibers, ethmozin again induced quiescence; in the seventh, it markedly reduced spontaneous rate. Hence, it appears that the sustained rhythmic activity of subendocardial fibers in the infarct is suppressed by ethmozin but not by lidocaine.

That both lidocaine and ethmozin did suppress in-
farct-induced delayed afterdepolarizations and triggered activity was shown in the only two experiments in which delayed afterdepolarizations and triggered activity occurred from the onset of the experiment. As shown in figure 5, when delayed afterdepolarizations and triggered activity occurred before administration of either lidocaine and ethmozin, these were suppressed by both drugs.

Response to pacing of the heart in situ. Fifteen dogs with sustained VT were studied with pacing alone 24 hr after infarction. Five of these were monomorphic tachycardias, and 10 were polymorphic. The spontaneous VT cycle length was 330 ± 16 msec, and the response to pacing is reviewed in figure 6. Single premature depolarizations, pacing for 10 beats, and pacing for 1 min did not result in the occurrence of significant overdrive suppression or of an increase in spontaneous rate. Rather, only a delay of the recovery cycle consistent with reset of the first recovery beat and/or a modest degree of overdrive suppression was seen.

Included in this group were the four dogs with complete atrioventricular block induced by formalin and 11 with an intact atrioventricular conducting system. To decrease the likelihood that sinus beats might induce a ventricular response after pacing in the latter group, both the atria and the left ventricle were paced simultaneously (see Methods). Hence the recovery beats shown in figure 6 are all ventricular. No apparent difference in the ventricular response was seen in the four dogs with complete atrioventricular block and those with intact conducting systems.

Response to drugs of the heart in situ. We studied the effects of sequential administration of lidocaine and ethmozin in seven dogs (table 3). The plasma lidocaine level before administration of ethmozin was 1.6 ± 0.1 μg/ml. Whereas lidocaine in therapeutic and toxic concentrations (the latter demonstrated by the occurrence of emesis) had no significant effect on the spontaneous cycle length of the VT, this was significantly prolonged by ethmozin. Furthermore, it should be noted that all seven animals initially reverted to normal sinus rhythm after administration of ethmozin. The VT cycle length shown in table 3 was that recorded 30 min after administration of ethmozin, at which time the tachycardia had recurred in all dogs.

The effects of lidocaine and of ethmozin during pacing are shown in figure 7. Pacing was performed 30 min after the start of lidocaine administration and 30 min after the ethmozin bolus, at which time all dogs were in VT. As shown in table 3 and in the right side of each panel in figure 7, there was no change from the control VT cycle length in the presence of lidocaine, but there was significant slowing with ethmozin. Figure 7, A, demonstrates that interruption of the VT by a single premature stimulus in the presence of either therapeutic or toxic concentrations of lidocaine did not significantly change the cycle length of the first recov-
There were two readily identifiable variables that might have accounted for this difference: one was the fact that in the isolated tissue studies, all effects of the sympathetic nervous system and of circulating catecholamines were eliminated; the second was that whereas the rectal temperature of the intact animals was 39.4° ± 0.3° C, the bath temperature for the isolated tissues was 37.5° C. Both sympathetic effects and temperatures can modify pacemaker function importantly, and so we studied the effects of both of these on the rhythms that occurred.

In the studies of sympathetic modulation, we used the short-acting β-adrenergic blocker ACC 8052, infused in six conscious dogs 24 hr after infarction. The control VT cycle length in these animals was 312 ± 20 msec. This increased to 382 ± 79 msec (p < .01) in the presence of the drug. As shown in figure 8, the β-adrenergic blocker significantly prolonged the recovery cycle length, shifting the curve relating recovery cycle length to overdrive pacing cycle length upward and to the right. After washout of the β-adrenergic blocker, the heart was removed from the animal and the infarct was studied in the tissue bath, as described above. The cycle length of the sustained rhythmic activity in the isolated infarct 10 min after placement in the tissue bath was 758 ± 72 msec. Upon superfusion with epinephrine, this decreased to 478 ± 29 msec (p < .001). Moreover, the addition of epinephrine to the superfusate significantly shifted the curve relating recovery cycle length to pacing cycle length downward and to the left (figure 8). Hence, β-adrenergic blockade in the intact animal shifted both the VT cycle length and the response to pacing in a direction similar to that seen in isolated tissue; the addition of epinephrine to the superfusate for the isolated tissue shifted the curve in a direction similar to that seen in the intact animal.

