Age-Related Changes in Purkinje Fiber Action Potentials of Adult Dogs

MICHAEL R. ROSEN, ROBERT F. REDER, ALLAN J. HORDOF, MARK DAVIES, AND PETER DANIO, JR.

SUMMARY To determine the effects of age on electrophysiological properties of Purkinje fibers (PF), we used standard microelectrode techniques to study PF from normal beagles of five age groups: 19.1 ± 0.8, 63.7 ± 2.6, 88.3 ± 1.9, 107.2 ± 1.6, and 132.3 ± 4.3 months (mean ± se). Maximum diastolic potential (MDP) did not change over this age range. Action potential (AP) amplitude and maximum upstroke velocity of phase 0 (V max) attained peak values at 63.7 months and then declined. As age increased there was an increase in phase 1 repolarization, a prolongation in the time to the peak of the plateau, and a decrease in plateau height. Fibers were superfused with the slow channel blocker, AHR-2666, and the fast channel blocker, tetrodotoxin (TTX). AHR-2666 had age-related effects on AP duration, phase 1 repolarization, plateau height, and time to the peak of the plateau. There were no age-related changes in TTX effects on phases 0-2. In another series of experiments, Purkinje fibers from dogs 23 ± 1.2 to 106 ± 1.4 months old were superfused with a calcium-rich solution in which Na + was replaced by tetraethylammonium (TEA +). The resultant slow response action potentials, which are calcium dependent, showed an age-related decrease in AP amplitude, V max, and time to peak amplitude. When these fibers were superfused with the slow channel blocker, verapamil, there was an age-related effect on AP amplitude. Our studies indicate that in the normal aging heart, major changes occur in repolarization that appear, in the main, to result from change in the slow inward current carried by Ca ++.

IN RECENT YEARS there has been an increasing appreciation of the effects of aging on electrophysiological and contractile function and pharmacological responses of the mammalian heart.1-8 However, only limited studies are available describing the changes in electrophysiological function of cardiac cells that occur with increasing age. These studies have been confined chiefly to the rat, in which the spectrum of age reported has encompassed the neonate through the elderly.4 In addition, studies have been performed on fetal, newborn, and adult sheep,6 on young and elderly guinea pigs,5 and on newborn, young, and adult dogs.7 Studies of left ventricular papillary muscles have shown that in the old rat there is an increase in time to peak tension, a lower velocity of contraction, and a lesser degree of shortening than in the young.1 In electrophysiological studies of rat atri, Cavoto et al.4 showed an age-related decrease in maximum upstroke velocity (V max) of phase 0 and an increase in the duration of the action potential and its plateau. No changes in resting potential or action potential amplitude and overshoot were seen.

Based on the information mentioned above, it seems likely that for any animal species normal electrophysiological properties will change with age, and that arrhythmias, when they occur, will be superimposed on a different "baseline" of electrical activity. Therefore, we believe it important to ascertain how normal cardiac electrophysiological properties change with age. For this reason we studied adult dogs ranging in age from 16 to 140 months and determined normal Purkinje fiber action potential characteristics. To aid in identifying the changes in ionic currents that contribute to the action potential, experiments were done using the fast Na + channel blocker, tetrodotoxin (TTX),9 and the slow-channel blockers, verapamil10 and AHR-2666.11

Methods

Experimental Protocol

We studied healthy beagles of both sexes obtained from three breeders who provided accurate information on age and medical history. Only dogs with a normal ECG, medical history, and physical examination were used in this study. The dogs were anesthetized with Na pentobarbital, 30 mg/kg, intravenously. The heart was removed rapidly through a right lateral thoracotomy and placed in cold Tyrode's solution containing (mM/liter): NaCl, 137; NaHCO₃, 12; KCl, 4.0; NaH₂PO₄, 1.8; CaCl₂, 2.7; MgCl₂, 0.5; dextrose, 5.5. Purkinje fiber bundles and attached myocardium were removed from both ventricles. The type of preparation studied has been described previously by Myerburg et al.13 The tissues were placed in a Lucite chamber perfused with

From the Departments of Pharmacology and Pediatrics, Columbia University College of Physicians and Surgeons, New York, New York. Supported by U.S. Public Health Service-National Heart, Lung, and Blood Institute Grant HL-12738 and a grant from the New York Heart Association.

