Effects of Calcium on Canine Purkinje Fiber Action Potential Duration in the Presence of Agents Affecting Potassium Permeability

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SUMMARY We used intracellular microelectrodes to study the effect of changes in extracellular calcium ion concentration \([\text{Ca}^{2+}]_o\), on the transmembrane potentials of canine cardiac Purkinje fibers in control Tyrode’s solution and in the presence of agents thought to modify membrane permeability to potassium. In Tyrode’s solution, decreasing \([\text{Ca}^{2+}]_o\), from 2.7 to 0.9 mM increased action potential duration measured at −60 mV (APD_{−60}) and at full repolarization (APD_{100}) but did not significantly modify the normal linear relationship between cycle length and APD between cycle lengths of 500 to 4000 msec. We used 9-aminoacridine (9-AA) to decrease potassium permeability. At concentrations between 0.01 and 1.5 × 10^{-5} M, 9-AA caused a concentration-dependent increase in APD_{−60} and APD_{100} and a significant increase in the slope of the line relating APD to cycle length. The usual effects of changes in \([\text{Ca}^{2+}]_o\), on APD were potentiated by 9-AA. We then used lidocaine (L) to increase potassium permeability. At concentrations between 0.75 and 1.5 × 10^{-5} M, L significantly increased the slope of the line relating APD and cycle length. In the presence of L an increase in \([\text{Ca}^{2+}]_o\), increased both APD_{−60} and APD_{100}, and a decrease in \([\text{Ca}^{2+}]_o\), decreased both parameters. 9-AA thus potentiated and L reversed the effects of changing \([\text{Ca}^{2+}]_o\), on APD. The results suggest that changes in \([\text{Ca}^{2+}]_o\), modify repolarization by modifying both slow inward current and potassium permeability and that the extent to which the latter changes can determine the effect of \([\text{Ca}^{2+}]_o\), on APD.

to potassium. Such a relationship has been demonstrated for other types of cells (Meech, 1972; Clusin et al., 1974). With respect to the heart, it has been shown that intracellular injection of Ca$^{2+}$ hyperpolarizes and shortens the action potential of Purkinje fibers (Isenberg, 1975) and that increases in [Ca$^{2+}$], markedly increase potassium conductance of ventricular muscle fibers (Bassingthwaite et al., 1976). Recently, the effects of changes in [Ca$^{2+}$], on potassium conductance have been modeled for Purkinje (Kass and Tsien, 1976) and ventricular muscle fibers (Beeler and Reuter, 1977).

Because most of the data explaining the effects of changes in [Ca$^{2+}$], on action potential duration have been obtained by use of voltage-clamp techniques, we thought it might be of use to examine the question by a different method. We attempted to demonstrate both the direct effect of changing [Ca$^{2+}$], (i.e., that resulting from a change in i$_{si}$) and the indirect effect (i.e., that resulting from a change in potassium conductance) by using pharmacological agents to modify potassium conductance of canine Purkinje fibers. The two agents we used to study the problem were lidocaine and 9-aminoacridine (9-AA). Lidocaine is believed to exert a number of its effects on the transmembrane potentials of Purkinje fibers by increasing potassium conductance (Bigger and Mandel, 1970; Weld and Bigger, 1976); 9-aminoacridine, a member of a large structural family of fluorescent dyes, decreases potassium conductance of frog skeletal muscle (Volle, 1971). Our studies show that, after exposure to lidocaine, a higher [Ca$^{2+}$], increases, and a lower [Ca$^{2+}$], decreases, action potential duration. In contrast, after exposure to 9-AA, the usual effects of changes in [Ca$^{2+}$], on action potential duration are potentiated.

