Electrophysiologic Properties and Response to Pharmacologic Agents of Fibers from Diseased Human Atria

ALLAN J. HORDOF, M.D., RICHARD EDIE, M.D., JAMES R. MALM, M.D., BRIAN F. HOFFMAN, M.D., AND MICHAEL R. ROSEN, M.D.

SUMMARY We used standard microelectrode techniques to record action potentials of human right atrial fibers obtained during cardiac surgery, and correlated these potentials with clinical and preoperative ECG data. Human atrial fibers were classified as follows: Group A (ten patients) had a maximum diastolic potential (MDP) of \(-71.4 \pm 5.1\) mV (mean \(\pm\) SD), and action potentials that were primarily fast responses. These atria were normal or slightly dilated. In group B (12 patients) MDP was \(-50.3 \pm 5.7\) mV; action potentials were slow responses and the atria were moderately to markedly dilated. Atrial arrhythmias occurred in four group B and no group A patients. The ECG revealed a significant difference \((P < 0.005)\) in P wave duration: group A, 89 \(\pm\) 3.0 msec; group B, 111 \(\pm\) 6.0 msec. Verapamil, 0.1 mg/L, markedly depressed group B action potentials. Verapamil, 0.1-1.0 mg/L, depressed only the action potential plateau of group A. Procainamide 1-100 mg/L had equivalent effects on fibers of both groups A and B, effects which were small at dosages of less than 40 mg/L. Procainamide did not depress slow response automaticity, but verapamil \((0.1-1 \text{ mg/L})\) did.

STUDIES OF THE ELECTROPHYSIOLOGIC PROPERTIES of human cardiac fibers have described the action potential characteristics of ventricular and atrial tissues taken from both relatively healthy and diseased hearts.1-7 Markedly disparate action potential characteristics have been reported and it has been shown that fibers from severely diseased hearts—regardless of the type of cardiac disease—have lower levels of resting membrane potential and develop action potentials with reduced amplitude and phase 0 upstroke velocity in comparison to fibers from more normal hearts.

It has been our goal, in the past few years, to evaluate the relationships between the presence and extent of cardiac disease and clinical electrocardiographic abnormalities in patients to abnormalities in cardiac cellular electrophysiologic properties. As a first step in this direction, several years ago we reported the action potential characteristics of right atrial myocardial and specialized fibers in tissues from the hearts of cyanotic patients with normal atria. For all of these patients there was no history of cardiac arrhythmias, no electrocardiographic evidence of P wave abnormalities, no evidence of atrial dilatation at catheterization or cardiac surgery, no shunts at the atrial level, and all had normal right atrial pressures. In these fibers maximum diastolic potentials (MDP) were \(-86 \pm 5\) (mean \(\pm\) SD) mV, action potential amplitudes, 102 \(\pm\) 12 mV, and maximum upstroke velocities of phase 0 depolarization \((V_{\text{max}})\), 226 \(\pm\) 14 V/sec. There were no significant differences in these variables between specialized and ordinary myocardial fibers.

Recently, there has been heightened awareness of the role that partially or markedly depolarized fibers may play in the genesis of cardiac arrhythmias.8 With this in mind, we selected patients who had evidence of atrial disease (see below for criteria) in an attempt to 1) observe the effects of atrial dilatation on the cellular electrophysiologic properties of atrial fibers; 2) correlate the clinical history of arrhythmias and electrocardiographic characteristics of individual patients with electrophysiologic properties of their atrial fibers; and 3) study cellular electrophysiologic responses to two antiarrhythmic drugs: procainamide, which is only moderately effective in treating atrial arrhythmias,8 and verapamil, which is reported to be highly effective in treating such arrhythmias.9

Methods

The atrial tissues studied were obtained from the hearts of 22 patients undergoing corrective cardiac surgery requiring cardiopulmonary bypass. All had been followed by our division of Pediatric Cardiology for at least one year before surgery. They had standard ECGs and physical examinations as indicated clinically. None had ECG evidence of pre-excitation. No His bundle studies were performed on these patients nor were their hearts mapped at the time of surgery. As in our previous study1 all patients had congenital heart disease. None had a history of rheumatic fever, rheumatic heart disease, bacterial, viral or idiopathic myocarditis, pericarditis, or endocarditis. In addition, the patients selected were in the same age range as those in our previous study \((<16\) years). None of the patients had received cardioactive drugs for two days prior to surgery. None of the patients was taking digitoxin or diphenylhydantoin.

