Effect of Calcium Concentration on the Transmembrane Potentials of Purkinje Fibers

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ABSTRACT

The effects of variation in calcium concentration on the action potential of Purkinje fibers isolated from the dog heart were studied. Action potentials recorded during perfusion with Tyrode solution containing 2.7 mM calcium chloride were compared with those recorded during subsequent perfusion with solutions containing 0.675 (1/4x), 1.35 (1/2x), 5.4 (2x), or 10.8 (4x) mM calcium chloride. In both 1/2x and 1/4x solutions, the time required to repolarize to minus 60 mv and the duration of the action potential were increased. There were significant decreases in the slopes of phases 2 and 3 and the terminal phase of repolarization, while the slope of phase 1 increased. In 2x or 4x solutions, repolarization was speeded mainly by an earlier onset of phase 3. As a result the time to repolarize to minus 60 mv and the duration of the action potential were decreased significantly. The slope of phase 1 decreased significantly in 4x solution. In both high calcium solutions the rate and magnitude of diastolic depolarization increased. It was shown that the rate of the calcium-enhanced diastolic depolarization was dependent on the stimulus rate. The possible role of these changes in transmembrane potential in causing the ventricular arrhythmias following CaCl₂ infusion in intact animals is discussed.

ADDITIONAL KEY WORDS

calcium ions
ventricular action potentials
diastolic depolarization

The effect of calcium on the transmembrane action potential has been described for several types of mammalian cardiac fibers. Weidmann (1) found no appreciable change in the contour of the action potential of spontaneously beating Purkinje fibers isolated from calf or sheep hearts when the calcium concentration was varied between 0.675 and 10.8 mM. The main effect was that high calcium lowered (moved toward zero) the level of the threshold potential while low calcium had the opposite effect. Hoffman and Suckling (2) showed that similar calcium concentrations do produce changes in the contour of the action potential of atrial and ventricular fibers of the dog. Increased calcium concentration accelerated repolarization, and decreased concentration slowed repolarization in both types of muscle fibers. In addition, they showed that reducing calcium to one-tenth of normal (0.27 mM) caused the action potential of Purkinje fibers to assume a shape similar to that normally recorded from fibers of the sino-atrial node. Seifen and co-workers (3) studied the effects of calcium on fibers of the sino-atrial node of rabbits and found that increased calcium caused a faster rate of diastolic depolarization.

On the basis of the above studies it appeared that Purkinje tissue was unique among the cardiac tissues in that its action potential was unaffected by relatively wide changes in calcium concentration. However, unlike atrial and ventricular muscle fibers, Purkinje fibers often beat spontaneously, and the study...
by Weidmann (1) was carried out on fibers whose spontaneous rate varied with different calcium concentrations. Since changes in rate alone can alter the contour of the action potential (4) we have investigated the effect of variation in calcium concentration on the action potential of Purkinje fibers driven at constant rates.

Methods

Hearts were excised from dogs anesthetized with cyclopropane (33% in O2) or sodium pentobarbital (30 mg/kg, iv). They were immersed while still beating in warm oxygenated Tyrode solution. Papillary muscles with attached false tendons containing Purkinje fibers were removed from the right and left ventricles. Several suitable preparations were obtained from each heart and were stored until ready for use in a reservoir containing Tyrode solution that was continuously oxygenated. For study, a muscle was pinned under slight tension to a paraffin block in a tissue bath of 15 ml volume. Tyrode solution equilibrated with 95% O2-5% CO2 flowed continuously through the bath at a rate of 30 ml/min. The composition of the control Tyrode solution in mM was NaCl, 137; dextrose, 5.5; KCl, 2.7; CaCl2, 2.7; MgCl2, 0.5; NaH2PO4, 0.9; and NaHCO3, 24.0. The pH of the perfusion fluid anaerobically collected from the tissue bath was 7.2 to 7.3. Determinations of pH were made with an Instrumentation Laboratories Model 113 gas analyzer. In these experiments, cardiac tissue was subjected to four concentrations of calcium ions in addition to that in the control solution. Solutions containing 0.675 (1/4x Ca-Tyrode), 1.35 (1/2x Ca-Tyrode), 5.4 (2x Ca-Tyrode) and 10.8 (4x Ca-Tyrode) mM calcium were made. In some experiments calcium acetate was used to increase calcium ion concentration. No corrections were made for the small changes in osmotic pressure. Temperature of the bath was maintained between 35° and 37° C but remained constant during any experiment.