Address for reprints: Michael R. Rosen, M.D., Department of Pharmacology, Columbia University College of Physicians and Surgeons, 630 W. 168th Street, New York, New York 10032.

Original manuscript received April 20, 1978; accepted for publication July 14, 1978.
Tyrode's solution, maintained at 37°C and equilibrated with 95% O2-5% CO2. The superfusate flow rate was 10-12 ml/min. The fiber bundles were stimulated at a cycle length of 500 msec through Teflon-coated bipolar silver wire electrodes, using previously described techniques. Fibers were impaled with 3 m KCl-filled glass capillary microelectrodes with tip diameters of <1 μm and resistances of 10-25 MΩ. An Ag-AgCl function was used to couple the microelectrodes to a preamplifier with high input impedance and input capacity neutralization, and the signal was displayed on oscilloscopes. The tissue chamber was connected to ground through a 3 m KCl-Ag-AgCl junction. The methods used to calibrate the equipment and to measure the maximum upstroke velocity of phase 0 (Vmax) have been described previously. The preparations were stimulated, impaled with microelectrodes, and allowed to equilibrate for 1 hour before control measurements were made. The following control measurements then were recorded: maximum diastolic potential (MDP); action potential (AP) amplitude (measured from the MDP to the peak of the overshoot); action potential duration to 50% repolarization (APD50); action potential duration to full repolarization (APD100); Vmax; the magnitude of phase 1 (PH1) repolarization; the peak of phase 2 (PH2) repolarization; and the time from the midpoint of the AP upstroke to the peak of phase 2 (tpho-ph2). These measurements were made by previously described methods or with a Nicolet 1090 digital processing oscilloscope. To ensure that comparable recording sites were studied in dogs of all ages, multiple microelectrode impalements were made in each fiber bundle to identify the area of maximum action potential duration or "gate." Impalements then were maintained at the gate for the duration of the experiment. Only values for these action potentials are reported here.

Following the recording of control action potential characteristics, fibers were superfused with the slow channel blocker, AHR-2666, or the fast channel blocker, verapamil. Thirty-minute superfusion periods for each drug concentration were used, and the effects of the drug on the action potentials were recorded. The variables recorded here were AP amplitude, MDP, Vmax, of phase 0, and time from the onset of phase 0 (abbreviated AV, for activation voltage) to the peak of the action potential (tAV-Peak). Here Vmax was measured by recording the action potential at a rapid oscilloscope sweep speed, laying a straight edge across the fastest portion of the upstroke and calculating the upstroke velocity from this portion of the depolarization phase.

**Data Analysis**

Assessment of aging effects on control action potential characteristics was obtained by applying a one-way, fixed-effects (age) analysis of variance (ANOVA). Age differences in control superfusates were globally tested by using the F statistic from the ANOVA tables. Significant F statistics for an age effect imply at least one difference in the means between any two age groups. Specification of which age groups are significantly different was accomplished by t-tests between groups under question, with protection of the α confidence maintained by Scheffe's procedure. The variance term in the denominator of the t statistic is the MSq due to error in the ANOVA table.

Age-related differences in dose-response to experimental solutions can be characterized in two ways. First, the shapes of the dose-response curves for different ages may be identical, but the magnitude of the response for each dose shifts as a function of age. This characterization occurs when the age x dose interaction in the ANOVA is not significant and there are initial age differences. Second, the shapes of the dose-response curves at different ages may not be identical. In these studies, age-related differences in response to experimental solutions were assessed by application of a nested (dose within age) analysis of variance. This statistical procedure explicitly tests for age-related differences in dose-response curves. The unit of analysis for slow and fast response experiments was the individual fiber's response.

