Mechanisms for atrial arrhythmias associated with cardiomyopathy: a study of feline hearts with primary myocardial disease

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ABSTRACT The cellular electrophysiologic and structural characteristics of arrhythmic and non-arrhythmic atria isolated from feline hearts with spontaneously occurring cardiomyopathy were studied. The animals were divided into three groups according to the degree of left atrial enlargement: mild (group I), moderate (group II), and severe (group III). The right atria were of relatively normal size. Microelectrode recordings showed that inexcitable cells were present in both left and right atria of all groups but were most numerous in the left atria of group III animals. Most inexcitable cells had low resting membrane potentials. There was also a significant reduction in resting membrane potentials, maximum rate of phase 0 depolarization, and action potential amplitude of excitable cells in left atria of animals in groups II and III, whereas action potentials of excitable cells in the right atria were normal. Acetylcholine or norepinephrine often restored excitability to cells that originally did not generate action potentials. Norepinephrine also caused slow-response action potentials as well as abnormal automaticity and triggered activity due to delayed afterpotentials. The diseased atria showed marked structural abnormalities, which were most pronounced in group III cats, including large amounts of interstitial fibrosis, cellular hypertrophy and degeneration, and thickened basement membranes. Therefore electrophysiologic abnormalities and concurrent changes in cell structure may be involved in the genesis of atrial tachyarrhythmias caused by cardiomyopathy.


CONGESTIVE or hypertrophic cardiomyopathy is sometimes associated with atrial tachyarrhythmias. The electrophysiologic mechanisms of these arrhythmias are unknown. Arrhythmias might be related to significant alterations in the transmembrane potentials of atrial fibers associated with marked structural abnormalities characteristic of cardiomyopathy. They might also be caused by the enlargement of the atria that occurs secondary to diminished ventricular function. Abnormal transmembrane potentials need not be a causative factor.

To determine the mechanisms of atrial arrhythmias associated with cardiomyopathy, we studied the electrophysiologic and ultrastructure characteristics of the atria of domestic cats with naturally occurring primary myocardial disease. The gross and microscopic pathologic changes in the heart associated with this disease mimic those of human hearts with hypertrophic and congestive cardiomyopathy. Cardiomyopathy in the cat, like that in humans, causes atrial enlargement and arrhythmias.

Methods

We selected 34 cats with clinical characteristics indicative of feline cardiomyopathy. The initial diagnosis was made after a physical examination, auscultation, and radiographic and electrocardiographic evaluations as described by Tilley et al. We also determined the left atrial volume of 27 of the cats with cardiomyopathy from nonselective angiocardiograms as previously described. Atrial size in cats that did not undergo angiocardiography was estimated from plain thoracic radiographs. In addition, 10 cats with normal hearts and no atrial enlargement were included in the study as a control group. Left atrial size was determined from nonselective angiocardiograms in four of these cats. The diagnosis of cardiomyopathy was always confirmed by gross and histologic examination of the hearts at the end of an experiment. This examination showed that 14 of the cats had congestive cardiomyopathy and 20 had...
hypertrophic cardiomyopathy (see figures 9B and 11 in ref. 7; see also ref. 8). The cats were anesthetized with 30 mg/kg sodium pentobarbital. The hearts were removed and placed in a cool oxygenated Tyrode’s solution, and tissue was then removed from both the right and left atria. Preparations for study included most of the free wall and some of the appendage (each right and left atrial preparation was approximately two-thirds free wall and one-third appendage). Each preparation, which was approximately 4 cm², was placed with the endocardial surface up in a separate 5 ml chamber of a dual-chambered Lucite superfusion bath. The samples were superfused with oxygenated Tyrode’s solution, and stimulated at a basic cycle length of 800 msec through Teflon-coated bipolar silver-wire electrodes. The stimuli were isolated rectangular pulses, 2 to 4 msec in duration and 2 times diastolic and threshold voltage. Temperature in the bath was maintained at 36⁰ ± 0.5⁰ C in each experiment.