Microelectrodes were pulled from capillary tubing and filled with 3 M KCl. Resistance of suitable electrodes was 8 to 10 megohms. An indifferent electrode filled with 3 M KCl made contact with the fluid in the bath. Both electrodes were connected by chloride-silver spirals to the input of a Bioelectrics (Type DS2C) cathode follower-amplifier which in turn was connected to the differential amplifier of a Tektronix Type 502 oscilloscope. A 100-mv d-c calibrator was interposed between the indifferent electrode and the amplifier.

The rising velocity of the action potential upstroke was determined from the amplitude of a differentiated record (Fig. 1). The voltage output of the differentiator was calibrated by introducing sawtooth pulses of known amplitude and duration through the microelectrode-cathode follower stage. The cathode follower provided for neutralization of input capacitance; the connection of the microelectrode with the cathode follower input was made short and with unshielded wire.

FIGURE 1

Methods used to measure different features of the action potential of Purkinje fibers. Top (a fast sweep record at 50 msec/cm): A = resting potential; A - B = magnitude of diastolic depolarization; C = magnitude of action potential; C - B = overshoot; D = slope of phase 1; E = slope of phase 2; F = slope of phase 3; G = slope of terminal repolarization; H = time to repolarize to -60 mv; I = duration of action potential; the magnitude of I is proportional to the maximum rate of rise of the action potential upstroke and is recorded at a sweep speed of 50 msec/cm; K = stimulus artifact. Bottom (a slow sweep record at 100 msec/cm): A = the slope of phase 4 and gives the rate of diastolic depolarization; B = maximum diastolic potential; C = duration of action potential.
Under these conditions, the output of the differentiator was linear, with rates of potential change from 100 to 1,000 V/sec.

Rhythmic contractions were maintained by applying supramaximal square-wave pulses of 5-msec duration at 95/min to the muscle. Single Purkinje cells and, on occasion, ventricular muscle cells were impaled by advancing the micro-electrode into the tissue until characteristic resting and action potentials were obtained (5). Only cells on the surface of the preparation were studied. The transmembrane resting and action potentials were monitored continuously on the Tektronix type 502 oscilloscope. Representative potentials were displayed on a Tektronix type 565 oscilloscope and photographed with a Grass kymograph camera.

The general procedure of the experiment was as follows: Perfusion with control Tyrode solution was maintained until a single Purkinje cell was penetrated. Perfusion was changed to Tyrode solution with altered calcium concentration and maintained for 10 min. The addition or removal of calcium ions resulted in changes that reached maximal values in this time, and there were no further changes when calcium perfusion was continued for an additional 10 min. A given tissue preparation was subjected to one altered calcium concentration and then discarded.

Photographic records of action potentials were taken on clear base film before and at intervals during the test perfusions. These records were projected onto the reading surface of a Benson-Lehner X-Y film reader-decimal converter system which magnified the original record approximately 13 times. The X-Y reader-converter was calibrated in milliseconds and millivolts from a film strip containing time and voltage deflections from a Tektronix type 180 A time mark generator and a 100 mv d-c calibrator. The abscissa and ordinate at a given point on the action potential were measured to three digits and automatically punched on tabulating cards by an IBM 026 printing key punch. Different features of the action potential were measured, as shown in Figure 1. These data were reduced by a program on a CDC 3600 digital computer. In addition to the printer output, a punched output was obtained and the represented data were analyzed statistically by a Student's paired t-test program. The printout consisted of mean values, mean differences and t values for a number of different features of the action potential (Fig. 1). The only data used for statistical comparison were from experiments in which there was reason to believe that the microelectrode remained in the same cell throughout the control and test experiments.

Results

EFFECTS OF DECREASED CALCIUM CONCENTRATION

Twenty-four cells from 10 hearts were treated with calcium ion concentrations below normal. In 11 experiments the cells were perfused with 1/2× Ca-Tyrode. Typical changes are shown in Figure 2. Records A and B illustrate action potentials recorded at two sweep speeds during perfusion with control-Tyrode. Records C and D show action potentials from the same cell after 10 min of perfusion with 1/2× Ca-Tyrode. In record E, records A and C have been superimposed by aligning the upstrokes of the action potentials and the lines of zero potential difference. Figure 3 contains records of one of the 13 experiments in which perfusion with
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1/4Ca-Tyrode was maintained for 10 min. Unless specified, the labeling of this and all subsequent records is identical to that in Figure 2. Detailed analysis of all data obtained in 1/2Ca and 1/4Ca calcium experiments is presented in Tables 1 and 2.