For all patients, the following information was available: history (including that of atrial arrhythmias), standard 12-lead electrocardiograms, and right and left heart catheterization data. The P wave characteristics reported here are from lead II. At the time of surgery, atrial size was estimated by the surgeon as normal, or slightly, moderately, or markedly dilated.

Prior to surgery, informed consent was obtained. At surgery approximately 1 cm² of atrial myocardium that was removed from the anterior free wall of the right atrium as part of the routine cannulation procedure for cardiopulmonary bypass was made available for study. The tissue was immersed in iced Tyrode's solution immediately after excision from the atrium and rapidly transported to the

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DISEASED HUMAN ATRIAL FIBERS/Hordof et al.

TABLE 1. Transmembrane Potential Characteristics of Human Atrial Fibers

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>N</th>
<th>MDP (mV)</th>
<th>Amp (mV)</th>
<th>Vmax (V/sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelband et al., A</td>
<td>1-16</td>
<td>22</td>
<td>86.0 ± 5.0</td>
<td>102.0 ± 12.0</td>
<td>226 ± 14</td>
</tr>
<tr>
<td></td>
<td>1-13</td>
<td>10</td>
<td>71.4 ± 5.1*</td>
<td>91.7 ± 8.3**</td>
<td>208 ± 38</td>
</tr>
<tr>
<td></td>
<td>(5.8 ± 3.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.75-13</td>
<td>12</td>
<td>50.3 ± 5.7†</td>
<td>63.3 ± 11.3†</td>
<td>5-20</td>
</tr>
</tbody>
</table>

All values are means ± standard deviations.
*P < 0.001 compared to Gelband study.1
**P < 0.005 compared to Gelband study.1
†P < 0.001 compared to group A.

Abbreviations: N = number of patients; AP = action potentials; MDP = maximum diastolic potential; Amp = amplitude;
Vmax = maximum upstroke velocity of phase 0 depolarization.

Electrophysiologic Characteristics of Atrial Fibers

Prior studies of Purkinje fiber transmembrane potentials have revealed that at membrane potentials greater than approximately -60 mV the major ionic current causing the action potential upstroke is a rapid inward sodium ion current.13 Action potentials dependent on the rapid inward Na+ current have been referred to as fast responses. At membrane potential more positive than -60 mV, a slow inward current, carried to a great extent by Ca2+, is the major current causing the action potential upstroke.13 Such action potentials are referred to as slow responses. Based upon this information we divided the atrial fibers into two groups, dependent on whether the mean resting potential for the 10-20 impalements for each preparation was less than or greater than -60 mV. The transmembrane potential characteristics and clinical and electrocardiographic data for each group then were compared to the data for the other, and both groups were compared to the normal values we previously had reported (table 1).1

All patients corresponded in age to those in the previous study.1 In our group A, (MDP: more negative than -60 mV) one patient had no history of atrial arrhythmias, normal P

TABLE 2. Preoperative Electrocardiographic Data

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>HR (beats/min)</th>
<th>P-R Int (msec)</th>
<th>P wave Amp (mV)</th>
<th>P wave duration (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>10</td>
<td>102 ± 6</td>
<td>156 ± 5</td>
<td>2.8 ± 0.3</td>
<td>89 ± 3</td>
</tr>
<tr>
<td>B</td>
<td>12</td>
<td>111 ± 5</td>
<td>164 ± 11</td>
<td>2.4 ± 0.3</td>
<td>111 ± 8*</td>
</tr>
</tbody>
</table>

All values are means ± standard deviations.
*P < 0.005 compared to group A.