After an equilibration period of at least 20 min, during which time the tissue was constantly stimulated, transmembrane potentials were recorded with 3M KCl-filled microelectrodes from atrial fibers at sites separated by 5 to 8 mm over the entire endocardial surface. An effort was made to collect data equally from all regions of each preparation. However, occasionally the increased thickness of the endocardium of the cardiomyopathic atria prevented microelectrode recordings from being obtained at some sites. Transmembrane potentials were recorded at each site from the most superficial fibers. The mean values for resting and action potential parameters were measured by techniques previously described for each of the right and left atrial preparations (see figures 2 and 3). In each group of animals (see below) we determined the mean of the mean values for preparations within the group. We then compared the mean values of the different experimental groups. A statistical difference was evaluated first with an analysis of variance F test on the groups of data and then with either an unpaired Student’s t test or a modified t test for populations of unequal variances.

In some studies, the effects of acetylcholine, catecholamines, and the slow channel-blocking drugs verapamil and AHR-2666 were investigated. Aliquots from stock solutions were added to the Tyrode’s solution to achieve the desired concentrations. When the effects of catecholamines were determined, NaEDTA (5 × 10⁻⁵M) was also added to the Tyrode’s solution to prevent oxidation.

Pieces of tissue approximately 1 cm² were also taken for electron microscopic study from at least two places on the endocardial surface adjacent to the regions used for the cellular electrophysiologic study. Tissue specimens were processed and sectioned by methods described in previous reports. The size of the atrial cells (diameter at the level of the nucleus) was also determined.

Results

To determine whether electrophysiologic abnormalities were related to atrial size, the cats with cardiomyopathy were subdivided into three groups on the basis of left atrial enlargement (group I, mild; group II, moderate; group III, severe). The left atrial volumes determined by nonselective angiography in these cats are shown in table 1 and compared with those in the four normal cats. No quantitative measurements were made of right atrial size in these cats, but generally only slight-to-mild right atrial enlargement occurs in cats with cardiomyopathy. In addition, on the basis of estimates of left atrial size from thoracic radiographs, two cats were added to group I (n = 10), four cats to group II (n = 15), and one cat to group III (n = 9).

The cats with congestive and hypertrophic cardiomyopathy were not put into separate groups because preliminary data analysis showed that the atrial cellular electrophysiologic characteristics for both types of pathologic changes were identical. In addition the histologic and ultrastructural abnormalities of atrial fibers in both groups were identical.

The cardiac rhythm of cats in the three groups are also shown in table 1. Although most of the cats in groups I and II were in sinus rhythm, only two cats in group III were in sinus rhythm; four had chronic atrial fibrillation.

Cellular electrophysiology

Nondriven rhythmic activity. Immediately after the right and left atrial preparations were mounted in the tissue chamber, we determined whether nondriven rhythmic activity was present by recording transmembrane potentials in the absence of electrical stimulation. The right and left atrial preparations from the normal cats were quiescent. Only four atrial preparations (two right and two left) of the 68 from cats with cardiomyopathy beat spontaneously (cycle length range 350 to 600 msec). Three of these preparations were from animals that were in sinus rhythm before death and one was from an animal with atrial premature depolarizations. All other diseased atrial preparations, including 10 from the five hearts with atrial flutter and fibrillation (see table 1), were quiescent. Spontaneous diastolic depolarization was not found in the atrial fi-

<table>
<thead>
<tr>
<th>Group</th>
<th>LA volume</th>
<th>ECG</th>
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<tbody>
<tr>
<td>Control (n = 10)</td>
<td>1.53 ± 0.41 ml (n = 4)</td>
<td>NSR (n = 10)</td>
</tr>
<tr>
<td>Group I (n = 10)</td>
<td>5.7 ± 2.3 ml (n = 8)</td>
<td>APC (n = 7)</td>
</tr>
<tr>
<td>Group II (n = 15)</td>
<td>11.54 ± 4 ml (n = 11)</td>
<td>AT (n = 1)</td>
</tr>
<tr>
<td>Group III (n = 9)</td>
<td>29.6 ± 10 ml (n = 8)</td>
<td>APC (n = 2)</td>
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NSR = normal sinus rhythm; APC = atrial premature depolarizations; AT = atrial tachycardia; AF = atrial flutter; LA = left atrial.

*p < .05 vs control value.
bers at this time. Application of electrical stimuli at a wide range of cycle lengths for brief periods of time (5 to 20 sec) did not elicit nondriven repetitive activity (reentrant or triggered activity) in any atrial preparation.