In the presence of a lowered concentration of calcium ions, consistent changes occurred in the contour of the action potential. In most instances those features of the action potential that changed during treatment with 1/2Ca-Tyrode changed to an even greater extent during treatment with 1/4Ca-Tyrode. The most prominent change was the increased duration of the action potential. This amounted to mean increases of 29.4 and 46.2 msec ($P < .001$) in 1/2Ca-Tyrode and 1/4Ca-Tyrode, respectively. The rate of repolarization and the duration of the plateau (phase 2) are the main factors determining the duration of the action potential of Purkinje fibers (6). In the unmagnified records the increased duration appears to result principally from prolongation of the plateau. However, a quantitative measure of the rates of repolarization performed on mag-

![Figure 3](image)

**FIGURE 3**

Effect of reducing calcium to one-fourth normal on the action potential of Purkinje fibers. A and B, in control Tyrode solution. C and D, after 10 min in 1/4Ca-Tyrode solution. (The differentiated records and the action potential overshoots were retouched.)

**TABLE 1**

Effect of One-Half Normal Ca-Tyrode (1.35 mM Ca$^+$) on the Transmembrane Action Potentials in Eleven Purkinje Fibers

<table>
<thead>
<tr>
<th></th>
<th>Control Tyrode (Mean)</th>
<th>1/2Ca-Tyrode (Mean)</th>
<th>Mean difference and confidence interval*</th>
<th>Percent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum diastolic potential (mv)</td>
<td>95.3</td>
<td>92.5</td>
<td>$2.8 \pm 2.0$</td>
<td>$-2.9^t$</td>
</tr>
<tr>
<td>Overshoot (mv)</td>
<td>36.6</td>
<td>35.7</td>
<td>$0.9 \pm 1.9$</td>
<td>$-2.4$</td>
</tr>
<tr>
<td>Magnitude of action potential (mv)</td>
<td>131.6</td>
<td>128.4</td>
<td>$3.2 \pm 2.4$</td>
<td>$+2.4^t$</td>
</tr>
<tr>
<td>Slope of phase 1 (mv/sec)</td>
<td>4407.6</td>
<td>5064.9</td>
<td>$1197.3 \pm 915.6$</td>
<td>$+27.2^t$</td>
</tr>
<tr>
<td>Slope of phase 2 (mv/sec)</td>
<td>131.5</td>
<td>119.0</td>
<td>$12.5 \pm 11.0$</td>
<td>$-9.5^t$</td>
</tr>
<tr>
<td>Slope of phase 3 (mv/sec)</td>
<td>1066.3</td>
<td>946.0</td>
<td>$120.3 \pm 79.8$</td>
<td>$-11.3^t$</td>
</tr>
<tr>
<td>Slope of terminal phase of repolarization (mv/sec)</td>
<td>519.3</td>
<td>460.1</td>
<td>$59.2 \pm 45.5$</td>
<td>$-11.4^t$</td>
</tr>
<tr>
<td>Rate of diastolic depolarization (mv/sec)</td>
<td>6.8</td>
<td>4.9</td>
<td>$1.9 \pm 4.0$</td>
<td>$-27.9$</td>
</tr>
<tr>
<td>Magnitude of diastolic depolarization (mv)</td>
<td>1.2</td>
<td>0.1</td>
<td>$1.1 \pm 1.2$</td>
<td>$-91.7$</td>
</tr>
<tr>
<td>Time to $-60$ mv (msec)</td>
<td>260.3</td>
<td>287.4</td>
<td>$28.2 \pm 12.8$</td>
<td>$+10.8^t$</td>
</tr>
<tr>
<td>Duration of action potential (msec)</td>
<td>343.8</td>
<td>373.2</td>
<td>$29.4 \pm 12.7$</td>
<td>$+8.6^t$</td>
</tr>
<tr>
<td>Maximum rate of rise of upstroke (v/sec)</td>
<td>751.0</td>
<td>750.0</td>
<td>$1.0 \pm 25.0$</td>
<td>$-0.1$</td>
</tr>
</tbody>
</table>

*Confidence intervals were computed at the 99% level.

$P < .01$.

$P < .001$. 

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TABLE 2

Effect of One-Fourth Normal Ca-Tyrode (0.675 mM Ca²⁺) on the Transmembrane Action Potentials of Thirteen Purkinje Fibers