The studies of temperature changes were carried out in 14 preparations from isolated infarcts. Two preparations initially were superfused at 36° C. Their maximum diastolic potentials were −40 and −46 mV, and the action potentials appeared to be induced by triggered activity. After the temperature had been increased to 38.5° to 39° C for 15 to 30 min, both preparations had automatic activity with maximum diastolic potentials of −54 and −57 mV, respectively. Then, when the temperature was decreased to 36° again, the preparations showed triggered activity with maximum diastolic potentials of −53 and −60 mV, respectively.

In five preparations that were equilibrated initially at 38.5° to 39° C, the maximum diastolic potential was

![Figure 3](http://circ.ahajournals.org/)

**FIGURE 3.** Effects of pacing with single extrastimuli, for 10 beats, and for 1 min on sustained rhythmic activity of 15 isolated 24 hr infarcts, 30 to 60 min after excision from the heart. The horizontal axis is the pacing cycle length or the coupling interval of the extrastimuli. The vertical axis is the cycle length of the first action potential after cessation of pacing. Single extrastimuli and 10 paced beats induced no overdrive suppression or enhancement of the rhythm. One minute of pacing induced overdrive suppression that was variable from one experiment to another. The extent of overdrive suppression increased as pacing cycle length decreased.

Every beat. The same phenomenon occurred with two premature beats (not shown), pacing for 10 beats (panel B), and pacing for 1 min (panel C). In contrast, ethmozin significantly delayed the first recovery cycle with single and double premature stimuli and brief bursts of pacing (panels A and B). Moreover, there was significant overdrive suppression in the presence of ethmozin (panel C), such that for six of the seven animals the recovery cycle reflected sinus escape rather than ventricular escape. In other words, the delay of recovery would have been even longer had there not been sinus node escape.

Effects of autonomic interventions and of temperature on the arrhythmia at 24 hr. One of the problems in relating the isolated tissue data to those obtained in intact animal experiments was the difference in the cycle length of the VT in the animals (approximately 300 to 400 msec) and that of the sustained rhythmic activity in the isolated tissues (approximately 600 to 800 msec).
$-55 \pm 0.9$ mV and all preparations were automatic. When the temperature was decreased to 36°C, the maximum diastolic potential was $-57 \pm 0.9$ mV. Two of the preparations showed delayed afterdepolarizations at the lower temperature, and three continued to show automaticity. Finally, when the bath temperature was returned to 38.5° to 39°C, all five preparations were again automatic, having maximum diastolic potentials of $-59 \pm 0.4$ mV. Typical results are shown in figure 9.

In the other seven preparations the initial maximum diastolic potentials were greater than $-60$ mV ($-76 \pm 1.9$ mV). The results here were consistent with those at the lower range of membrane potentials: six

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| TABLE 2 |
| Effects of lidocaine and ethmozin on the transmembrane potential and cycle length of sustained rhythmic activity in infarcted preparations$^A$ |