**Methods**

Mongrel dogs weighing 15–30 kg were anesthetized with sodium pentobarbital, 30 mg/kg, iv. The heart was removed rapidly through a right lateral thoracotomy and placed in cool oxygenated Tyrode’s solution (Isenberg, 1975) which the preparation was superfused for 45 minutes with Tyrode’s solution containing 9-AA first at 0.1 X 10$^{-5}$ M and then at 1.0 X 10$^{-5}$ M. The control tracings are shown on the left. Changing from a CL of 1000 to 4000 msec and were isolated from ground. Stimuli were approximately 1.5 times threshold and 1–1.5 msec in duration; for any given experiment, stimulus strength and duration were not varied once control action potentials had been recorded. For each experiment, the tissue was stimulated at several different cycle lengths (CL). Transmembrane potentials were recorded through microelectrodes filled by boiling under reduced pressure in 3 m KCl. Electrode resistance was between 10 and 30 MΩ. The electrodes were coupled by a 3 m KCl interface to a Ag-AgCl bar connected to an operational amplifier with high input impedance and input-capacity neutralization. The maximum rate of rise of phase 0 of the action potential ($V_{max}$) was measured by recording the first time derivative through an operational amplifier calibrated against a linear sawtooth pulse (Bigger et al., 1968).

9-Aminoacridine (9-AA) (K & K Laboratories, Inc.) (mol wt = 194) was prepared in a concentrated stock solution (10 mg/ml in 40% ethanol), and aliquots were diluted and added to the Tyrode’s solution to give the desired concentrations. Volumes of ethanol equivalent to those added during drug superfusion were shown to produce no discernible effects on transmembrane potentials. For experiments with lidocaine (L), we used a commercially available preparation of the hydrochloride salt (Astra Pharmaceutical Products Inc.) (mol wt = 270), 40 mg/ml. For drug superfusion, aliquots of the appropriate solution were added to Tyrode’s solution prepared in a separate gassed reservoir.

Records of the transmembrane potential and $V_{max}$ were made with a Tektronix RM565 oscilloscope and a Polaroid camera. Transmembrane potentials were recorded both at low and high amplification to demonstrate changes in the entire action potential and in the slope of phase 4 depolarization.

All results were recorded from single impalements maintained throughout the experiment. In several cases, two impalements of different cells at least 1 cm apart were maintained in the same preparation. Thus, for different experimental sequences, both the number of experiments and the total number of continuously monitored cells are reported. Data were analyzed using Student’s paired $t$-test. Regression lines were calculated using the method of least squares, and the regression coefficients were compared by analysis of covariance.

**Results**

**Effects of 9-AA**

Figure 1 shows the results of an experiment in which the preparation was stimulated at two different cycle lengths, 1000 and 4000 msec, and superfused for 45 minutes with Tyrode’s solution containing 9-AA first at $0.1 \times 10^{-5}$ M and then at $1.0 \times 10^{-5}$ M. The control tracings are shown on the left. Changing from a CL of 1000 to 4000 msec...
FIGURE 1  Transmembrane potentials at cycle lengths of 1000 (A) and 4000 (B, C) msec under control conditions and during superfusion with low (0.1 x 10^-5 M) and high (1.0 x 10^-5 M) concentrations of 9-AA. In A and B, each division represents 20 mV and 100 msec. C is recorded at 1/10 sweep speed and double gain to show phase 4 depolarization. For the tracing of V_max the calibration in this and subsequent figures is 200 V/sec. In this figure and others showing records of transmembrane potential and its derivative, rapid transients have been retouched for clarity.

caused no change in maximum diastolic potential (MDP), action amplitude, or V_max. Action potential duration, measured both as the time necessary for repolarization to -60 mV (APD-60) or for 100% repolarization (APD100), increased on changing to the longer cycle length. The middle panel shows the transmembrane potentials of the same cell after superfusion for 45 minutes with Tyrode’s solution containing 0.1 x 10^-5 M 9-AA. At a CL of 1000 there is a slight prolongation of APD-60 and APD100 that reflects a decrease in slope of phase 4. At a CL of 4000 the increases in APD-60 and APD100 are more pronounced. The contour of phase 4 depolarization is not altered appreciably. The panel on the right shows the responses of the same cell after superfusion for 45 minutes with Tyrode’s solution containing 1.0 x 10^-5 M 9-AA. At both CL there is a further prolongation of APD. In this preparation there is also a slight decrease in the slope of phase 4 depolarization due primarily to a 3 mV shift of the activation voltage (AV) toward more negative potentials.

