Electrophysiologic Characteristics of Human Ventricular and Purkinje Fibers

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SUMMARY We studied the electrophysiologic characteristics of ventricular muscle and Purkinje fibers from the hearts of five patients undergoing cardiac transplantation. All five patients had congestive failure and coronary artery disease before surgery and were receiving digitalis therapy. Ventricular muscle had a maximal diastolic potential (MDP) of \(-78 \pm 1\) mV (mean \(\pm\) SEM), an action potential (AP) amplitude of \(104 \pm 2\) mV, a phase 0 upstroke velocity (V_{max}) of \(297 \pm 19\) V/sec and an AP duration at 50% repolarization (APD_{50}) of \(190 \pm 4\) msec. Purkinje fibers had an MDP of \(-80 \pm 2\) mV, an AP amplitude of \(107 \pm 2\) mV, a V_{max} of \(388 \pm 25\) V/sec and an APD_{50} of \(195 \pm 9\) msec. Fibers from infarcted sections of the heart had significantly longer APD than those from noninfarcted, which resulted in marked dispersion of APD in infarcted and adjacent zones. Both epinephrine and ouabain induced delayed afterdepolarizations in Purkinje fiber. This suggests that delayed afterdepolarizations and resultant triggered activity can occur in the human ventricle.

DURING the last 2 years we have obtained ventricles from patients undergoing cardiac transplantation. These cardiac tissues were well preserved and not subjected to local trauma at cardiac surgery. In this paper, we report the cellular electrophysiologic properties of myocardial and Purkinje fibers from these hearts and the response of these fibers to certain cardioactive drugs.

Methods

Hearts were obtained from five patients at cardiac transplantation. Informed consent was obtained from each patient.

Patient 1 was a 49-year-old female who had acute rheumatic fever at 19 years of age and an acute myocardial infarction at 45 years of age, followed by coronary artery bypass surgery. Congestive heart failure developed and was treated with digoxin, but her health continued to deteriorate; during the 6 months before her cardiac transplantation she had two episodes of ventricular fibrillation. Before transplantation, severe biventricular failure with poor contractility of both ventricles was demonstrated. Her medications included digoxin, quinidine and, just before surgery, lidocaine.

Patient 2 was a 15-year-old female in whom tetralogy of Fallot was diagnosed at 3 months of age. At 4 months of age she underwent a shunt procedure to increase pulmonary blood flow. Approximately 2 years before cardiac transplantation she began having episodes of hemoptysis. Cardiac catheterization revealed an occluded shunt and she underwent open heart repair of tetralogy of Fallot, during which she had a large anterolateral myocardial infarction. Subsequently, cardiac catheterization revealed severe right and left ventricular dysfunction and a large anterolateral aneurysm. Because of persistent severe congestive failure, cardiac transplantation was performed. Her medications before transplantation were digoxin and furosemide.

Patient 3 was a 48-year-old male who had an acute myocardial infarction at 41 years of age. One year later, he had left ventricular failure that required digitalis and diuretics. Five months before transplantation, coronary angiography showed complete occlusion of the right coronary artery and a 60% proximal occlusion of the left anterior descending artery. Because of severe coronary artery disease deemed not remediable by bypass techniques, and severe cardiomyopathy, cardiac transplantation was performed.

Patient 4 was a 48-year-old male who had his first myocardial infarction at the age of 43 years. This was

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complicated by recurring ventricular arrhythmias and congestive heart failure. One year before transplantation, he had an anterolateral myocardial infarction. Recurrent ventricular arrhythmias required treatment with procainamide. Cardiac catheterization, done because of severe congestive failure, showed severe ventricular dyskinesia, a 50% proximal occlusion of the left anterior descending coronary artery and total occlusion of the right coronary artery. His medications included digitalis and furosemide. While waiting for a suitable donor, he developed low cardiac output and required intraaortic balloon counterpulsation.

Patient 5 was a 49-year-old male who had an anteroseptal myocardial infarction followed by atrial fibrillation and left ventricular failure at the age of 45 years. Two years before transplant, coronary angiography showed inoperable coronary artery disease. One year before transplantation he developed complete atrioventricular block and required a pacemaker. He subsequently had increasing ventricular failure. His medications included digoxin, furosemide and isosorbide dinitrate. Because of severe congestive failure, cardiac transplantation was performed.