Abbreviations: N = number of patients; HR = heart rate; Int = interval.

laboratory. Here it was transferred to a Lucite chamber and superfused with Tyrode's solution warmed to 37°C and equilibrated with 95% O2-5% CO2. The superfuse flow rate was 12 ml/min. For some studies, drive stimuli were delivered to the preparation through Teflon-coated silver wire bipolar electrodes at cycle lengths of 800-1000 msec. For other studies the fibers were allowed to beat spontaneously. The methods for stimulating the preparations have been described previously.11,13

The tissues were impaled with machine-pulled glass capillary microelectrodes that had tip diameters of less than 1µ and were filled with 3 M KCl. The electrodes were coupled by a 3 M KCl interface to an Ag-AgCl bar which led to an amplifier having a high input impedance and input capacity neutralization. The output was displayed on a cathode ray oscilloscope (Tektronics Model 565) and photographed using Polaroid film. The tissue chamber was connected to ground through a salt bridge and an Ag-AgCl junction. The methods for calibrating the recording system and for determining the maximum rate of rise of phase 0 depolarization (Vmax) have been described previously.11,13

After the tissue had stabilized in Tyrode's solution for 20 min, measurements were made of action potential amplitude, MDP, and Vmax. For fibers having markedly depressed action potentials with slow upstroke velocities (<50 V/sec), the rate of rise of the action potential was measured by hand. We did not attempt to measure conduction velocity in the isolated tissues because, in preparations with depressed cardiac fibers, it was not possible to identify the geometry or length of the pathway traversed by an impulse traveling between two points.

Control measurements for each tissue sample were obtained from 10-20 impalements in the first subendocardial cell layer. All impalements were made at least 3 mm away from the cut edges of the preparation. A single impalement then was maintained for the duration of the study. The tissue then was superfused with the drug to be studied and on-line measurements of resulting changes in the aforementioned electrophysiologic properties were made.

For these studies either procainamide (Promestyl, ER, Squibb & Sons, New Brunswick, N.J.) or verapamil (Knoll Pharmaceutical Co., Whippany, N.J.) was dissolved in separate reservoirs of Tyrode's solution. For procainamide the concentrations were 1, 10, 40, and in some studies 100 mg/L. For verapamil the concentrations were 0.1, 1.0, and 10 mg/L. The tissues were superfused with Tyrode containing each drug concentration for 30 minutes. Because procainamide could be washed out of the tissues readily and verapamil could not, the fibers were superfused first with procainamide in 14 experiments, and then—after Tyrode superfusion had brought a return to control action potential characteristics—with verapamil. For the remainder of the studies, the fibers were superfused only with verapamil. There was no significant difference in the response to verapamil between the two groups of fibers. All results of pharmacologic studies reported from atrial fibers in which a single impalement was maintained throughout the duration of the experiment. Statistical analysis was performed using Student's t-test.

Results
waves, no shunt at the atrial level or elevated atrial pressures, and no history of cyanosis. For this patient, the MDP (−81 mV) and action potential amplitude (100 mV) were within the range described in our earlier study of normal atria. The other patients in this group had cyanosis, shunts at the atrial level, slight atrial dilatation, and in two cases, moderately dilated atria. It should be emphasized that the action potential characteristics for each atrium in this group were variable, and occasional slow responses were recorded from preparations in which the fast response predominated. For the most part, however, this group can be classified as having normal (one case) or depressed (nine cases) fast response action potentials.

In group B, ten of the 12 atria were moderately to markedly dilated and there was a history of arrhythmias in four of the ten patients. For almost all fibers impaled in these atria MDP was less negative than −60 mV, and the action potentials appeared to be slow responses. The phase 0 upstroke velocities for ten of these preparations were 5–20 V/sec; in the other two, the maximum upstroke velocities were 80 and 95 V/sec.

The major anatomical difference between the atria in groups A and B appeared to be the greater chamber dilatation in group B. Mean right atrial pressures for both groups were normal to slightly elevated (4.2 ± 1.5 [mean ± SD] mm Hg), and patients in both groups had cyanosis, shunts at the atrial level, and a similar incidence of congenital lesions (tetralogy of Fallot [A and B], endocardial cushion defects [B], transposition of the great vessels [A and B], Ebstein’s anomaly [B], ventricular septal defects [A], pulmonic stenosis [A and B], atrial septal defects [A and B], congenital mitral insufficiency [B]). On the basis of these observations we concluded that the decrease in resting membrane potential and the progressive alteration of electrophysiologic properties of the atrial fibers largely could be explained on the basis of chamber dilatation.