<table>
<thead>
<tr>
<th></th>
<th>Control Tyrode (Mean)</th>
<th>1/4 X Ca-Tyrode (Mean)</th>
<th>Mean difference and confidence interval*</th>
<th>Percent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum diastolic potential (mv)</td>
<td>98.6</td>
<td>94.5</td>
<td>2.3 ± 0.9</td>
<td>-2.3t</td>
</tr>
<tr>
<td>Overshoot (mv)</td>
<td>36.4</td>
<td>36.1</td>
<td>0.3 ± 0.2</td>
<td>-0.8</td>
</tr>
<tr>
<td>Magnitude of action potential (mv)</td>
<td>132.9</td>
<td>130.6</td>
<td>2.3 ± 0.1</td>
<td>-1.7t</td>
</tr>
<tr>
<td>Slope of phase 1 (mv/sec)</td>
<td>3986.1</td>
<td>6392.8</td>
<td>2406.7 ± 4187.3</td>
<td>+60.4t</td>
</tr>
<tr>
<td>Slope of phase 2 (mv/sec)</td>
<td>140.5</td>
<td>99.9</td>
<td>40.6 ± 10.8</td>
<td>-28.9§</td>
</tr>
<tr>
<td>Slope of phase 3 (mv/sec)</td>
<td>1000.0</td>
<td>840.1</td>
<td>160.8 ± 113.5</td>
<td>-10.1§</td>
</tr>
<tr>
<td>Slope of terminal phase of repolarization (mv/sec)</td>
<td>548.1</td>
<td>439.6</td>
<td>108.5 ± 95.0</td>
<td>-19.8t</td>
</tr>
<tr>
<td>Rate of diastolic depolarization (mv/sec)</td>
<td>0.4</td>
<td>3.7</td>
<td>2.7 ± 4.0</td>
<td>-42.2</td>
</tr>
<tr>
<td>Magnitude of diastolic depolarization (mv)</td>
<td>0.3</td>
<td>0.1</td>
<td>0.2 ± 0.9</td>
<td>-66.7</td>
</tr>
<tr>
<td>Time to —60 mv (msec)</td>
<td>267.3</td>
<td>311.8</td>
<td>44.5 ± 11.3</td>
<td>+16.6§</td>
</tr>
<tr>
<td>Duration of action potential (msec)</td>
<td>341.5</td>
<td>387.7</td>
<td>46.2 ± 14.0</td>
<td>+13.5§</td>
</tr>
<tr>
<td>Maximum rate of rise of upstroke (v/sec)</td>
<td>835.0</td>
<td>830.0</td>
<td>5.0 ± 14.0</td>
<td>-0.6</td>
</tr>
</tbody>
</table>

*Confidence intervals were computed at the 99% level.
†P < .01.
‡P < .005.
§P < .001.

nified records showed that the slope of the plateau decreased significantly (1/2× Ca, 9.5% decrease; 1/4× Ca, 28.9% decrease; P < .01). In addition, the slopes of the rapid limb of repolarization (phase 3) and the terminal portion (terminal phase of repolarization) of the action potential were significantly decreased. These changes in phases 2 and 3 resulted in 10.8% and 16.6% (P < .001) increases in the time to repolarize to −60 mv in 1/2× Ca and 1/4× Ca, respectively.

During these experiments it was noted that calcium affected the initial reversal of the upstroke (phase 1). A quantitative measure of the rate of repolarization during phase 1 showed that it increased 27.2% and 60.4% (P < .01) in 1/2× Ca and 1/4× Ca, respectively. When the level of calcium was decreased, small but significant decreases occurred in the magnitude of the maximum diastolic potential and the action potential. Other features of the action potential remained unchanged.

EFFECTS OF INCREASED CALCIUM

Twenty-three cells from 10 hearts were subjected to increased concentrations of calcium ions. All data are presented in Tables 3 and 4. In the presence of increased calcium, alterations occurred in the action potentials (Figs. 4 and 5). The most prominent changes were an increase in rate and magnitude of diastolic depolarization and the shortening of the plateau. The duration of the action potential was shortened a small but significant amount (2× Ca, 3.9%; 4× Ca, 6.7%; P < .01). The rate of repolarization during phase 1 was decreased (4× Ca, 28.1%; P < .05). No significant differences were found in the slopes of phase 2, phase 3, or the terminal phase of repolarization. The small decrease in duration of the action potential occurred because of earlier onset of phase 3. Thus, phase 3 and the terminal phase of repolarization occurred earlier, resulting in significant decrease in time to repolarize to −60 mv.

Perhaps the most significant finding was the effect of increased calcium ion concentration on the rate and magnitude of diastolic depolarization. Purkinje fibers perfused with control Tyrode usually had a small amount of diastolic depolarization. When the calcium ion concentration was increased, the magni-
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The amplitude of diastolic depolarization increased significantly (2x Ca, 148%; 4x Ca, 650%; P < .001).

A quantitative measure of the rate of diastolic depolarization was provided by measuring the slope of a line tangent to phase 4 (Fig. 1). When calcium ion concentration was increased, the rate of diastolic depolarization significantly increased (2x Ca, 118.5%; 4x Ca, 344.8%; P < .001).