Cellular Electrophysiologic Methods

Immediately after removal from the chest, the heart was brought to the laboratory in iced Tyrode's solution that contained NaCl, 137 mM; KCl, 4.0 mM; CaCl₂, 2.7 mM; MgCl₂, 0.5 mM; NaH₂PO₄, 1.8 mM; NaHCO₃, 12 M; and dextrose, 5.5 M/l. The heart was dissected within 10 minutes of its removal and tissue samples were obtained for microelectrode study. Grossly normal as well as scarred fibrotic tissues were prepared for study. These tissues were cut into sections that were as large as 4 x 4 cm and as small as 1 x 1 cm. The larger samples were used to "map" activation; the smaller samples were used to study drug superfusion. Transmembrane potentials from both types of tissue samples were comparable. In both types of experiments, the tissue was sliced 2–3 mm thick and the sections were placed in a tissue bath. The bath for the larger tissue samples held a volume of 20 ml that was changed 3 times/min by perfusion with Tyrode's solution. The bath for the smaller tissues held a volume of 3 ml that was also changed 3 times/min. The superfusate was maintained at a temperature of 37°C and was gassed with a mixture of 95% oxygen and 5% carbon dioxide. The tissue baths were connected to ground using a 3-M KCl-Ag-AgCl junction. The tissues were stimulated with bipolar extracellular electrodes made of silver wire and insulated to the tips with Teflon. The methods for stimulating the tissues have been described.¹

Microelectrodes fabricated from Pyrex glass were filled with 3 M KCl. Tip resistances were 10–20 MΩ. The microelectrodes were connected by a 3 M KCl-Ag-AgCl junction to amplifiers that had high-input impedance and capacity neutralization. Methods for calibrating the recording system, displaying the transmembrane potentials, calibrating and measuring the maximum upstroke velocity of phase 0 depolarization (Vₘₐₓ) and determining membrane responsiveness (by inducing premature stimuli at progressively shorter intervals during phases 4 and 3 of the transmembrane potential) have been described.²

We studied the endocardial surfaces of normal and abnormal appearing tissues in the tissue bath. In preparations in which a clear demarcation between normal and abnormal areas was visible, we studied the junction between normal and diseased tissues. Multiple microelectrode impalements one or two cell layers below the endocardial surface were made in each preparation to determine its electrophysiologic characteristics. Thereafter, single impalements were maintained in individual Purkinje or myocardial fibers. In experiments in which drugs were not superfused, transmembrane potentials were stable for 8–10 hours. All other experiments were completed in 5–8 hours.

The drugs studied included the fast-channel blocker tetrodotoxin³ (Sigma), the slow-channel blockers verapamil⁴ (Knoll) and AHR-2666 ⁷ (A.H. Robins), and 1-epinephrine (Sigma) and ouabain (Lilly). AHR-2666 was used in those experiments in which we planned to wash out the slow-channel blocking drug. Both AHR and verapamil were used in concentrations shown to abolish slow-response action potentials in canine Purkinje fibers.⁵ In the present study, no differences in the effects of the two drugs were seen. The drugs were dissolved in Tyrode's solution immediately before administration. During and after equilibration with each drug, the transmembrane potential characteristics of maintained impalements were recorded. Superfusion with drugs was continued until a steady-state effect was seen. This required 10 minutes for tetrodotoxin and epinephrine and 30 minutes for verapamil and AHR-2666. The actions of ouabain were determined after a 30-minute superfusion period.

Data are presented as mean ± SEM. Data were analyzed by analysis of variance and Scheffé's test or a paired or group t test. In experiments on cycle length effects on the action potential, the data were analyzed by linear regression.¹⁰

Results

Transmembrane Potential Characteristics of Noninfarcted Tissues

Control data for action potentials recorded in all noninfarcted tissues are shown in table 1. Two types of action potentials were recorded: those in the first group showed an upstroke with a sharp "spike" and a relatively high Vₘₐₓ; those in the second group showed a significantly lower Vₘₐₓ and no phase 0 spike. For the latter fibers there was no phase 1 repolarization and the plateau often was shorter, with phases 2 and 3 not being clearly separable. In the first group of fibers, phase 4 depolarization and automaticity occurred on discontinuation of the drive stimulus; in the second, it did not. (This was a major determinant in distinguishing Purkinje fibers from ventricular muscle.) The first group was therefore presumed to represent trans-
membrane potentials from Purkinje fibers, and the second, transmembrane potentials from ventricular muscle. Representative records of both are shown in figure 1. It must be emphasized that we could not always distinguish between Purkinje and myocardial fibers in normal tissues. The fibers characterized in table 1 are those for which the criteria of \( V_{\text{max}} \) phase 0 spike and phase 4 depolarization all were met.