**Results**

Age-Related Changes in Electrophysiological Characteristics of Fast Response Action Potentials

The dogs were divided into five groups, based on their ages (Table 1). Those in the youngest group (six female, three male) were approximately ½ years old; in the second (five female, four male), 5 years old; in the third (three female, one male), 7 years old; in the fourth (four female, one male), 9 years old; and in the fifth (two female, two male), 11 years old. All females were at least 6 weeks postpartum. The data in Table 1 show that, for normal adult dogs, as age increased from 19.1 to
132.3 months, there were no significant changes in maximum diastolic potential and action potential duration measured to 50% or full repolarization. There were, however, significant changes in the amplitude, $V_{\text{max}}$, phase 1, and phase 2, as follows: AP amplitude attained a maximum value of 130.8 mV in the 63.7-month-old dogs. For older dogs, amplitude was significantly lower ($P < 0.01$). The value for $V_{\text{max}}$ was significantly lower ($P < 0.05$) for groups in which the dogs were 107 and 132 months old than it was for those at 63.7 months. Finally, there were significant age-related changes in phases 1 and 2 of repolarization that resulted in an increasingly negative voltage for phase 1, a decreasing plateau height, and a prolongation of the interval from the rapid phase 0 upstroke to the peak of phase 2. Characteristic action potentials for dogs from two age groups are shown in Figure 1, A and B.

**Effects of TTX and AHR-2666 on Fast Response Action Potentials**

In 17 experiments on Purkinje fibers from dogs of three age groups, we determined the effects of TTX and AHR-2666 on fast response action potentials. The results are shown in Figures 2 and 3. TTX (Fig. 2) significantly depressed all variables studied except MDP and the time to the peak of the plateau in a concentration-dependent fashion. When we tested the relationship of age to the effects of TTX, no apparent relationship was found, with the exception of a decrease in $\text{APD}_{50}$; that is, all TTX-induced changes in transmembrane potential characteristics except for $\text{APD}_{50}$ occurred to an equivalent extent at the different ages studied. AHR-2666 significantly decreased $\text{APD}_{50}$.

### Table 1

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>n</th>
<th>Amp (mV)</th>
<th>MDP (mV)</th>
<th>$V_{\text{max}}$ (V/sec)</th>
<th>$\text{APD}_{500}$ (msec)</th>
<th>$\text{APD}_{500}$ (msec)</th>
<th>$p_{\text{th}}$ (mV)</th>
<th>$p_{\text{th}}$ (mV)</th>
<th>$t_{\text{pho-ph2 peak}}$ (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.1</td>
<td>18</td>
<td>127.2</td>
<td>±0.8</td>
<td>589.8</td>
<td>172.3</td>
<td>5.6*</td>
<td>5.4</td>
<td>20.3*</td>
<td></td>
</tr>
<tr>
<td>63.7</td>
<td>18</td>
<td>130.8</td>
<td>±1.6</td>
<td>634.3</td>
<td>185.4</td>
<td>0.1</td>
<td>1.3</td>
<td>16.7</td>
<td></td>
</tr>
<tr>
<td>88.3</td>
<td>8</td>
<td>125.1</td>
<td>±0.8</td>
<td>577.1</td>
<td>182.7</td>
<td>0.2</td>
<td>0.9</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>107.2</td>
<td>10</td>
<td>123.3</td>
<td>±1.2</td>
<td>520.4</td>
<td>162.0</td>
<td>0.1</td>
<td>0.9</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>132.3</td>
<td>8</td>
<td>122.4</td>
<td>±1.2</td>
<td>508.0</td>
<td>183.8</td>
<td>0.1</td>
<td>0.9</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

Values expressed as mean ± se. Numbers in parentheses = number of dogs. Transmembrane potential characteristics two Purkinje fiber bundles (posterior division of the left bundle and the right bundle) were used. Hence "n" (number of fibers) for all transmembrane potential characteristics measured is twice the number of dogs in the first column. Significant age-related changes are seen in action potential amplitude, $V_{\text{max}}$, phase 1, phase 2, and $t_{\text{pho-ph2 peak}}$. For the levels of significance presented here, the 63.7-month group is used as the standard for comparison.