<table>
<thead>
<tr>
<th>MDP (mV)</th>
<th>AV (mV)</th>
<th>OS (mV)</th>
<th>APD (msec)</th>
<th>SRA CL (msec)</th>
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<tbody>
<tr>
<td>A: Lidocaine followed by ethmozin</td>
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<tr>
<td>Control (n = 12)</td>
<td>$-65.4 \pm 1.5$</td>
<td>$-54.6 \pm 3.5$</td>
<td>$11.9 \pm 1.6$</td>
<td>$441 \pm 20$</td>
</tr>
<tr>
<td>Lidocaine 5 mg/l (n = 12)</td>
<td>$-72.5 \pm 1.5^b$</td>
<td>$-57 \pm 3.5$</td>
<td>$18.5 \pm 2.7^b$</td>
<td>$419 \pm 16$</td>
</tr>
<tr>
<td>Washout (n = 9)</td>
<td>$-71.1 \pm 1.7^b$</td>
<td>$-56 \pm 4.2$</td>
<td>$19 \pm 3.4^b$</td>
<td>$414 \pm 12$</td>
</tr>
<tr>
<td>Ethmozin 2 mg/l (n = 9)</td>
<td>$-74 \pm 2.4^b$</td>
<td>—</td>
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<td>—</td>
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<tr>
<td>B: Ethmozin alone</td>
<td></td>
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</tr>
<tr>
<td>Control (n = 7)</td>
<td>$-62.4 \pm 1.7$</td>
<td>$-51.3 \pm 2.4$</td>
<td>$10.1 \pm 3.4$</td>
<td>$391 \pm 20$</td>
</tr>
<tr>
<td>Ethmozin 2 mg/l (n = 7)</td>
<td>$-73.2 \pm 2.7^b$</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations as in table 1.

$^A$Results expressed as mean ± SE; n values represent number of experiments.

$^b$p < .01 vs control.
preparations showed delayed afterdepolarizations at the low temperatures and all showed automaticity at the high temperatures.

Studies performed 48 to 96 hr after infarction. As demonstrated above, the occurrence of triggered activity resulting from delayed afterdepolarizations in the 24 hr isolated infarcts appeared to be related, in part, to the membrane potentials of the preparations. Moreover, in none of the intact animals 24 hr after infarction was there a response to pacing or to drugs that was consistent with triggered activity as its cause. This led us to conclude that whereas triggered activity was inducible in the isolated infarct when its membrane potential increased, it would not be expected to occur spontaneously in the intact heart at a time when the tissues in the infarct were markedly depolarized. Therefore, it seemed reasonable to ask whether healing of the infarct and attendant hyperpolarization of the subendocardial fibers in situ might not be associated with the occurrence of delayed afterdepolarizations and triggered activity. For this reason we studied eight dogs 48 to 96 hr after infarction. In only three of these animals was there a spontaneous VT. However, in three others, a tachycardia was inducible that, in its response to pacing, was consistent with triggered activity (figure 10). The transmembrane potentials recorded from the infarcted tissues taken from these animals were as follows: maximum diastolic potential, $-73.0 \pm 1.5\, \text{mV}$; activation voltage, $-62.5 \pm 2.8\, \text{mV}$; overshoot, $22.0$

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**TABLE 3**

<table>
<thead>
<tr>
<th></th>
<th>Lidocaine</th>
<th>Ethmozin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 2 to 5 (\mu)g/ml</td>
<td></td>
</tr>
<tr>
<td>CL (msec)</td>
<td>355 $\pm$ 10</td>
<td>374 $\pm$ 12</td>
</tr>
</tbody>
</table>

CL = cycle length.

$^a$ Plasma levels and changes in cycle length 30 min after onset of lidocaine administration and 30 min after ethmozin bolus.

Ethmozin initially induced a return to sinus rhythm in all dogs. The values presented here are 30 min after ethmozin, at which time VT had recurred.

$^b$ $p < .001$ vs control.
± 4.1 mV. The five infarcts studied at 48 hr and the one at 72 hr showed automaticity. The two studied at 96 hr were quiescent. Hence the transmembrane potential recordings were derived from eight samples for the maximum diastolic potential and from six samples for the other variables. Note that the values for maximum diastolic potential are significantly higher than those seen in the isolated tissues at 24 hr (cf tables 1 and 2). In addition, three of the infarcts showed pacing-induced delayed afterdepolarizations and triggered activity similar to those in figure 1.

Discussion

Previous studies of the VT that occurs 24 hr after myocardial infarction have shown it to be focal in origin and probably not reentrant. This conclusion is based on mapping experiments that localized the site of origin of the tachycardia to specific foci and demonstrated a pattern of activation emanating from that site rather than a circus movement. Hence, the major question with regard to this tachycardia was not whether it is reentrant, but rather whether the mechanism for the focal tachycardia is automaticity, as has been held to be the case, or triggered activity, as has been suggested more recently.