Excitable and inexcitable cells. The atria were stimulated at a basic cycle length of 800 msec. In the 20 preparations from normal hearts (10 right and 10 left) all the fibers from which transmembrane potentials were recorded in both atria were excitable and generated action potentials in response to the extracellular stimulus when it was in the range of 0.5 to 2 msec duration and 2 to 4 μA amplitude (table 2). In contrast, preparations from hearts with cardiomyopathy contained both excitable and inexcitable cells. Inexcitable cells did not generate action potentials in response to the extracellular stimulus even though resting potentials were recorded. We increased the stimulus duration to 10 msec and amplitude to 10 μA, but these strong stimuli did not elicit action potentials. The increased stimulus intensity and/or duration very often caused a "local" membrane response in the inexcitable cells, a very small and presumably nonregenerative depolarization of up to 20 mV. Inexcitable cells were found in 18 of the 34 right atrial and 32 of the 34 left atrial preparations. In 10 left atrial preparations (two from group I, five from group II, and three from group III) all fibers impaled with the microelectrode were inexcitable.

The percentage of inexcitable cells in the enlarged left atria (percentage of all cells impaled) was significantly greater than that of cells in the right atria (table 2). The percentage of inexcitable left atrial cells (but not right atrial cells) also increased with atrial size, so that a majority of fibers in the moderately and severely enlarged atria of groups II and III were inexcitable.

The results in table 2 and those presented below on transmembrane potential characteristics are at best semiquantitative, since the number of attempts at impalement, successful impalements, and impalements per unit area varied form one preparation to another and from one group to another. In some regions, perhaps where connective tissue was most abundant, we were not able to penetrate cardiac fibers that may have been viable. Membrane potential characteristics of such fibers, if recorded, might have altered the quantitative assessment of our data.

Transmembrane potential characteristics. The excitable right atrial fibers in all three groups had normal resting and action potentials, an example of which is shown in figure 1, A. The resting potentials and action potentials of excitable left atrial fibers in group I were also normal. The resting potentials and the action potentials of excitable left atrial fibers in groups II and III were abnormal and depressed. Representative examples of these abnormal membrane potentials are shown in figure 1, B to E. Figure 1, C to E, shows transmembrane potentials that we would classify as depressed fast responses; resting potential is decreased into the range

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**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>Right atria</th>
<th>Left atria</th>
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<tbody>
<tr>
<td></td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Control</td>
<td>0%</td>
<td>265</td>
</tr>
<tr>
<td>Group I</td>
<td>19%</td>
<td>329</td>
</tr>
<tr>
<td>Group II</td>
<td>15%</td>
<td>359</td>
</tr>
<tr>
<td>Group III</td>
<td>24%</td>
<td>210</td>
</tr>
</tbody>
</table>

n = total number of cells impaled.

* p < .05; p value was obtained by comparing the values from cardiomyopathic animals with control values by means of Student's t test.

* kep < .05; p value was obtained by comparing the left atrial values from cardiomyopathic animals with the right atrial values by means of Student's t test.

FIGURE 1. Representative transmembrane potentials recorded from atrial fibers in preparations from cats with cardiomyopathy and left atrial enlargement. In each panel zero potential is represented by the horizontal black line to the left. The lower trace in panels A, C, D, and E is the differentiated upstroke velocity preceded by a 100 V/sec calibration. A, Normal atrial transmembrane potential. B, Slow-response action potential. C thru E, Depressed fast-response action potentials.
of −65 to −70 mV, and the maximum rate of phase 0 depolarization (V_max) is decreased to values between 20 and 80 V/sec. Most of the abnormal action potentials fell into this group. Some excitable cells (n = 53) had transmembrane potentials like the one shown in figure 1. B. Resting potentials of these cells were less than −60 mV and action potentials had a V_max of less than 10 V/sec. Resting potentials and upstroke velocities were in the range characteristic of slow responses. However, only 5% of the cells from which membrane potentials were recorded generated these kinds of action potentials.

Figures 2 and 3 show the quantitative analyses of the data obtained from all the impalements of right and left atrial fibers in the control and cardiomyopathic groups. The mean resting potentials of atrial fibers in the right (−74 ± 5 mV) and left atria (−73 ± 4 mV) from normal cats were similar to values previously reported in the literature and were not significantly different from each other (figure 2, A). The mean resting potentials of all right atrial fibers in the three groups of cardiomyopathic cats and of left atrial fibers in group I animals were not significantly different from control (p > .05) (figure 2, A). Left atrial fibers in groups II and III had resting potentials that were significantly reduced (p < .001) (figure 2, A). These data for resting potentials of fibers from cardiomyopathic cats shown in figure 2, A, include both excitable and inexcitable cells. We also compared the resting membrane potentials of the inexcitable cells with those of the excitable cells for both the right and left atria in all groups. Inexcitable cells had a significantly lower resting potential as shown in figure 3.