Effects of 9-AA (0.01 to 1.5 x 10^-5 M) were studied in 31 experiments by monitoring a total of 60 cells at CL of 500, 1000, 2000, and 4000 msec. Steady state effects were reached after 45 minutes of superfusion with drug-containing Tyrode’s solution. Effects of 9-AA were partially reversible by long periods of washout (>1½ hour) with drug-free Tyrode’s solution. Results of these experiments are shown in Figure 2. At all but the shortest cycle lengths, APD-60 and APD100 show statistically significant and dose-dependent increases at concentrations of 9-AA that did not change the other measured parameters. At higher concentrations of 9-AA, there is a small but statistically significant decrease in overshoot, V_max, and AP amplitude as well as a small decrease in MDP at CL = 4000 msec for 9-AA = 1.5 x 10^-5 M. Except at the highest concentration examined, there was no statistically significant change in the slope of phase 4 depolarization; at 9-AA = 1.5 x 10^-5 M the slope decreased from 1.38 ± 0.30 mV/sec to 0.64 ± 0.35 mV/sec (P < 0.05). Superfusion with higher concentrations of 9-AA (5.0 x 10^-4 M to 1.0 x 10^-3 M) caused a significant decrease in MDP (10-30 mV) and eventually lead to inexcitability.

Effects of 9-AA on the Rate Dependence of APD

In normal Tyrode’s solution, APD100 increases by approximately 32% as CL increases from 500 to 4000 msec and APD-60 increases by 38%. Figure 3 shows plots of cycle length vs. AP duration for APD-60 (panel A) and APD100 (panel B) under control conditions and during superfusion with several concentrations of 9-AA. Least square best fits are plotted for 9-AA = 0.1, 1.0, and 1.5 x 10^-5 M, with indicated regression coefficients and the statistical significance of the difference in slope between control conditions and during superfusion with 9-AA. This analysis suggests that higher concentrations of 9-AA significantly modify the rate dependence of action potential duration.

Effects of Decreased [Ca^2+], on APD

Figure 4 shows the results of an experiment in which a preparation stimulated at a CL of 1000 and 4000 msec was superfused with Tyrode’s solution containing one-third the control concentration of calcium. The panel on the left shows the control tracings. Superfusion for 20 minutes with Tyrode’s solution containing 0.9 mM Ca^2+ (right panel) leads to an increase in APD-60 and APD100 at both cycle lengths. Effects of a decrease in [Ca^2+]o on APD are summarized in Table 1A. The increases in APD-60 and APD100 when [Ca^2+]o is decreased appear to result from a decrease in the slope of phase 2, rather than from a change in the slope of phase 3, as was the case during superfusion with 9-AA (Fig. 2). A slight decrease in activation voltage also is seen at both CL.

Figure 5 shows plots of cycle length vs. AP duration (APD-60 and APD100) for PF superfused with control Tyrode’s solution in which [Ca^2+]o is 2.7 mM, and then with Tyrode’s in which [Ca^2+]o is 1.35 or 0.9 mM. Least square best fits are plotted with the regression coefficients. There was no statistically significant difference among the slopes of the derived regressions. This analysis suggests that a decrease in [Ca^2+]o does not significantly modify
Figure 2 Effects of 9-AA on Purkinje fiber transmembrane potentials. Concentration of 9-AA × 10^-5 M shown on abscissas; c = control Tyrode’s solution. Cycle length = 500 msec (A), 1000 msec (B), 2000 msec (C), and 4000 msec (D). MDP = maximum diastolic potential; AV = activation voltage (i.e., the value of transmembrane potential at the onset of phase 0); amplitude = action potential amplitude; $V_{\text{max}}$ = maximum slope of phase 0, $\text{APD}_{\text{rep}}$ = time to full repolarization; $\text{APD}_{\text{rep}} = 60$ mV = time to repolarize to $-60$ mV; n = number of continuous impalements for each concentration and for control observations. For each variable and cycle length, the concentration of 9-AA causing a significant change from the control value is shown. Horizontal bars show ± one standard error. * = P < 0.05; ** = P < 0.01; *** = P < 0.001.
the dependence of action potential duration on cycle length.