Membrane responsiveness and the effects of potassium on membrane potential were studied in myocardial fibers only. Figure 2 demonstrates the relationship of maximum upstroke velocity of phase 0 (\( V_{\text{max}} \)) and overshoot to the membrane potential at which an action potential is initiated (i.e., membrane responsiveness). As expected, there was a sigmoid relationship of \( V_{\text{max}} \) to membrane potential. For the curve relating overshoot to membrane potential, there was a small increase in overshoot as membrane potential decreased from -90 to -80 mV.

Figure 3 shows the relationship of extracellular potassium ([K\(^+\)]\(_o\)) to resting potential for one of five normal human ventricular muscle fibers studied. There was a linear decrease in membrane potential as [K\(^+\)]\(_o\) increased from 4 to 6, 10, 20, 40 and 80 mM. Membrane potential decreased 52 mV per 10-fold increase in [K\(^+\)]\(_o\), a change slightly less than that which would be expected from the Nernst equation at 37°C.

When [K\(^+\)]\(_o\) was increased from 4 to 10 mM, the decrease in RMP was accompanied by the expected reduction in action potential amplitude and \( V_{\text{max}} \) and by an acceleration of repolarization (not shown). At a \([K^+]_o\) of 10–20 mM, the preparations became inexcitable.

The effect of changes in drive cycle length (from 1000–320 msec) on ventricular myocardial repolarization are shown in figure 4. There was a linear relationship between the logarithm of the cycle length and \( \text{APD}_{50} \) (\( r = 0.992 \)). When the preparations were driven at cycle lengths longer than 1000 msec, \( \text{APD}_{50} \) further increased, but it no longer was related linearly to the logarithm of the cycle length.

Transmembrane Potential Characteristics of Infarcted Tissues

Table 2 displays the transmembrane action potential characteristics from a section of myocardium (patient 4) in which there was an area of marked fibrosis and infarction, an adjacent area of noninfarcted tissue and an intermediate border zone. The maximal dia-

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**Table 1. Ventricular Myocardial and Purkinje Fiber Action Potentials from Noninfarcted Segments of Human Ventricle (Basic Cycle Length 800 msec)**

<table>
<thead>
<tr>
<th>Purkinje fiber</th>
<th>Myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 10)</td>
<td>(n = 24)</td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>79.6 ± 2.0 (67–93)</td>
</tr>
<tr>
<td>Amp (mV)</td>
<td>107.1 ± 2.2 (95–124)</td>
</tr>
<tr>
<td>( V_{\text{max}} ) (V/sec)</td>
<td>387.9 ± 25.0* (300-500)</td>
</tr>
<tr>
<td>APD_{50} (msec)</td>
<td>195.2 ± 8.9 (155–223)</td>
</tr>
<tr>
<td>APD_{100} (msec)</td>
<td>377.0 ± 13.3 (310–427)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; the range is given in parentheses.

\( V_{\text{max}} \) of Purkinje fibers was significantly greater than \( V_{\text{max}} \) of myocardial fibers (group \( t \) test).

*p < 0.02 compared with myocardium.