The values for action potential amplitude, V_max, and the time to 50% and 100% repolarization (APD_{50} and APD_{100}) for right atrial fibers in all the cardiomyopathic groups and left atrial fibers in group I were not significantly different from those of normal right and left atrial fibers (p > .05) (figure 2, B and C; data for APD_{50} and APD_{100} not shown). However, action potential amplitude and V_max of excitable left atrial fibers in groups II and III were significantly reduced (figure 2, B and C) and the time course of repolarization was significantly prolonged in group III (control APD_{50} 65
Effects of autonomic neurohumors on transmembrane potentials. Continuous recordings of transmembrane potentials were maintained in these experiments. The effects of acetylcholine (5.7 x 10^{-5}M to 1.4 x 10^{-4}M) on membrane potentials recorded from cardiomyopathic preparations depended on the initial membrane potential characteristics. Some atrial fibers with normal resting and action potentials were found in right and left atrial preparations in all three groups of cardiomyopathic animals. These cells responded to acetylcholine in a manner identical to that of the cells in the control preparations. Acetylcholine increased resting potential from -76 ± 4 to -81 ± 3 mV (n = 24), action potential amplitude from 94 ± 8 to 104 ± 6 mV, and V_{max} from 139 ± 44 to 200 ± 46 V/sec. Total action potential duration was reduced from 178 ± 34 to 137 ± 33 msec. Likewise, resting potential, action potential amplitude, and V_{max} of cardiomyopathic fibers that had significantly depressed resting and action potentials (resting membrane potential -64 ± 5 mV, action potential amplitude 47 ± 12 mV, V_{max} 19 ± 25 V/sec; n = 14) were increased by acetylcholine (to -72 ± 8 mV, 78 ± 13 mV, and 83 ± 58 V/sec, respectively) (figure 4, A). These values were lower than those of normal fibers after exposure to acetylcholine. The action potential duration (APD_{100}) of these cells also decreased from 182 ± 49 to 159 ± 42 msec. All changes were statistically significant (p < .05).

We also determined the effects of acetylcholine on inexcitable fibers with low resting potentials in both the right and left atria and observed three different types of response: (1) Acetylcholine increased resting membrane potential (from -63 ± 12 to -78 ± 7 mV; p < .05) and restored excitability in 34 of the 95 fibers studied (figure 4, B). The action potentials generated by these cells in the presence of acetylcholine were similar to normal (action potential amplitude 89 ± 12 mV, V_{max} 119 ± 49 V/sec, APD_{100} 186 ± 44 msec, APD_{50} 63 ± 23 msec). (2) Acetylcholine increased the resting potential in 20 fibers (from -55 ± 6.9 to -66 ± 11 mV; p < .05) but did not restore excitability. (3) Acetylcholine had no effect on the resting potential (-58 ± 8 mV) in 11 other fibers (figure 4, C).

The effects of norepinephrine were also dependent on the characteristics of the transmembrane potential. In the 31 cells that had initial values within the range of fibers in normal preparations, norepinephrine (2.5 x 10^{-5}M to 3.3 x 10^{-5}M) slightly increased the resting membrane potential from -77 ± 5 to -79 ± 6 mV, action potential amplitude from 95 ± 10 to 102 ± 9 mV, and V_{max} from 164 ± 65 to 183 ± 75 V/sec. When these values were low, norepinephrine also increased resting potential from -68 ± 6 to -70 ± 7 mV, action potential amplitude from 68 ± 19 to 86 ± 11 mV, and V_{max} from 60 ± 57 to 107 ± 59 V/sec. It did not significantly alter action potential duration.