Figure 6 illustrates an experiment in which [Ca\(^{2+}\)]\(_o\) was decreased to 0.9 mM in the presence of 9-AA (1 \(\times\) 10\(^{-5}\) M). The control tracings at CL = 1000 and 4000 msec are shown on the left and are action potentials from the same cell as in Figure 4, after return to Tyrode's solution with [Ca\(^{2+}\)]\(_o\) = 2.7 mM. The middle panel shows the transmembrane potentials after superfusion with Tyrode's containing 9-AA for 45 minutes. In addition to the increases in APD\(_{90}\) and APD\(_{100}\), there is a slight loss of MDP, a small decrease in \(V_{\text{max}}\), and a disappearance of the notch marking the transition from phase 1 to phase 2. Superfusion with Tyrode's solution containing the same concentration of 9-AA but with [Ca\(^{2+}\)]\(_o\) reduced to 0.9 mM leads to greater increases in APD\(_{90}\) and APD\(_{100}\), increases nearly twice those

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**Figure 3** Relationship between APD\(_{90}\) (A) and APD\(_{100}\) (B) and CL or frequency of stimulation under control conditions and during superfusion with five concentrations of 9-AA. Regression lines calculated for control conditions and 9-AA = 1.5, 1.0, and 0.1 \(\times\) 10\(^{-5}\) M.

**Figure 4** Effects of decreasing [Ca\(^{2+}\)]\(_o\) from 2.7 to 0.9 mM on Purkinje fibers stimulated at cycle lengths of 1000 (A) and 4000 (B) msec. In A, APD\(_{100}\) increases by 15% and in B by 13% in the low calcium superfusate. Calibration grid represents 20 mV, 200 V/sec, and 100 msec.

**Figure 5** Effect of a decrease in [Ca\(^{2+}\)]\(_o\) on the relationship between APD\(_{90}\) (A) and APD\(_{100}\) (B) and CL or frequency of stimulation. [Ca\(^{2+}\)]\(_o\) = 2.7 mM is the control condition.
Effects of Decreasing [Ca\textsuperscript{2+}]\textsubscript{o} on APD

For these experiments the concentration of 9-AA was 1.5 \times 10^{-5} \text{ M}, one which maximally increased APD. The results of 11 experiments at CL of 500, 1000, and 2000 msec are presented in Table 2. The decrease in APD caused by increased [Ca\textsuperscript{2+}]\textsubscript{o} is statistically significant for both values of increased [Ca\textsuperscript{2+}]\textsubscript{o} at all three CL. In a study of canine PF in normal Tyrode's solution and stimulated at a CL of 630 msec, Temte and Davis (1967) noted decreases in APD\textsubscript{50} and APD\textsubscript{100} of -7.4% and -3.9% on changing [Ca\textsuperscript{2+}]\textsubscript{o} from 2.7 to 5.4 mM and decreases of -10.8% and -6.7% when [Ca\textsuperscript{2+}]\textsubscript{o} was increased from 2.7 to 10.8 mM. On comparing these data with ours (Table 2), it appears that the degree to which an increased [Ca\textsuperscript{2+}]\textsubscript{o} shortens APD\textsubscript{50} and APD\textsubscript{100} is augmented in the presence of 9-AA.

Effects of Lidocaine

Figure 7 shows the result of an experiment in which a preparation, stimulated at CL of 1000 and 4000 msec, was superfused with Tyrode's solution containing lidocaine (2 \mu g/ml; 0.75 \times 10^{-5} \text{ M}). The control tracings are shown on the left. Superfusion with Tyrode's solution containing lidocaine leads to a decrease in APD\textsubscript{50} and APD\textsubscript{100}, a slight decrease in V\textsubscript{max} at CL = 1000, and a decrease in the slope of phase 4 depolarization, without significant change in MDP. This results in a shift in the activation voltage toward a more negative value at both CL. Superfusion with a higher concentration of lidocaine (4 \mu g/ml; 1.5 \times 10^{-5} \text{ M}) leads to a slightly greater shift in activation voltage and a more pronounced decrease in APD\textsubscript{50}. Data on the effects of