**Abbreviations:** MDP = maximum diastolic potential; Amp = action potential amplitude; \( V_{\text{max}} = \) maximum upstroke velocity of phase 0; APD_{50} and APD_{100} = action potential duration measured at 50% and 100% repolarization; \( n \) = the number of impalements.
TABLE 2. Action Potential Characteristics from an Infarcted Human Ventricle (Basic Cycle Length 800 msec)

<table>
<thead>
<tr>
<th></th>
<th>Normal zone</th>
<th>Border zone</th>
<th>Infarct zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDP (mV)</td>
<td>63.4 ± 2.4</td>
<td>56.0 ± 1.6</td>
<td>54.8 ± 4.3</td>
</tr>
<tr>
<td>Amp (mV)</td>
<td>82 ± 2.1</td>
<td>80.3 ± 1.7</td>
<td>80.0 ± 4.5</td>
</tr>
<tr>
<td>(\dot{V}_{\text{max}}) (V/sec)</td>
<td>168 ± 15</td>
<td>194 ± 43</td>
<td>164 ± 15</td>
</tr>
<tr>
<td>APD(_{50}) (msec)</td>
<td>177 ± 6</td>
<td>203 ± 14</td>
<td>224 ± 5*</td>
</tr>
<tr>
<td>APD(_{100}) (msec)</td>
<td>274 ± 3</td>
<td>280 ± 5</td>
<td>313 ± 7*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 7).
*\(p < 0.02\) vs normal zone.
The cells in the infarcted zone demonstrate a significantly longer APD\(_{50}\) and APD\(_{100}\) than those of the normal zone. The data were obtained from tissue from patient 4.
Abbreviations: See table 1.

The diastolic potential of the noninfarcted myocardium was lower than that shown in table 1 for a normal ventricle. There were no apparent differences in maximal diastolic potential, action potential amplitude and duration, perhaps reflecting its action on the rapid inward sodium current. It also shortened the action potential duration, perhaps reflecting its action on background inward sodium currents. Therefore, AHR-2666 only altered the repolarization phase of the action potential, presumably reflecting its action on the slow inward calcium current.

TABLE 3. Pharmacologic Effects on Noninfarcted Human Myocardial Fibers (Basic Cycle Length 630 msec)

<table>
<thead>
<tr>
<th></th>
<th>MDP (mV)</th>
<th>Amp (mV)</th>
<th>(\dot{V}_{\text{max}}) (V/sec)</th>
<th>APD(_{50}) (msec)</th>
<th>APD(_{100}) (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrodotoxin (n = 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>84.3 ± 0.6</td>
<td>117 ± 1</td>
<td>320 ± 90</td>
<td>160 ± 10</td>
<td>380 ± 25.2</td>
</tr>
<tr>
<td>Tetrodotoxin (1 mg/l)</td>
<td>-1.3 ± 0.3</td>
<td>-4 ± 0.5*</td>
<td>-66 ± 0.9*</td>
<td>-18.3 ± 1.5†</td>
<td>-15 ± 2.5†</td>
</tr>
<tr>
<td>AHR-2666 (n = 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>84.3 ± 1.5</td>
<td>115 ± 3.6</td>
<td>336 ± 77</td>
<td>163 ± 5</td>
<td>373 ± 32</td>
</tr>
<tr>
<td>AHR-2666 (45 mg/l)</td>
<td>0</td>
<td>-1.3 ± 0.6</td>
<td>-6 ± 2.3</td>
<td>-45 ± 6.6†</td>
<td>-12 ± 3.8</td>
</tr>
</tbody>
</table>

Values are mean (control) and mean change (drug) ± SEM.
*\(p < 0.025\).
†\(p < 0.01\).
‡\(p < 0.05\).
Abbreviations: See table 1.
The effects of tetrodotoxin and verapamil on the action potentials of spontaneously firing cells from a markedly depressed segment of human ventricle are shown in figure 5. Both drugs suppressed automaticity in these preparations. Neither showed any effect on the action potential before abolishing the spontaneous rhythm.

Both epinephrine (fig. 6) and ouabain (fig. 7) induced delayed afterdepolarizations after the Purkinje fiber action potential. These occurred in three of six fibers with epinephrine and three of four with ouabain. When the delayed afterdepolarizations were of sufficient magnitude, they attained threshold potential and induced action potentials (fig. 6).

**Discussion**

Our results do not reflect the electrophysiologic properties of completely normal hearts. All patients had hearts so diseased that they required transplant procedures. Moreover, all five patients had received digoxin and other cardioactive drugs. Nonetheless, our studies confirm the occurrence, in the human ventricle, of cellular electrophysiologic properties previously described extensively in other mammalian and, to a lesser extent, in human tissues. For example, when relatively normal human ventricular myocardial fibers were exposed to increasing extracellular concentrations of potassium, resting potential decreased to a level approximating that predicted by the Nernst equation. As shown for other species and for human atrium, at a [K+]o of 1–4 mM there was no further change in resting potential.