Norepinephrine affected inexcitable fibers in preparations from cardiomyopathic cats in several different ways (figure 5). One type of response we observed in 14 experiments was an increase in resting potential (from -61 ± 6 to -68 ± 5 mV) and restoration of excitability. These fibers generated action potentials with fast upstroke velocities after exposure to norepinephrine, although V_{max} (69 ± 29 V/sec) and the ac-

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**FIGURE 4.** Effects of acetylcholine (Ach) on fibers in atrial preparations from cats with cardiomyopathy. In each panel the top trace is the reference zero potential and the second trace is the transmembrane voltage. In panels A and B the bottom trace is the differentiated upstroke velocity preceded by 100 V/sec calibration. A. Left trace shows a depressed fast-response action potential before superfusion with Ach. At the right is the membrane potential recorded from the same cell after 1 min of superfusion with Ach (1.4 x 10^{-5}M). Resting membrane potential is increased, action potential amplitude and V_{max} are increased, and repolarization is accelerated. B. Left trace shows the resting membrane potential of a fiber during superfusion with normal Tyrode’s solution. An extracellular stimulus was applied but the cell did not generate an action potential. At the right is the recording from the same fiber 30 sec after superfusion with Ach (1.4 x 10^{-5}M) was begun. Resting membrane potential has increased and the cell now generates an action potential. C. Left trace shows the resting potential of another inexcitable cell in normal Tyrode’s solution. Superfusion with Ach (5 x 10^{-6}M) for 3 min did not alter the resting membrane potential or restore excitability.
A potential in cardiomyopathic cats. The lower panel traces were recorded from the atrial preparations during phase 2 of superfusion with the catecholamine. Nine fibers had low maximum diastolic potentials of $-50$ to $-65$ mV and had previously been inexcitable before exposure to norepinephrine (figure 6, B). In three preparations the automatic activity in these fibers with low membrane potentials was slowed and/or abolished by verapamil (0.5 to 1.0 mg/ml). Maximum diastolic potential of the other automatic fibers was in the range of $-65$ to $-86$ mV. Delayed afterdepolarizations and triggered activity occurred in an additional nine preparations (three in the right and six in the left atria) in which norepinephrine did not cause automaticity. To produce triggered activity the preparations were stimulated at rates of 60 to 120 impulses/min for 10 to 15 impulses and the stimulator was then turned off. Action potentials followed by delayed afterdepolarizations were recorded in 15 fibers (eight in the right and seven in the left atria) during the procedure (figure 7, A). These fibers had low resting potentials and slow upstrokes and were inexcitable before exposure to catecholamines. In each fiber the afterdepolarization amplitude increased at the more rapid stimulation rates until triggered activity occurred at rates of 100 to 120 impulses/min (figure 7, A). Triggered activity lasted usually for less than 2 min and then stopped spontaneously. In three experiments the delayed afterdepolarizations and triggered activity were abolished by the slow channel-blocking drugs verapamil (1 mg/ml) an AHR-2666 (50 μg/ml) (figure 7, B).

Automatic and triggered activity also occurred in atrial preparations from normal cats after exposure to norepinephrine, although in those preparations, fibers that exhibited phase 4 depolarization or delayed afterdepolarizations had normally high maximum diastolic potentials. Seven of nine atrial preparations (three of four right atrial, four of five left atrial) beat spontaneously when superfused with norepinephrine and not stimulated electrically. Pacemaker cells from which transmembrane potentials were recorded had maximum diastolic potentials of $-78$ ± 3 mV and rapid upstrokes of 130 ± 41 V/sec. Triggered activity was induced by stimulation in three (left atrial) out of four preparations (one right atrial, three left atrial) during superfusion with catecholamines. The delayed after-

**FIGURE 5.** Effects of norepinephrine on inexcitable cells in atria from cardiomyopathic cats. Traces are as in figure 4. Extracellular stimulation did not elicit an action potential during the control period. In panel A, a local response occurred. Norepinephrine increased the resting potential in each example and enabled the extracellular stimulus to elicit an action potential. The characteristics of the action potentials in each of the panels are different (see text).
depolarizations recorded from fibers in these preparations occurred at membrane potentials more negative than \(-75 \text{ mV}\) and increased in amplitude as the rate of stimulation increased; triggered activity occurred at critical stimulation cycle lengths.

**Ultrastructure of the atria in cardiomyopathy.** The muscle bundles in both the right and left atria were widely separated by unusually large amounts of interstitial connective tissue. Interstitial fibrosis and fiber disarray appeared to be more pronounced in the left atria than in the right. The nerves and blood vessels did not appear unusual.