### Table 1

<table>
<thead>
<tr>
<th>[Ca\textsuperscript{2+}]\textsubscript{o} (mM)</th>
<th>Cycle length = 1000 msec</th>
<th>Cycle length = 2000 msec</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7</td>
<td>1.35</td>
<td>0.9</td>
</tr>
<tr>
<td>A. Control</td>
<td>(18)</td>
<td>(14)</td>
</tr>
<tr>
<td>APD\textsubscript{50}</td>
<td>448.0\pm8.6</td>
<td>+9.1\pm1.1</td>
</tr>
<tr>
<td>APD\textsubscript{100}</td>
<td>306.9\pm2.1</td>
<td>+14.5\pm3.3</td>
</tr>
<tr>
<td>B. 9-AA, 1 \times 10^{-5} M</td>
<td>(10)</td>
<td>(10)</td>
</tr>
<tr>
<td>APD\textsubscript{50}</td>
<td>556.7\pm12.0</td>
<td>+16.2\pm2.6</td>
</tr>
<tr>
<td>APD\textsubscript{100}</td>
<td>340.7\pm6.8</td>
<td>+28.8\pm2.0</td>
</tr>
</tbody>
</table>

Values in control [Ca\textsuperscript{2+}]\textsubscript{o} (2.7 mM), expressed in msec as mean \pm SE; values for [Ca\textsuperscript{2+}]\textsubscript{o} = 1.35 and 0.9 mM as percent change from control \pm SE. Numbers in parentheses show n for each determination.

\( \dagger P < 0.01; \ddagger P < 0.001 \) comparing reduced [Ca\textsuperscript{2+}]\textsubscript{o} to control. \( \S P < 0.05; \| P < 0.01 \) comparing 9-AA to control.
supersition with Tyrode's solution containing lidocaine, 4 \mu g/mL (1.5 \times 10^{-5} \text{ M}), for CL of 500, 1000, 2000, and 4000 msec are shown in Table 3 and summarize the results of 10 experiments. Figure 8 shows plots of cycle length vs. APD_{90} and APD_{100} for PF superfused with control Tyrode's solution and then with Tyrode's solution containing lidocaine, 1.5 \times 10^{-5} \text{ M}. Least square best fits are plotted and regression coefficients indicated. There are statistically significant differences between the slopes in drug-free and lidocaine-containing superfusate. This suggests that lidocaine significantly modifies the rate dependence of the APD.

### Table 2 Effects of Increasing $[\text{Ca}^{2+}]_o$ during Exposure to 9-AA (1.5 \times 10^{-5} \text{ M})

<table>
<thead>
<tr>
<th>$[\text{Ca}^{2+}]_o$ (mM)</th>
<th>CL = 500 msec</th>
<th>CL = 1000 msec</th>
<th>CL = 2000 msec</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
</tr>
<tr>
<td>5.4</td>
<td>5.4</td>
<td>5.4</td>
<td>5.4</td>
</tr>
<tr>
<td>10.8</td>
<td>10.8</td>
<td>10.8</td>
<td>10.8</td>
</tr>
</tbody>
</table>
| Values in control $[\text{Ca}^{2+}]_o$ (2.7 mM) expressed in msec ± SE. Results of 11 experiments expressed as mean ± SE. Numbers in parentheses represent the number of continuously monitored impalements. Changes in APD_{90} and APD_{100} are expressed as mean percent change ± SE.

#### Effects of Decreased $[\text{Ca}^{2+}]_o$ on APD

Figure 9 shows the results of an experiment in which $[\text{Ca}^{2+}]_o$ was decreased in the presence of lidocaine. Rather than causing an increase in APD, as had been observed in drug-free Tyrode's solution (Table 1A), decreasing $[\text{Ca}^{2+}]_o$ to 1.35 mM in the presence of lidocaine causes a small decrease in both APD_{90} and APD_{100}. A further decrease in $[\text{Ca}^{2+}]_o$ to 0.9 mM causes a further decrease in APD. We observed a similar decrease in APD, on decreasing $[\text{Ca}^{2+}]_o$, in three experiments monitoring six cells with lidocaine = 0.75 \times 10^{-5} \text{ M}, and in three experiments monitoring six cells with lidocaine = 1.50 \times 10^{-5} \text{ M}. Table 4 presents data from the experiments with lidocaine = 1.5 \times 10^{-5} \text{ M} at CL = 1000 and 4000 msec. APD_{90} decreases significantly at both cycle lengths and both decreased concentrations of $[\text{Ca}^{2+}]_o$; decreases in APD_{100} are statistically significant at $[\text{Ca}^{2+}]_o = 0.9 \text{ mM}$.