### TABLE 3
Effect of 2x Ca-Tyrode (5.4 mM Ca++) on the Transmembrane Action Potentials of Eleven Purkinje Fibers

<table>
<thead>
<tr>
<th></th>
<th>Control Tyrode (Mean)</th>
<th>2x Ca-Tyrode (Mean)</th>
<th>Mean difference and confidence interval</th>
<th>Percent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum diastolic potential (mv)</td>
<td>96.1</td>
<td>95.4</td>
<td>0.7 ± 2.1</td>
<td>- 0.7</td>
</tr>
<tr>
<td>Overshoot (mv)</td>
<td>31.3</td>
<td>33.5</td>
<td>2.2 ± 3.4</td>
<td>+ 7.0</td>
</tr>
<tr>
<td>Magnitude of action potential (mv)</td>
<td>124.9</td>
<td>122.8</td>
<td>2.1 ± 3.6</td>
<td>- 1.7</td>
</tr>
<tr>
<td>Slope of phase 1 (mv/sec)</td>
<td>2560.4</td>
<td>1947.2</td>
<td>613.2 ± 1332.9</td>
<td>- 23.9</td>
</tr>
<tr>
<td>Slope of phase 2 (mv/sec)</td>
<td>171.6</td>
<td>175.7</td>
<td>4.1 ± 43.8</td>
<td>+ 2.3</td>
</tr>
<tr>
<td>Slope of phase 3 (mv/sec)</td>
<td>1041.7</td>
<td>991.4</td>
<td>50.3 ± 123.1</td>
<td>- 4.8</td>
</tr>
<tr>
<td>Slope of terminal phase of repolarization (mv/sec)</td>
<td>486.9</td>
<td>459.3</td>
<td>27.6 ± 81.5</td>
<td>- 5.7</td>
</tr>
<tr>
<td>Rate of diastolic depolarization (mv/sec)</td>
<td>9.2</td>
<td>20.1</td>
<td>10.9 ± 2.2</td>
<td>+118.5†</td>
</tr>
<tr>
<td>Magnitude of diastolic depolarization (mv)</td>
<td>2.5</td>
<td>6.2</td>
<td>3.7 ± 2.3</td>
<td>+148.0†</td>
</tr>
<tr>
<td>Time to -60 mv (msec)</td>
<td>251.4</td>
<td>232.6</td>
<td>18.7 ± 17.2</td>
<td>- 7.4‡</td>
</tr>
<tr>
<td>Duration of action potential (msec)</td>
<td>341.8</td>
<td>328.6</td>
<td>13.2 ± 17.3</td>
<td>- 3.9‡</td>
</tr>
<tr>
<td>Maximum rate of rise of upstroke (v/sec)</td>
<td>635.0</td>
<td>612.0</td>
<td>23.0 ± 24.0</td>
<td>- 3.6</td>
</tr>
</tbody>
</table>

*Confidence intervals were computed at the 99% level.  
†P < .001.  
‡P < .05.  
§P < .01.

### TABLE 4
Effect of 4x Ca-Tyrode (10.8 mM Ca++) on the Transmembrane Action Potentials of Twelve Purkinje Fibers

<table>
<thead>
<tr>
<th></th>
<th>Control Tyrode (Mean)</th>
<th>4x Ca-Tyrode (Mean)</th>
<th>Mean difference and confidence intervals</th>
<th>Percent change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum diastolic potential (mv)</td>
<td>93.4</td>
<td>93.5</td>
<td>0.1 ± 2.4</td>
<td>+ 0.1</td>
</tr>
<tr>
<td>Overshoot (mv)</td>
<td>36.2</td>
<td>37.8</td>
<td>1.6 ± 3.2</td>
<td>+ 4.4</td>
</tr>
<tr>
<td>Magnitude of action potential (mv)</td>
<td>128.0</td>
<td>119.3</td>
<td>8.7 ± 6.7</td>
<td>- 6.8†</td>
</tr>
<tr>
<td>Slope of phase 1 (mv/sec)</td>
<td>3334.2</td>
<td>2308.4</td>
<td>935.8 ± 1989.8</td>
<td>- 28.1‡</td>
</tr>
<tr>
<td>Slope of phase 2 (mv/sec)</td>
<td>138.2</td>
<td>159.6</td>
<td>21.4 ± 36.4</td>
<td>+ 15.4</td>
</tr>
<tr>
<td>Slope of phase 3 (mv/sec)</td>
<td>878.5</td>
<td>861.7</td>
<td>16.8 ± 109.2</td>
<td>- 1.9</td>
</tr>
<tr>
<td>Slope of terminal phase of repolarization (mv/sec)</td>
<td>435.9</td>
<td>483.7</td>
<td>47.8 ± 99.1</td>
<td>+ 11.0</td>
</tr>
<tr>
<td>Rate of diastolic depolarization (mv/sec)</td>
<td>10.7</td>
<td>47.6</td>
<td>36.9 ± 4.9</td>
<td>+344.8§</td>
</tr>
<tr>
<td>Magnitude of diastolic depolarization (mv)</td>
<td>1.6</td>
<td>12.0</td>
<td>10.4 ± 3.6</td>
<td>+650.0§</td>
</tr>
<tr>
<td>Time to -60 mv (msec)</td>
<td>259.0</td>
<td>231.0</td>
<td>28.0 ± 17.2</td>
<td>- 10.8§</td>
</tr>
<tr>
<td>Duration of action potential (msec)</td>
<td>340.1</td>
<td>317.2</td>
<td>22.9 ± 20.4</td>
<td>- 6.7§</td>
</tr>
<tr>
<td>Maximum rate of rise of upstroke (v/sec)</td>
<td>693.0</td>
<td>605.0</td>
<td>88.0 ± 20.0</td>
<td>- 12.7§</td>
</tr>
</tbody>
</table>