Our results suggest that automaticity of the type that occurs at low membrane potentials (so-called abnormal automaticity, e.g., ref. 11) is the most frequent cause of VT in the 24 hr infarct. The evidence that leads us to this conclusion is the following: First, as shown in figure 6, the rhythm was not suppressed by overdrive pacing nor was its rate increased as overdrive rate increased. Were the rhythm the result of the normal automatic mechanism seen in fully polarized
Purkinje fibers, we would expect its recovery cycle length to prolong progressively as the pacing cycle length is shortened.20 That this did not occur might be explained as a result of (1) failure of the paced impulses to gain access to the site of origin of the arrhythmia or (2) inability of overdrive pacing to modify the mechanism. That the stimulus did, in fact, gain access to the arrhythmogenic focus was demonstrated in those experiments in which we recorded electrograms from the subendocardial Purkinje fibers in the infarct (see Methods). Therefore we conclude that the mechanism was one that simply was not overdrive suppressible by 1 min of pacing in the intact dog. This inability to be overdrive suppressed is far more consistent with abnormal automaticity than with triggered activity as the mechanism. The latter would be expected to increase in its rate as drive cycle length decreases,23 29 a phenomenon we did not see in the intact animal 24 hr after infarction.

The isolated tissue studies also suggested that the mechanism was automatic rather than triggered. As shown in figure 3, the rhythm was not overdrive suppressible by short periods of pacing, but after long periods of drive at short cycle lengths it could be suppressed. Only a small subset of preparations demonstrated overdrive-induced delayed afterdepolarizations and increases in spontaneous rate interpreted as triggered activity. Moreover, these were seen only in those preparations in which some hyperpolarization had occurred. Such membrane potential dependence of delayed afterdepolarizations has been demonstrated for other types of delayed afterdepolarizations, most notably those induced by digitalis.30

Our results in intact animals and in isolated tissues 48 to 96 hr after infarction are also consistent with the association between triggered activity and hyperpolarization of the membrane. In only three of the eight intact animals was an arrhythmia demonstrable in the absence of cardiac pacing. Nonetheless, pacing induced tachycardias that had a cycle length dependence consistent with triggered activity occurred in three dogs (figure 10). Moreover, the isolated tissues obtained from the infarcts at 48 to 96 hr showed high membrane potentials, delayed afterdepolarizations, and triggered activity.

To summarize our interpretation of our results to this point, in the 24 hr infarct the arrhythmia in the intact animal is automatic. However, when the isolated tissues obtained from these animals hyperpolarize in the tissue bath they can demonstrate triggered activity. In addition, at 48 to 96 hr after infarction, cells in the infarct also tend to hyperpolarize and triggered activity then is demonstrable in the intact animal as well as in the isolated tissue. Although there are discrepancies between the cycle lengths in the isolated tissue and intact animal experiments, these can be explained, at least in part, by differences in β-adrenergic input as suggested by the data in figure 8.

The effect of temperature also appears to be important in modulating the arrhythmia in the isolated tissues. The temperature of the intact animals was 39.4°C, almost 2°C higher than that routinely maintained in the tissue bath. Delayed afterdepolarizations were more readily demonstrable, and automaticity was least evident when temperature was reduced from 39.5°C to 36°C. The latter finding may help to explain an apparent discrepancy between our results and those of El-

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**FIGURE 8.** Effects of β-adrenergic interventions and pacing for 1 min on VT in six conscious dogs 24 hr after infarction and on sustained rhythmic activity of isolated preparations excised from these dogs. The horizontal axis is the pacing cycle length; the vertical axis is the recovery cycle length for the first beat after cessation of pacing. The discontinuous symbols on the right are the cycle lengths before pacing. The unfilled circles are data from the conscious control animals, and the filled circles are data from the conscious animals after infusion of 40 μg/kg/min ACC 8052. These two curves differ significantly (p < .05). The filled triangles are the controls for spontaneous rhythmic activity in the isolated tissues. The unfilled triangles represent data recorded in the presence of epinephrine, (1 × 10−6M). The two curves differ significantly (p < .001). The curves from epinephrine-treated isolated tissues (unfilled triangles) and ACC 8052-treated intact animals (filled circles) did not differ significantly (p > .05).
Sherif et al.,\textsuperscript{5} in which delayed afterdepolarizations were identified as a mechanism for the arrhythmia rather than automaticity. In the latter study, the temperature in the tissue bath was maintained at 36\degree C, which we have shown to favor the occurrence of delayed afterdepolarizations. Moreover, in our studies the rhythms of the isolated tissues at 39\degree C were not readily suppressible by overdrive pacing (see figure 9). This is consistent with the inability of pacing alone to terminate the rhythm in our intact animal studies.