We found both normal- and abnormal-appearing cells in both the right and left atria of all three groups. Normal cells were identical to cells in atria of cats without cardiomyopathy. The ultrastructure of these cells has previously been described in detail.\(^8\) The cells we identified as abnormal were either hypertrophied or degenerating. Hypertrophied cells had the following characteristics\(^7\) (figure 8): (1) transverse diameters ranging from 8 to 15 \(\mu\text{m}\), significantly larger than the range of diameters we found in normal atria (4 to 10 \(\mu\text{m}\)), (2) large and lobulated nuclei, (3) enlarged Golgi apparatus with increased rough endoplasmic reticulum profiles, (4) increased numbers of myofilaments and mitochondria of variable size (myofilaments often were not arranged in sarcomere units but showed marked changes in orientation), (5) tubular invaginations of the sarcolemma, some extending deep into the interior of cells, often accompanied by subsarcolemmal cisternae, (6) increased amounts of glycogen particles and ribosomes, and (7) focal thickening of Z band material. Unlike normal cylindrical atrial cells, hypertrophied cells were often irregularly shaped with multiple cellular processes joining several adjacent cells. Hypertrophied cells were found in the right atria of cats with cardiomyopathy in all three groups and in the left atria of cats in groups I and II. They were rarely seen in the severely enlarged left atria of cats in group III (figure 8).

Myocardial cells we classified as degenerating had the following characteristics (figure 9): (1) focal or uniform loss of myofilaments, with a preferential loss of thick filaments, (2) focal proliferation of agranular sarcoplasmic reticulum, (3) aggregation and alteration in the shape of the mitochondria, (4) increased amounts of glycogen granules, and (5) accumulation of Z band material. Although the ultrastructure of these cells is similar in some respects to the “myofibril-poor” cells described by Sherf and James,\(^8\) we interpret the changes we found to have resulted from the myocardial disease, since we never found this ultrastructure in atria from normal cats. In general, these degenerative changes were frequently present in left atrial cells in the group III cats (six of seven preparations studied). Only a small number of cells in the left

![Figure 6](http://circ.ahajournals.org/content/31/6/1042/F6)

**FIGURE 6.** A, Effects of verapamil on a slow-response action potential induced by norepinephrine in a left atrial preparation from a cat with cardiomyopathy. \(a\), Potential during superfusion with Tyrode’s solution containing norepinephrine. The resting membrane potential is \(-50 \text{ mV}\), the action potential amplitude is 58 mV, and \(V_{\text{max}}\) is 2 V/sec. \(b\) and \(c\). Membrane potential recorded from the same fiber 3 and 7 min after verapamil (1 mg/l) was added to the Tyrode’s solution. \(B\), Abnormal automaticity in an atrial fiber in a left atrial preparation from a cat with cardiomyopathy. In both panels, the upper line represents the reference zero potential and the lower trace is the transmembrane voltage. \(a\). During superfusion with normal Tyrode’s solution the fiber did not generate an action potential in response to the extracellular stimuli (note the stimulus artifacts). The stimulus was then turned off and superfusion with Tyrode’s solution containing epinephrine was begun. Within minutes \((b)\) this fiber spontaneously depolarized, giving rise to abnormal automatic activity.
FIGURE 7. Delayed afterdepolarization and triggered activity in fiber in a left atrial preparation from a cat with cardiomyopathy. The zero reference is indicated by the horizontal line at the right of each trace. A. Recordings made during superfusion with Tyrode’s solution containing norepinephrine (2.25 × 10⁻⁵M). The upper trace shows the response of this fiber when the preparation was stimulated at a cycle length of 1500 msec. The action potential has a slow upstroke and is followed by a delayed afterdepolarization. In the second trace, when the preparation was stimulated at a cycle length of 1000 msec, triggered activity started at the arrow. This nondriven activity continued for several minutes; the last five impulses during the nondriven activity are shown at the right. B. Recordings made while the same fiber was stimulated at 1500 msec cycle length 3 min after AHR-2666 (50 μg/ml) was added to the Tyrode’s solution containing norepinephrine. The stimulated action potentials have reduced upstroke velocities and action potential amplitudes. The delayed afterdepolarizations are almost totally abolished. In the lower trace, the stimulation cycle length was changed to 1000 msec. Triggered activity did not occur.