#### Effects of Increased $[\text{Ca}^{2+}]_o$ on APD

The results of an experiment in which $[\text{Ca}^{2+}]_o$ was increased during exposure to lidocaine are shown in Figure 10. Superfusion for 25 minutes with Tyrode's solution containing lidocaine, 4 \mu g/mL (1.5 \times 10^{-5} \text{ M}), leads to a shift in both MDP and activation voltage toward more negative values, a decrease in the slope of phase 4 depolarization, and a decrease in APD_{90} and APD_{100}. Increasing $[\text{Ca}^{2+}]_o$ during exposure to lidocaine leads to a prolongation of phase 1 and a lower take-off potential of the plateau, an increase in the slope of phase 4 depolarization, and an increase in both APD_{90} and APD_{100}. We observed the same increase in APD on increasing $[\text{Ca}^{2+}]_o$ in two experiments monitoring four cells with lidocaine = 2 \mu g/mL (0.5 \times 10^{-5} \text{ M}) and in six experiments monitoring 12 cells with lidocaine = 4 \mu g/mL (1.5 \times 10^{-5} \text{ M}). Effects on APD of increasing $[\text{Ca}^{2+}]_o$ during exposure to lidocaine (4 \mu g/mL) for CL of 1000 and 4000 msec are presented in Table 4.

### Reversal by Lidocaine of the Effects of 9-AA

In three experiments monitoring six cells, we studied the ability of lidocaine to reverse the effects of 9-AA. After superfusion with Tyrode's solution containing 1.5 \times 10^{-5} \text{ M} 9-AA, the tissue was superfused with Tyrode's solution containing the same...
## Table 3
**Effect of Lidocaine (1.5 × 10^{-5} M) on Transmembrane Potential**

<table>
<thead>
<tr>
<th></th>
<th>CL = 500 msec</th>
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<th>CL = 2000 msec</th>
<th>CL = 4000 msec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Lidocaine</td>
<td>Control</td>
<td>Lidocaine</td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>-95.3 ± 1.0</td>
<td>-97.5 ± 1.1</td>
<td>-94.9 ± 0.8</td>
<td>-96.8 ± 1.2</td>
</tr>
<tr>
<td>AV (mV)</td>
<td>-95.3 ± 1.0</td>
<td>-97.5 ± 1.1</td>
<td>-94.1 ± 1.0</td>
<td>-95.7 ± 1.3</td>
</tr>
<tr>
<td>Amplitude (mV)</td>
<td>136.1 ± 1.3</td>
<td>137.1 ± 1.8</td>
<td>136.5 ± 1.1</td>
<td>137.6 ± 1.9</td>
</tr>
<tr>
<td>Overshoot (mV)</td>
<td>40.7 ± 1.3</td>
<td>40.6 ± 1.5</td>
<td>40.8 ± 0.9</td>
<td>40.2 ± 1.6</td>
</tr>
<tr>
<td>V_{max} (V/sec^{-1})</td>
<td>541 ± 44</td>
<td>500 ± 31</td>
<td>531 ± 34</td>
<td>525 ± 35</td>
</tr>
<tr>
<td>APD_{endo} (msec)</td>
<td>419.5 ± 6.3</td>
<td>406.8 ± 7.1</td>
<td>500.3 ± 10.5</td>
<td>453.6 ± 11.2</td>
</tr>
<tr>
<td>APD_{endo} (msec)</td>
<td>276.0 ± 6.7</td>
<td>207.0 ± 7.6</td>
<td>321.7 ± 9.6</td>
<td>420.4 ± 6.3</td>
</tr>
</tbody>
</table>

CL = cycle length. Results of 10 experiments expressed as mean ± SE. Numbers in parentheses represent the number of continuously monitored impalements.

* P < 0.05; † P < 0.01; ‡ P < 0.001.

Concentration of 9-AA and, in addition, lidocaine (2 μg/ml; 0.75 × 10^{-5} M). Results of these experiments, for CL = 1000 and 4000 msec, are presented in Table 5. The addition of lidocaine caused no significant change in MDP, AV, amplitude, overshoot, V_{max}, or slope of phase 4 depolarization. There was, however, significant shortening of both APD_{endo} and APD_{endo}.