Table 2 is a summary of the preoperative electrocardiographic data for the group A and group B patients. All patients were in normal sinus rhythm when the ECG was recorded. For groups A and B there were no significant differences in heart rate (R–R interval), P wave amplitude, or the P–R interval. However, the duration of the P wave, reflecting intra-atrial conduction, was significantly longer (P < 0.005) for group B than for group A. This suggests that as membrane potentials decreased and slow response action potentials became dominant in the atria, conduction was slowed. The four group B patients with atrial arrhythmias all had markedly dilated atria and had MDP of less than −55 mV. Three of the patients had paroxysmal atrial tachycardia (one atrial septal defect, one Ebstein’s anomaly, one endocardial cushion defect) and one had paroxysmal atrial flutter and fibrillation (congenital mitral insufficiency).

Responses to Pharmacologic Agents

After recording control action potentials for each tissue sample, a single impalement was maintained in each preparation for the duration of the study. The tissues then were superfused with Tyrode containing either procainamide or verapamil.

Figure 1 shows the effects of procainamide, 1, 10, and 40 mg/L, on action potential characteristics of depressed fast responses and slow responses. There were small decreases in action potential amplitude and maximum diastolic potential which were of equal magnitude for both groups of fibers. In addition, there was a marked concentration-dependent decrease in Vmax for group A fibers. In figure 2 are shown the effects of verapamil, 0.1, 1, and 10 mg/L, on the action potential. For group A there were no significant changes in action potential amplitude, maximum diastolic potential, or Vmax with verapamil, 0.1–1 mg/L. Only at 10 mg/L were there significant decreases in these variables. However, verapamil, 0.1 mg/L, markedly depressed the slow response action potential.

Records from representative experiments showing the
effects of procainamide, 40 mg/L, on a fast response and a slow response action potential are shown in figures 3 and 4. Note that in both fast and slow response fibers exposed to procainamide there is a concentration-dependent decrease in action potential amplitude and upstroke velocity and prolongation of action potential duration.

The effects of verapamil on fast and slow response action potentials are shown in figures 5 and 6. Verapamil, 0.1-1.0 mg/L, induced no changes in maximum diastolic potential, action potential amplitude, and $V_{\text{max}}$ of the fast response fiber. The voltage-time course of repolarization was altered by a decrease in the duration of the plateau and a prolongation of the time to full repolarization. At a verapamil concentration of 10 mg/L, the fiber became quiescent and only by increasing stimulus strength and duration was a response elicited. When slow response fibers were superfused with Tyrode containing verapamil, 0.1 mg/L, (fig. 6), the fibers rapidly became inexcitable. The concentration of verapamil that depressed the slow response fibers was 1/100 of that required to achieve a similar effect on fast response fibers.

We also studied the effects of procainamide and verapamil on the automatic rhythms occurring in five preparations with slow responses. In none of these experiments did procainamide significantly depress the spontaneous rhythm. Moreover, at a procainamide concentration of 100 mg/L, the fibers often were further depolarized and the automatic rate increased (fig. 7). On the other hand, verapamil (0.1-1 mg/L) invariably suppressed automaticity and induced quiescence in the slow response fibers. After the individual fibers impaled became quiescent, mapping the preparation with the microelectrode revealed that the entire preparation had, in fact, become quiescent.

**Discussion**

One of our major purposes in this study was to determine the extent to which clinical evidence of atrial disease is associated with abnormalities in electrophysiologic function of atrial cells. The results reported in our earlier study established the guidelines for normal human atrial action potential characteristics (given a situation where surgery is required for cardiac disease). A number of factors must be considered as possibly contributing to the occurrence of the abnormal action potentials in the present study. Trauma during surgical excision, hypoxia during transport to the laboratory, and trauma during preparation of the tissue for study all might contribute artifactual distortion to our results. We rigidly adhered to the protocol described in the earlier study, discarded any preparations which appeared macerated or ecchymotic after excision, and made all impalements at sites distant from the cut ends of the tissue.

In one of the group A preparations the action potential characteristics were quite comparable to those reported in the earlier study. The patient, in this case, met the criteria for selection in the earlier study, that is, he did not have cyanotic heart disease (pulmonic stenosis), shunts at the atrial level, elevated atrial pressures, atrial dilatation, ECG abnormalities, or a history of arrhythmias. The other nine patients in group A all had at least one of the first three of these abnormalities listed. However, atrial arrhythmias were present in none and atrial dilatation was infrequent (moderate in two patients).