The ionic mechanisms responsible for the change from automatic to triggered activity as temperature was lowered from 39\degree C to 36\degree C are not known. It is possible that an increase in threshold potential and/or a decrease in the slope of phase 4 depolarization may accompany the lowering of temperature. We may speculate, as well, that changes in temperature modify intracellular Ca\textsuperscript{2+} metabolism, thereby modulating the transition between triggered and automatic activity.

Our pharmacologic studies support our interpretation of the 24 hr arrhythmia as being automatic. Therapeutic concentrations of lidocaine had no significant effect and ethmozin suppressed the sustained rhythmic activity in the isolated tissues (table 2) and the VT in the intact animals (table 3). Moreover, whereas ethmozin modified the response to pacing in both the intact animals and the isolated tissues, lidocaine did not. In keeping with the matrix of effects presented in Methods, this result suggests that the rhythm is automatic rather than triggered.

Another point to be stressed is that even in instances where delayed afterdepolarizations were seen, that they occurred was in itself not sufficient evidence to permit their acceptance as the cause of the arrhythmia. This is perhaps best demonstrated in figure 4, where

\textbf{FIGURE 9.} Effects of bath temperature on rhythms of two isolated infarcts. A. One preparation at 39\degree C. The rhythm appeared to be automatic, and pacing (black bar) induced no change in it. B. Same preparation at 36.2\degree C. The preparation hyperpolarized and pacing induced further hyperpolarization followed by a delayed afterdepolarization and 3 beats that appeared triggered. After quiescence there was warm-up of an apparently automatic rhythm. C. Another preparation, showing sustained rhythmic activity at 39.1\degree C. Pacing had no effect. D. At 36.1\degree C, pacing induced hyperpolarization that was followed by a delayed afterdepolarization and quiescence. There again was a gradual warm-up of an apparently automatic rhythm.

\textbf{FIGURE 10.} Effects of pacing for 10 beats on the heart of an intact dog 48 hr after infarction. During control the rhythm was sinus in origin, and few ventricular premature beats were seen. At relatively long pacing cycle lengths (unfilled circles), the first beat after cessation of pacing was sinus in origin and overdrive suppression occurred. At a critical cycle length (asterisk), pacing induced a ventricular premature beat, the coupling interval of which decreased as the pacing cycle length decreased. At pacing cycle lengths of 200 msec or less two ventricular beats followed the last stimulated beat.
overdrive pacing of sustained rhythmic activity resulted in suppression of the rhythm, the last action potential of which was followed by a delayed afterdepolarization. However, as the automatic focus resumed its function and increased its rate, the membrane depolarized and the afterdepolarization became smaller. This suggests that even the demonstration of delayed afterdepolarizations at the site of infarction in the heart in situ may not indicate that the afterdepolarizations are responsible for the arrhythmia.

It is clear, then, that in instances where myocardial infarction is induced by the Harris technique, both abnormal automaticity and delayed afterdepolarizations can occur. Our experimental results suggest that abnormal automaticity is responsible for most of the arrhythmias, even in instances where delayed afterdepolarizations are seen concurrently. Nonetheless, it is likely that the afterdepolarizations are responsible for some of the arrhythmias that occur in the 24 hr infarct and thereafter (eg., 48 to 96 hr after infarction), although it appears that these arrhythmias are induced, either by the basic automatic rhythm or by extrinsic pacing. The conditions that favor triggered rhythms include reduced temperature and higher membrane potentials. It may be that in instances where infarcts are small or where sufficient time after infarction has passed to permit membrane hyperpolarization of surviving tissues, the likelihood of triggered activity increases.

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