*Confidence intervals were computed at the 99% level.  
†P < .01.  
‡P < .05.  
§P < .001.

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Figure 6 illustrates the relationship between the rate of diastolic depolarization and calcium ion concentration. With the assumption of negligible variance in each of the five calcium concentrations, a least squares regression line was computed from all the data points. The means and standard deviations of the rate of diastolic depolarization at each concentration have been plotted in this figure. It can be seen that the linear fit is good in that the line approaches the mean values at the upper concentrations. At the lower concentrations used in this study, the fit is not close because the curve flattens in a region close to 0 mv/sec. Hoffman and Suckling (2) found that as the calcium concentration approached zero the rate of diastolic depolarization increased. Thus, if their data were plotted at concentrations between 1/10 and 0 mEq, the curve would rise rapidly in this concentration range. However, it would appear that a linear relationship exists between calcium concentration and diastolic depolarization in the upper range of concentrations used in this study.

Weidmann (1) found no change in the rate of diastolic depolarization in spontaneously beating Purkinje fibers treated with high calcium. It was felt that this finding might, in part, be explained by the decrease in frequency of beat. Figure 7 shows the results of two of five experiments in which the effect of frequency of stimulation on the rate of calcium-enhanced diastolic depolarization was studied in fibers subjected to 4x calcium. Frames A, B, and C show action potentials of one fiber at frequencies of 125, 95, and 75 per min, respectively, which had been treated with 4x Ca-Tyrode for 12 min. Frames D, E, and F are records from another experiment at the same respective frequencies. In all experiments, it was found that a decrease in frequency of stimulation
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Effect of varying extracellular calcium concentration on the rate of diastolic depolarization.

Rate of diastolic depolarization =

\[-2.68 + 4.50 (±0.27) [Ca]\]

The above equation gives the least squares regression line computed from 13, 11, 47, 11, and 12 experiments at 1/4 Ca, 1/2 Ca, control Ca, 2 Ca, and 4 Ca, respectively, where 0.27 represents the standard error of the coefficient and [Ca] is expressed in mm. The standard error of the estimate of the curve is 9.2. The simple correlation coefficient is 0.83. The dots are mean values (See Tables 1-4) and the bars are ±SD (1/4 x = 4.2; 1/2 x = 3.2; 2 x = 7.6; 4 x = 20.4 and normal calcium = 6.5 mv/sec).

resulted in a decrease in rate of diastolic depolarization.

A question that arose during the course of these experiments was: Can calcium-enhanced diastolic depolarization lead to spontaneous activity? In five experiments Purkinje fibers were perfused with 4 Ca-Tyrode for 10 min. At this time, when the rate and magnitude of diastolic depolarization had reached maximal values, the drive stimulus was stopped. Figure 8 presents results from a typical experiment. With cessation of stimulation following the last action potential, a small local depolarization was followed by repolarization to a steady resting level. Even though the membrane potential at this time was lower than control level, the fiber remained quiescent. With resumption of stimulation, the rate of diastolic depolarization was initially small but gradually increased with every action potential until a steady state was reached. Also it should be noted that the maximum diastolic potential attained in the driven preparation is greater than the resting potential during the quiescent period.

The final effect of calcium-enhanced diastolic depolarization was the decreased membrane potential at time of upstroke of the action potential. As the potential at the take-off of upstroke of the action potential decreased, the maximum rate of rise of the upstroke decreased (4 Ca, 12.7%; P < 0.001).