* $P < 0.05$ compared to 63.7 months.  † $P < 0.05$ compared to 63.7 months.
FIGURE 2  Effects of TTX on fast response action potentials. Results are expressed as increase or decrease from control. The control values are found in Table 1 and are expressed here as "0." There were no significant drug-induced changes in MDP or time from phase 0 to phase 2. The changes that were significant and age-related are discussed in the text. The asterisks indicate the minimum effective concentration for each drug in each age group. This was determined by using Scheffe’s procedure and testing each value against its own control. Note: the results are expressed as increase or decrease from control as a convenience. The statistical analysis was performed using the absolute numbers. Unfilled circles = 19.1 months; filled circles = 63.7 months; triangles = 107.2 months.

voltages for phase 1 repolarization and the peak of phase 2 at all ages, and increased APD\(_{100}\) and the interval from phase 0 to the peak of phase 2 (Fig. 3). All of these changes were related to the age of the dog, and as age increased there was a tendency for the drug’s effect to occur at a lower concentration. For example, the minimum concentration that significantly increased t\(_{0-\text{Ph2}}\) was 5 \(\mu\)g/ml for the oldest group tested and 30 \(\mu\)g/ml for the youngest group tested.

Changes in the Slow Response Action Potential with Age

The preceding experiments suggested to us that the major change in the plateau phase of the action potential probably resulted from changes in the secondary or slow inward current carried by calcium. We therefore studied Purkinje fibers from an additional 18 dogs using TEA solution as the superfusate (Table 2 and Fig. 1, C and D). As age increased there were significant decreases in the amplitude and \(V_{\text{max}}\) of phase 0 of the action potentials, and an increase in time to the peak of the upstroke. Values for APD are not reported because the occurrence of secondary depolarizations during repolarization in some preparations made it difficult to measure this variable accurately. Superfusion with verapamil (Fig. 4) induced concentration-dependent decreases in AP amplitude and \(V_{\text{max}}\) and an increase in time to peak amplitude. The change in amplitude was age-dependent. The change in time to peak amplitude also occurred at lower drug concentrations in the 2 older than in the two younger age groups. There was no apparent age-related relationship for the drug effect on \(V_{\text{max}}\), however.

Discussion

We have shown that as age increases from the young to the old adult there are consistent and significant changes in certain of the action potential characteristics that appear to be primarily related to the age of the dog.

The data in Table 1 demonstrate that as dogs age there is an increase in phase 1 repolarization, a prolongation of the interval from phase 0 to the peak of the plateau, and a diminution in plateau height. It is likely that the major contributor to this age-related change in the plateau is the slow or
EFFECTS OF AGING ON PURKINJE FIBER ACTION POTENTIALS

FIGURE 3  Effects of AHR-2666 on fast response action potentials. Results are expressed as in Figure 2; controls are found in Table 1. There were no significant drug-induced changes in AP amplitude, MDP, or Vmax. The significance of the changes that did occur is discussed in the text. For meaning of asterisks and method of statistical analysis, see Figure 2. Unfilled circles = 19.1 months; filled circles = 63.7 months; triangles = 107.2 months.

Secondary inward current which is carried largely by calcium in fibers with the fast response as well as in those with the slow response. The major evidence for an age-related change in Ca2+ effects on the action potential is obtained from the studies of the slow response. In the control situation (see Table 2) slow response amplitude, Vmax, and time to peak magnitude all decreased with age. Because calcium is the major contributor to the slow response action potential in the sodium-free, calcium-rich superfusate, it is likely that an inward current carried by this ion was decreasing in terms of rate of entry (Vmax) and total calcium entry (amplitude) with increasing age. The control observations of action potential characteristics for fibers with the fast response (Table 1) also support this interpretation. The decreasing amplitude of the fast response plateau as age increases is analogous to the change in amplitude of the slow response; the longer time from phase 0 to the peak of the plateau is consistent with the longer time to peak magnitude for the slow response.