This study and those of others have shown that "normal" human ventricular and Purkinje fibers have different transmembrane potential characteristics. The V_{max} of the Purkinje fibers is higher than that in ventricular muscle. The Purkinje fibers also show a prominent phase 0 spike and phase 1 repolarization, not seen in muscle. That the transmembrane potentials from human tissue are not as negative in resting potential nor as high in action potential amplitude and V_{max} as those in the dog heart probably reflects the fact that canine tissues are obtained from completely normal hearts, whereas our relatively normal values came from the less diseased segments of severely diseased hearts.

The relationship of V_{max} to membrane potential was similar to the membrane responsiveness relationships in other species. The relationship of overshoot to membrane potential — the former increasing as the latter decreased from −90 to −80 mV and then decreasing again as the membrane potential became less negative — was also similar to that described in canine Purkinje fiber and myocardium.

This small increase in overshoot has been attributed to the contribution of calcium to the phase 0 upstroke.

Our values for "normal" muscle show higher action potential amplitudes and V_{max} than reported by Spear et al. Also, our values for V_{max} of Purkinje fibers from relatively normal regions are significantly higher than those reported by Spear et al., probably because Spear and co-workers studied tissues in and

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**FIGURE 5.** Effect of graded concentrations of tetrodotoxin (TTX) (A) and verapamil (B) on spontaneously firing fibers from a section of diseased myocardium. Note the change of time base for records depicting superfusion with verapamil 0.1 and 0.5 μg/ml.
adjacent to excised ventricular aneurysms. Presumably, the higher $V_{\text{max}}$ in both myocardium and Purkinje fibers in our study are the result of having more normal tissues available for study. When we studied tissues in infarcted areas, the values were similar to those reported by Spear et al.

Both Spear et al.\(^1\) and we have shown that in diseased human ventricle the action potential characteristics are consistent with those of the slow response; that is, they have low levels of membrane potential and slowly rising upstrokes. Not only verapamil, which would be expected to abolish calcium-dependent potentials at low levels of membrane potential, but also tetrodotoxin depressed automaticity and impulse initiation in these tissues. That automaticity can occur in mammalian myocardial fibers at low membrane potentials was shown by Surawicz and Imanishi\(^2\) and by Katzung and Morgenstern.\(^3\) That tetrodotoxin abolishes automaticity in diseased cardiac fibers has been demonstrated in the human atrium by Mary-Rabine et al.\(^4\) The mechanism may be related to the action of tetrodotoxin on resting membrane conductance to sodium (which has been demonstrated in invertebrates by Narahashi\(^5\)) or to the action of tetrodotoxin on the background inward current carried by sodium (described in Purkinje fibers by Coraboeuf et al.\(^6\)). We could not determine whether tetrodotoxin also depressed the action potential upstroke in these experiments, as the event that invariably occurred was a prolongation of cycle length without any change in action potential amplitude followed abruptly by quiescence.

The relationship of drive cycle length to action potential duration of normal, infarcted and inter-

![Effect of epinephrine (10^-4 M) on human Purkinje fiber action potentials.](image)

**Figure 6.** Effect of epinephrine (10^-4 M) on human Purkinje fiber action potentials. During control studies at three cycle lengths (CL), discontinuation of the drive stimulus (arrows) is followed by quiescence. In the presence of epinephrine, delayed afterdepolarizations occur and induce action potentials. As the drive cycle length decreases, the delayed afterdepolarization amplitude increases. This effect is reversible by washing in epinephrine-free Tyrode’s.

mediate tissues is of importance with respect to the occurrence of arrhythmias. Action potential duration was significantly greater in infarcted than in nearby noninfarcted tissues (table 2). This disparity in repolarization, which presumably would be associated with dispersion of refractoriness, may predispose to slow conduction and reentry.

Finally, both epinephrine and digitalis induced delayed afterdepolarizations. This effect has not been shown to occur in tissues in the human ventricle. That it was seen in these studies provides further evidence that such afterdepolarizations and their resultant triggered activity can be elicited in the presence of spontaneously occurring cardiac disease (in the presence of digitalis) and warrant further consideration in the genesis of ventricular arrhythmias.

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