In group B, ten of 12 patients had at least moderate atrial dilatation and four of the ten had arrhythmias as well. It would appear reasonable to conclude, then, that while the occurrence of cyanotic heart disease and shunts at the atrial
level can result in variable depression of the fast response (group A), more marked atrial dilatation is required before a preponderance of slow response action potentials occur. Further strengthening the association between the slow response action potential and abnormal atrial function is the fact that in those atria with slow response action potentials intra-atrial conduction was significantly slower (prolonged P wave duration) than in those with depressed fast responses (table 2).

Although the difference in P wave durations between groups A and B was highly significant ($P < 0.005$), the slowing in conduction was not commensurate with what we might expect were slow responses occurring uniformly throughout the atrial mass. This observation can be explained in the following way: first, while the impalements reported were reasonably consistent within any one preparation, they were representative only of the subendocardial fibers themselves. The P wave reflects conduction through the entire atrial muscle mass. That conduction was not slowed (i.e., the P wave was not prolonged) to the extent one might expect if slow responses had, in fact, been activating the entire atrial mass would appear to indicate that the depression of the action potentials—and therefore of conduction—was nonuniform.

Another question to be considered with respect to the electrophysiologic characteristics reported here is whether the fibers we studied were specialized atrial fibers or myocardial fibers. For the group A fibers, as in the earlier study, the identification of specialized and myocardial fibers was relatively easy. Specialized fibers invariably were impaled in the first subendocardial layer of cells; their plateaus were of longer duration than in muscle fibers and, when the drive stimulus was discontinued, phase 4 depolarization occurred. For the fibers in group B it was impossible to state whether the fibers were specialized or not. Because we again impaled only the first subendocardial layer, we feel it is likely that many of the fibers were the specialized type. However, studies are available which indicate that markedly depolarized muscle can develop phase 4 depolarization and automaticity.[4] Hence, the major criterion for differentiating specialized from myocardial fibers was lacking for group B.

The identification of slow response action potentials in fibers from dilated human atria permits us to speculate on how such fibers might be involved in the initiation and propagation of atrial arrhythmias. A number of studies have documented the relationship between slow responses, slow conduction, unidirectional block, and re-entry.[6] The presence of slow response action potentials in the atria could therefore permit the initiation and propagation of re-entrant arrhythmias. Similarly, the spontaneous rhythms that can

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**Figure 5.** Effect of verapamil, 0.1–10 mg/L, on a group A fast response fiber. At verapamil, 0.1–1 mg/L the only change seen is in repolarization. At a verapamil concentration of 10 mg/L, MDP has decreased and a stimulus of increased magnitude and duration is required to excite the fiber.

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**Figure 6.** Effect of verapamil, 0.1 mg/L, on a group B slow response fiber. Note that at this drug concentration the fiber has become quiescent.
be generated by slow response action potentials could under appropriate conditions give rise to ectopic atrial rhythms.

Our second purpose in this study was to observe the response of atrial fibers to the antiarrhythmic drugs, procainamide and verapamil. Procainamide is effective in the therapy of approximately 50% of a broad spectrum of atrial arrhythmias.8 On the other hand, verapamil is reportedly effective in treating up to 90% of atrial arrhythmias.9 The effect of procainamide on the fast and slow response atrial fibers was to decrease action potential amplitude and $V_{\text{max}}$ and to prolong action potential duration. Such effects can be associated with suppression of arrhythmias: the first two by slowing conduction, the latter, by prolonging the effective refractory period. It is of interest that procainamide had a nearly equivalent effect on the fast and slow response fibers, and except for the depression of $V_{\text{max}}$ in the fast response fibers, no one of its actions was particularly prominent. Finally, procainamide not only had no effect on slow response automaticity, but at high concentrations, automaticity occasionally was enhanced.

The effects of procainamide offer a marked contrast to those of verapamil. The latter drug markedly and selectively depressed slow response action potentials; the effective concentrations (0.1–1 mg/L) modified only the repolarization phase of the fast response. However, these low concentrations of verapamil markedly depressed slow response automaticity. It would appear, then, that if the slow response is involved in the genesis of human atrial arrhythmias, verapamil offers a far more specific and selective means for its suppression than does procainamide. It is important to note that these observations do not take into consideration the role of the atrioventricular node in the genesis of certain atrial arrhythmias.10 However, evaluation of the extent to which the actions of both drugs on the atrioventricular node might explain their clinical efficacy was beyond the scope of this study.

**Acknowledgment**

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**References**

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