The superfusion of fibers having the fast and the slow response with slow-channel blockers further supports the view that there are age-related changes in the slow inward (calcium) current. The superfusion of fast response fibers with AHR-2666 induced age-related changes in action potential duration, phase 1 repolarization, plateau height, and time from phase 0 to the peak of the plateau. Although this observation does not rule out the possibility of another slow channel changing with age, actions on such channels have not to our knowledge been demonstrated (or studied) for AHR-2666. The studies in which the effects of another slow channel blocker, verapamil, were observed on the slow response are consistent with the observations made for the fast response. The effect of verapamil on slow response amplitude clearly was age-related. Although a clear age relationship was not obtained with respect to the time to peak of the slow response, the calculation of the minimum effective drug concentration for each age group shows that the minimum effective verapamil concentration for the two older groups is 1-2 orders of magnitude less than that for the two younger age groups (Fig. 4). This observation supports the concept of a lesser sensitivity of the fibers from the young dogs to a Ca2+ blocker. Although verapamil has been shown to block a slow inward current carried by Na+, there was no Na+ in the superfusate used, and studies by others have shown fibers studied in this fashion in TEA solution to be Na+ free.

The observation that the minimum effective con-
Superfused with Tetraethylammonium Chloride

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>Amp (mV)</th>
<th>MDP (mV)</th>
<th>$V_{\text{max}}$ (V/sec)</th>
<th>$t_{\text{AV-peak}}$ (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>23.0</td>
<td>12</td>
<td>82.6 ± 2.2</td>
<td>65.6 ± 0.9</td>
<td>5.1*</td>
<td>19.8 ± 1.7</td>
</tr>
<tr>
<td>±1.2</td>
<td>(6)</td>
<td>78.2 ± 3.1</td>
<td>62.3 ± 2.7</td>
<td>3.1</td>
<td>25.7 ± 3.3</td>
</tr>
<tr>
<td>66.0</td>
<td>6</td>
<td>68.6 ± 2.9</td>
<td>60.7 ± 2.5</td>
<td>1.8</td>
<td>62.9 ± 10.3</td>
</tr>
<tr>
<td>±3.2</td>
<td>(3)</td>
<td>68.6 ± 3.2</td>
<td>57.5 ± 2.1</td>
<td>2.6</td>
<td>40.6 ± 2.7</td>
</tr>
<tr>
<td>106.2</td>
<td>10</td>
<td>68.6</td>
<td>57.5</td>
<td>2.6</td>
<td>40.6</td>
</tr>
<tr>
<td>±1.4</td>
<td>(5)</td>
<td>68.6</td>
<td>57.5</td>
<td>2.6</td>
<td>40.6</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SE. Numbers in parentheses = number of dogs. For transmembrane potential characteristics two fiber bundles from each dog were used (see Table 1), so "n" for the transmembrane potentials is twice the number of dogs in the first column. Significant age-related decreases are seen in action potential amplitude and $V_{\text{max}}$, and a significant increase occurs in $t_{\text{AV-peak}}$. For the levels of significance presented here, the 66-month group is used as the standard for comparison.

* $P < 0.05$ compared to 66 months.
† $P < 0.01$ compared to 66 months.

Regardless of the role of the $Cl^-$ current, the results of the present study attest to the importance of an age-related change in the slow inward current. For example, the long time required to attain peak magnitude for the plateau of the fast response and the smaller plateau magnitude that occur with age are consistent with a change in the slow inward current. The experiments on the slow response, in which Ca$^{2+}$ is the major determinant of the action potential, provide important support for this view in that the amplitude, time to peak amplitude, and $V_{\text{max}}$ of the slow response all decrease with age. Another possibility that must be considered is that the age-related changes seen in phase 1 and the plateau of the action potential are not only the result of changes in inward current, but also may reflect changes in magnitude or kinetics of outward currents. Important to these considerations would be information on whether AHR-2666, like verapamil, modifies outward current. In summary, the complexity of current changes that are faced in considering phase 1 and the plateau is such that one would suggest the use of voltage clamp techniques as one means for further study here.