In five experiments, action potentials from a ventricular muscle fiber and a Purkinje fiber were recorded simultaneously during treatment with 4 Ca-Tyrode. The results

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of a typical experiment are illustrated in Figure 9. It can be seen that typical high calcium effects on the ventricular fiber occur at a time when the Purkinje fiber is responding as described above. One difference was noted between the present experiments and those of Hoffman and Suckling (2). In the present experiments the maximum rising velocity of the upstroke of ventricular fibers, as shown in the differentiated record in Figure 9, was decreased. A second observation that may indicate a decreased rising velocity was an increase in the time between application of the drive stimulus and the onset of the

![Figure 8](image1)

**Figure 8**

Effect of stopping the drive stimulus during treatment with 4× Ca-Tyrode. A = action potentials recorded after 11 min in 4× calcium solution. At the point indicated, the drive stimulus was stopped. B is a continuation of A and shows the events which occurred upon resumption of stimulation at the point indicated. Note the progressive increase in rate and magnitude of diastolic depolarization with each succeeding action potential in B.

![Figure 9](image2)

**Figure 9**

Effect of increasing calcium to four times normal on the simultaneously recorded action potentials of Purkinje fiber (upper potential) and a ventricular muscle fiber (lower potential). A and B, in control Tyrode solution. C and D, after 10 min in 4× Ca-Tyrode solution. The differentiated records in A and C are of the ventricular fiber.
ventricular action potential recorded at a distance. Such an increase would be expected because a decrease in rising velocity should decrease conduction velocity. However, it should be noted that conduction velocity is not the only factor that contributes to the time between stimulus and response. To show that the response of Purkinje fibers obtained in the high calcium experiments was due mainly to calcium ions, several different experiments were done. To rule out the effects of chloride ion, in three experiments 4x calcium solution was prepared by adding calcium acetate to the control Tyrode solution. Following 10 min of perfusion with this solution, the contour of the action potential was indistinguishable from the typical response in 4x CaCl₂-Tyrode. Also the response of Purkinje fibers to high calcium solutions prepared with two different brands of analytical reagent grade calcium chloride was not significantly different.

Discussion

This study demonstrates that variation in extracellular concentration of calcium ions causes changes in configuration of the action potential of Purkinje fibers. The predominant changes were in repolarization and diastolic depolarization. Repolarization occurred earlier in high calcium solutions and later in low calcium solutions, mainly because of changes in duration of the plateau phase of the action potential. The rate and magnitude of diastolic depolarization increased consistently when calcium concentration was raised to two or four times normal. Some of these changes have been shown to occur in other cardiac fiber types subjected to altered calcium concentrations. As early as 1913, Mines (7) showed that the ventricular complex of the electrogram lengthens during perfusion with a solution containing a lowered calcium concentration. Recently Surawicz and associates (8), using isolated perfused rabbit hearts, showed that calcium-deficient solutions lengthen the S-T segment and the Q-T interval of the electrocardiogram and that there is a concomitant prolongation of the plateau of the monophasic action potential. Also, repolarization of atrial and ventricular fibers of the dog heart is accelerated in high and delayed in low calcium solution (2). Similar alterations in the contour of the action potential have been observed in ventricular fibers of the frog heart (9,10). Seifen and co-workers (3) have shown that elevation of calcium concentration causes an increase in rate of diastolic depolarization of sino-atrial nodal fibers of the rabbit heart.

The results of our study differ from those of Weidmann (1), who noted no appreciable change in contour of the action potential of Purkinje fibers when the calcium concentration was varied over the range we used. A likely explanation is that in our study the frequency of beat was the same throughout control and test perfusions, whereas in Weidmann's experiments the fibers were allowed to beat spontaneously, with the result that frequency of beat increased in low, and decreased in high, calcium solutions. It is well known that changes in heart rate alter the contour of the cardiac action potential (4). The effects of heart rate changes are predominantly on duration of the plateau phase, which shortens as heart rate increases and lengthens with decrease in heart rate. Therefore, as noted by Shanes (11), it is necessary to keep frequency constant if contour of the action potential is an object of study. Use of such a technique, as in the present study, allows detection of even minor changes in configuration of the action potential. It also may be noted that the slowing of spontaneous rate that occurs in high calcium solution (1) would tend to mask the demonstrated effect of high calcium on the duration of the plateau. Similarly, the increased rate during treatment with low calcium would tend to mask the increase in duration of the plateau caused by this procedure.

It was shown here that the rate of the calcium-enhanced diastolic depolarization is dependent on the frequency of beat. Thus, as the rate of beat was decreased, the rate of diastolic depolarization decreased, whereas, within limits, as stimulus frequency was in-
creased, the rate of diastolic depolarization also increased. Therefore it is possible that slowing of the rate of spontaneously beating fibers in high calcium solution could prevent the appearance of detectable increases in rate of diastolic depolarization during treatment with high calcium.