atria of the cats in group I (three of five preparations) and group II (three out of six) and cells in the unenlarged diseased right atria had these abnormalities. Degenerating cells were not usually grouped together but rather were connected to normal or hypertrophied cells by desmosomes and nexi. Many of the normal-appearing, hypertrophied, and degenerating cells had focally thickened basement membranes. Whereas the myocardial fibers in the normal atria had 500 to 600 Å thick basement membranes, including the sarcolemma, a sarcolemmal space, and the mucopolysaccharide coat, the basement membrane around many of the fibers in myopathic atria was up to 10,000 Å thick (figure 9, C). We determined the frequency of occurrence of cells with thickened basement membranes (>1000 Å) by measuring their thickness in consecutive fibers (ranging from 67 to 118 depending on specimen) in each of the three groups. The right atria from group I cats had the smallest mean percentage of cells with thickened basement membranes (38%), and the left atria from group III cats had the largest (85%). The mean percentage of cells with thickened basement membranes in right atria from groups II (55%) and III (56%) and the left atria from groups I (56%) and II (52%) were similar.

Discussion

Atrial pathology and electrophysiologic properties. Atrial tachyarrhythmias are often associated with atrial enlargement.¹⁹, ²⁰ In our previous studies on atrial enlargement and arrhythmias secondary to mitral valvular disease or tricuspid insufficiency, we did not find highly unusual atrial cellular ultrastructure or transmembrane potentials and concluded that abnormalities in the membrane potentials are probably not the causes of the arrhythmias.³ ⁶ ²³ In the experiments reported here, the abnormal transmembrane potentials and ultrastructure in hearts with cardiomyopathy may have contributed to the occurrence of the arrhythmias. Possible mechanisms are discussed below. Cellular electrophysiologic mechanisms for atrial arrhythmias associated with cardiomyopathy may be very different from those associated with mitral or tricuspid insufficiency, although electrocardiographically the arrhythmias are similar (i.e., atrial flutter and fibrillation). The difference in mechanisms may be related to the difference in pathology.²¹

Although the results of the studies discussed above suggest that abnormal transmembrane potentials are not caused by atrial enlargement in the absence of myocardial disease, in the presence of cardiomyopathy enlargement may contribute to the occurrence of abnormal membrane potentials. This is suggested by our observations that the severity of the electrophysiologic abnormalities was greater in the enlarged left atria in the cats with cardiomyopathy in groups II and III than in the more normal-sized right atria in the same groups. Some of the electrophysiologic abnormalities are also secondary to the disease process itself, as indicated by the significant number of inexcitable cells in the unenlarged diseased right atrial preparations.

Alterations in membrane properties that might cause abnormal transmembrane potentials. A major abnormality we found in the diseased fibers was the reduction in the maximum diastolic or resting potential. Although the extracellular ionic environment provided by the Tyrode’s solution was normal, the extracellular envi-
vironment immediately adjacent to the sarcolemma might have been abnormal and influenced the resting membrane potential. This is suggested by the markedly thickened basement membranes we observed (see figure 9). Langer has suggested that negatively charged acid mucopolysaccharides, which constitute the material coating the external membrane surface, play a crucial role in the control of transmembrane ionic fluxes. Cations such as Ca and K ions bind to these mucopolysaccharides. The composition of the basement membrane might also be abnormal in cardiomyopathy and might therefore contribute to the reduction in the resting potential.

Another possible cause of the low resting potential is
a decrease in intracellular [K], which is suggested by the results of some of our experiments with acetylcholine. Acetylcholine increases the resting potential of atrial fibers by increasing the permeability of the cell membrane to K ions, causing the membrane potential to approach the K' equilibrium potential. The increase of depressed resting membrane potentials of some fibers in the atria from cats with cardiomyopathy

**FIGURE 9.** Abnormal ultrastructure of atrial fibers from cats with cardiomyopathy. A, Degenerating cell (DC) between two more normal-appearing atrial cells (AC). In the degenerating cell, myofibrils (mf) are scant, mitochondria (M) are pleomorphic and scattered throughout the myoplasm, and glycogen and smooth endoplasmic reticulum (ser) are present. A thickened basement membrane (curved arrow) surrounds cells in this illustration, (×8750). B, Long segment of junction (arrows) that occurs between a degenerating cell (DC) and a normal-appearing cell (AC), (×11,200). C, Thickening of the basement membrane (curved arrows) around degenerating left atrial cells. There is a large accumulation of Z-bund material (Z) and reduced number of myofibrils (mf). The cell-to-cell junction is intact (straight arrow). Large amounts of interstitial fibrosis (F) surround these cells. (×15,265.)
to −80 to −90 mV in the presence of acetylcholine suggests that intracellular [K] of these cells was normal or nearly normal. On the other hand, in a number of experiments the same concentration of acetylcholine caused only a small increase in the depressed resting membrane potential, which remained significantly positive to the K+ equilibrium potential. If it is assumed that membrane conductance to K+ is maximally increased by the acetylcholine, then the results of these experiments may indicate that intracellular [K'] is low. However, the effective [K'], may also be increased in the thick basement membranes (see above) or the membrane conductance of these fibers may not respond in the usual way to acetylcholine. The results of these experiments are thus not easily interpreted.