FIGURE 4 Effects of verapamil on slow response action potentials. Results, asterisks, and statistical analysis as in Figure 2. Control values are found in Table 2. For discussion of significance, see text. Unfilled circles = 23 months; filled circles = 66 months; filled triangles = 90 months; unfilled triangles = 106.2 months.
To place these data on age-related changes in the action potential of adult dogs into a proper perspective, it is useful to compare them to the action potential characteristics we have reported for neonatal and adult dogs in the past. In one study of Purkinje fiber action potentials recorded at the area of maximum action potential duration, we reported that, for neonatal mongrels, AP amplitude is 118.6 ± 1.5 mV (mean ± SE); MDP, −83.7 ± 1.3 mV; V_{max}, 455 ± 29 V/sec; APD_{50}, 153 ± 4 msec; and APD_{100}, 231 ± 5.5 msec (at a cycle length of 500 msec). For 4- to 7-week-old puppies, these values had changed to AP amplitude, 119.5 ± 0.8 mV; MDP, −84.4 ± 0.9 mV; V_{max}, 511 ± 19 V/sec; APD_{50}, 176 ± 4 msec; and APD_{100}, 268 ± 4 msec. If we view these values in light of the information presented in Table 1, it is apparent that, as age increases from the neonate to the old dog, a number of changes occur. Maximum diastolic potential increases significantly from the neonate to the adult (see reference 7), and the peak value for MDP (−91.5 mV) is attained at 63.7 months. Thereafter, values diminish to the range of from −85 to −88 mV. In a related study we have shown that the change in MDP comparing neonates to adults is attributable to an increase in intracellular K+ activity (a_{K}) from 117.4 ± 1.1 (mean ± SE) mm in the newborn to 130.0 ± 2.3 mm in the adult. The idea that an increase in a_{K} is responsible for the age-related increase in MDP is also borne out by the ionic flux studies of Goldberg et al.

The changes in action potential amplitude and V_{max} that are seen as age increases from neonate to adult appear closely related to those in MDP. As for MDP, peak values for both amplitude and V_{max} occur at 63.7 months of age and thereafter diminish. If, for the adults, age were the only or major variable that determines the changes in phase 0 amplitude and V_{max}, we might expect that the studies using TTX would show age-related differences in response to this Na+ blocker. In our present study of adults, this did not occur. Nonetheless, it is possible that extension of our studies to the neonate may show an age-related basis for the changes in amplitude and V_{max} that occur from neonates to adults.

Comparing the action potential duration of neonatal to adult animals, it is apparent that significant changes occur in both APD_{50} and APD_{100}. Although some variability in action potential duration is seen among the adult age groups, it appears that once adulthood is reached there are no consistent age-related changes here. However, the changes in phase 1 and the plateau are consistent and significant.

In summary, significant changes occur in action potential characteristics in apparently healthy adult dog hearts. The repolarization changes seen appear largely to reflect a change in the slow inward current, although other causes must be considered. Awareness of the fact that the aging process is associated with such cellular electrophysiological changes should make us increasingly cognizant of the extent to which we must consider what is normal for cardiac cellular electrophysiological function for animals of any age group as a conditioning factor in studies of impulse initiation, conduction, and arrhythmogenesis.

Acknowledgments

We wish to thank Alicia Hart for her excellent technical assistance, Cynthia Brandit for her careful preparation of the manuscript, and Dina Rosen for assisting with the data processing.

References

19. Aronson RS, Cranefield PF: The electrical activity of canine
cardiac Purkinje fibers in sodium-free, calcium-rich solutions. J Gen Physiol 66: 786-808, 1973


Age-related changes in Purkinje fiber action potentials of adult dogs.
M R Rosen, R F Reder, A J Hordof, M Davies and P Danilo, Jr

doi: 10.1161/01.RES.43.6.931

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1978 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/43/6/931

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

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

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/