We found that the maximum rate of rise of the upstroke of Purkinje fibers decreased during perfusion with high calcium. Weidmann (1) has not noted such an effect. The development of diastolic depolarization could result in such an effect because of the lowered membrane potential at the upstroke (partial inactivation of sodium carriers) (12). However, it is known that calcium ions reduce the degree of carrier inactivation at lower levels of membrane potentials (1), and this should tend to negate the effect on rising velocity. That this did not occur is evident, and there is no satisfactory explanation for the differences in experimental findings.

Production of hypercalcemia in intact animals (13-17) or elevation of calcium in the fluid perfusing isolated heart preparations (18) frequently causes cardiac arrhythmias, notably ventricular extrasystoles, tachycardia and fibrillation. In the intact animal, such arrhythmias may be caused in part by effects mediated by cardiac nerves (16). However, there is evidence from experiments with isolated tissue that a direct action of calcium on ventricular tissues is involved, and it has been suggested, specifically, that an action on ventricular conduction tissues is important (18). Hypotheses advanced to explain occurrence of calcium-induced ventricular arrhythmias (19) have relied largely on the demonstrated effect of calcium ions on the threshold potential (1, 20). Presumably a decrease in level of the threshold potential would favor occurrence of local blocks of conduction with consequent reentry of excitation. In the present study, increased calcium concentration caused several changes in the transmembrane potential of Purkinje fibers, which, consistent with current theories on the origin of cardiac arrhythmias (21), could contribute to production of ventricular arrhythmias during periods of hypercalcemia.

For purposes of discussion it is convenient to divide the mechanisms capable of originating cardiac arrhythmias into two general categories: (1) those that act by causing changes in automaticity and (2) those that act by predisposing to the development of circus movement and reentry of excitation. The increase in rate and magnitude of diastolic depolarization is then doubly important because it may cause changes in both automaticity and in impulse conduction.

1. Changes in automaticity. It is well established that the presence of slow diastolic depolarization during the period of electrical diastole confers upon a cardiac fiber the ability to become a pacemaker (22). Whether such a fiber actually is or becomes a pacemaker depends not only on the rate of such diastolic depolarization but on other factors such as the levels of the maximum diastolic potential and the threshold potential (22). That high levels of calcium in the extracellular fluid increase the rate of diastolic depolarization has been established in this study. However, in most experiments in which the calcium concentration was increased, ectopic pacemakers did not appear, nor did the fibers beat in the absence of extrinsic stimuli. There are at least two possible explanations for this failure of spontaneous activity in the presence of increased diastolic depolarization. First, the magnitude of depolarization attained in high calcium solution might not be sufficient to reach even the normal level of threshold potential. Second, the level of the threshold potential declines in a high calcium solution which would require an even larger magnitude of diastolic depolarization for discharge of an action potential. Presumably in our experiments one or both of these possibilities prevented spontaneous beating. If such conditions occur uniformly in all parts of the ventricle subjected to rising calcium concentrations, development of ectopic foci would not be expected.

2. Conduction disturbances favorable for reentry of excitation. Singer and co-workers (23) have shown that development of dia-
EFFECT OF CALCIUM ON PURKINJE FIBERS

Dystolic depolarization in preparations of isolated Purkinje fibers is accompanied by decremental conduction, local blocks of conduction and changes in sequence of fiber activation. Recently Hoffman and Cranefield (21) have presented a detailed discussion of the mechanisms by which diastolic depolarization can give rise to cardiac arrhythmias. Of main importance is the fact that the diastolic depolarization carries the membrane potential to a lower level at the time of the upstroke of the action potential, which in turn reduces the maximum rate of rise of the upstroke and overshoot (12). Each of these changes in the action potential can slow the velocity of impulse propagation (24). In the present study, perfusion with high calcium solution caused a reduction in maximum rate of rise of the upstroke. This event, coupled with the decrease in level of the threshold potential noted above, which also slows conduction velocity (25), makes decremental conduction and occurrence of local block a distinct possibility in Purkinje fibers subjected to hypercalcemia.

It was demonstrated here that elevation of calcium concentration hastened repolarization, with the result that the time required to repolarize to −60 mv decreased. This measurement was made because this is the approximate minimal level of transmembrane potential necessary to elicit conducted action potentials in Purkinje fibers by cathodal stimuli (12) or by propagating action potentials (26). Therefore, the time taken to repolarize to −60 mv is a measure of the duration of the functional refractory period of Purkinje fibers. On this basis, one would predict a shortening in duration of the functional refractory period of Purkinje fibers subjected to hypercalcemia. Such an effect, especially when coupled with a decrease in speed of conduction, favors the development and maintenance of reentrant excitation (19).

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