We have no data on the resting membrane Na+ or K+ permeability of atrial fibers from hearts with cardiomyopathy. In studies on human atrial fibers and on atrial fibers in the coronary sinus, it has been suggested that net inward depolarizing current resulting from a relatively high PNa/PK ratio results in the low resting potential.

Just as there may be several different causes for the low resting potential, the decrease in Vmax and action potential amplitude may also have several different causes. In some fibers these changes were largely secondary to the reduced membrane potentials of the diseased cells (due to partial inactivation of the Na channel conductance), since membrane hyperpolarization by either acetylcholine or norepinephrine increased Vmax and action potential amplitude. Some additional, more specific effects of disease on the conductance mechanisms underlying the action potential upstroke, however, cannot be ruled out, since in some experiments hyperpolarization did not increase the action potential upstroke as much as expected.

Relationship of abnormal membrane potentials to atrial arrhythmias. The decreased resting potential, Vmax, and action potential amplitude (both slow and depressed fast responses, shown in figure 1) are expected to cause slow conduction and block, necessary prerequisites for reentry. Regions in which cells are inexcitable (table 2) may form functional obstacles around which reentry might occur. Either vagal or sympathetic activation may also contribute to arrhythmogenesis. The vagus may speed conduction in some areas by increasing resting potential (figure 4, A and B) while block persists in others (figure 4, C), thereby increasing heterogeneity. Sympathetic stimulation may cause slow conduction by producing slow-response action potentials, which results from the tendency of norepinephrine to increase slow inward current (figure 5, C). Norepinephrine also caused automatic activity in the cells with low membrane potentials (figure 6) and triggered activity dependent on delayed afterdepolarizations (figure 7). Although nondriven activity could also be induced in some normal atria in the presence of catecholamines, abnormal automaticity (at a low membrane potential) did not occur and is therefore a result of the cardiac disease. The alterations in structure of the atria may also contribute to the occurrence of arrhythmias. The separation of myocardial fibers by connective tissue may slow conduction by decreasing disc connections or by physically uncoupling cells, thereby increasing intercellular resistivity. This effect might also cause reentry.

References
LABORATORY INVESTIGATION–MYOCARDIAL DISEASE


Erratum


The left hand columns of tables 9 and 10 should have appeared as shown below:

**TABLE 9**

<table>
<thead>
<tr>
<th>Difference</th>
<th>HDL(_c)/LDL(_c)</th>
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</thead>
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<td>&gt; .0594</td>
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<tr>
<td></td>
<td>.0154–.0594</td>
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<tr>
<td></td>
<td>&lt; .0154</td>
</tr>
<tr>
<td>Linear trend p value</td>
<td></td>
</tr>
<tr>
<td>HDL(_c)/TC</td>
<td>&gt; .0285</td>
</tr>
<tr>
<td></td>
<td>.0057–.0285</td>
</tr>
<tr>
<td></td>
<td>&lt; .0057</td>
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<tr>
<td>Linear trend p value</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 10**

<table>
<thead>
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<th>Difference</th>
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</thead>
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<tr>
<td>Placebo (n = 57)</td>
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<tr>
<td>.0057–.0285</td>
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<tr>
<td>&lt; .0057</td>
<td></td>
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<tr>
<td>Linear trend p value</td>
<td></td>
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<tr>
<td>Cholestyramine (n = 59)</td>
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<tr>
<td>&gt; .0285</td>
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<tr>
<td>.0057–.0285</td>
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<tr>
<td>&lt; .0057</td>
<td></td>
</tr>
<tr>
<td>Linear trend p value</td>
<td></td>
</tr>
</tbody>
</table>
Mechanisms for atrial arrhythmias associated with cardiomyopathy: a study of feline hearts with primary myocardial disease.
P A Boyden, L P Tilley, A Albala, S K Liu, J J Fenoglio, Jr and A L Wit

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