### Discussion

9-AA is a planar molecule with dimensions comparable to those of the hydrated potassium ion (4.5 × 8.5 Å); it has a pK_a of 9.99 and therefore exists predominantly in the ionized form at pH = 7.4. Studies of the effects of 9-AA (1.0 – 100.0 × 10^{-5} M) on frog sartorius muscle (Voile, 1971; Henderson and Voile, 1972) suggest that it causes an asymmetrical blockade of K^+ exchange, depressing efflux significantly more than influx. It has no effect on resting Na^+ exchange, extrusion, or content. In chloride-containing solutions with [K^+]_o ≤ 2.5 mM, 9-AA does not alter the resting membrane potential of skeletal muscle fibers; 9-AA causes changes in K^+ flux comparable to those produced by 5 times greater concentrations of tetraethylammonium (Voile, 1971; Henderson and Volle, 1972). Because of our interest in the modulation of APD by Ca^{2+}

![Figure 8](http://circres.ahajournals.org/)

**Figure 8** Relationship between APD_{endo} (A) and APD_{endo} (B) and CL and frequency of stimulation under control conditions and during superfusion with lidocaine (4 μg/ml; 1.5 × 10^{-5} M).
and the possible effects of Ca\(^{2+}\) on potassium conductance, we chose to characterize the effects of 9-AA on PF action potential parameters and to observe the effects of varying [Ca\(^{2+}\)]\(_o\) on fibers pretreated with it. Since lidocaine appears to have effects on membrane potassium conductance qualitatively opposite from those of 9-AA (Weld and Bigger, 1976; Volle, 1971), we compared responses to changes in [Ca\(^{2+}\)]\(_o\) in the presence of each.

Our finding that prolongation of the action potential by decreasing [Ca\(^{2+}\)]\(_o\) does not alter the rate dependence of APD is in agreement with recently reported observations (Mary-Rabine and Rosen, 1978); these, along with evidence from voltage clamp studies, (Kass and Tsien, 1976) have been interpreted as suggesting that calcium-dependent changes in APD are secondary to changes in time-invariant background potassium permeability. The effects of 9-AA on transmembrane potentials of Purkinje fibers also may reflect an effect on membrane conductance to K\(^+\). However, the dose-dependent increase in APD\(\_\infty\) and APD\(\_\infty\) with minimal effects on MDP, AV, or slope of phase 4 at all but the highest concentrations studied, and the significant modification in the rate dependence of APD, suggest that 9-AA may effect a time-varying dynamic current rather than a background current. If 9-AA is effecting the magnitude and kinetics of some membrane K\(^+\) conductance in Purkinje fibers, as it has been shown to do in frog sartorius muscle, the potentiated effects of increasing or decreasing [Ca\(^{2+}\)]\(_o\) on APD in fibers pretreated with 9-AA suggest that the control value of K\(^+\) conductance modulates the magnitude of the response to changes in [Ca\(^{2+}\)]\(_o\). Although we can not rule out effects of 9-AA on the kinetics of i\(_h\), it should be noted that superfusion with 9-AA neither modified plateau voltage, as might be expected if it blocked the secondary inward channel, nor markedly influences contractility.

Our findings for the effects of lidocaine on the transmembrane potential are similar to those which have been described previously (Bigger and Mandel, 1970; Weld and Bigger, 1976; Mary-Rabine and Rosen, 1978). Action potential shortening secondary to an increase in [Ca\(^{2+}\)]\(_o\) appears to cause no change in the rate dependence of APD. To the extent that this reflects effects of Ca\(^{2+}\) on background, as opposed to time-varying dynamic currents, our finding of a significant change in rate-dependence of APD after superfusion with lidocaine (1.5 x 10\(^{-5}\) M) suggests that, unlike 9-AA, lidocaine

### Table 4 Effects of [Ca\(^{2+}\)]\(_o\) on APD in the Presence of Lidocaine (1.5 x 10\(^{-5}\) M)

<table>
<thead>
<tr>
<th>[Ca(^{2+})](_o) (mM)</th>
<th>CL = 1000 msec</th>
<th>CL = 4000 msec</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>APD(_\infty)</td>
<td>APD(_\infty)</td>
</tr>
<tr>
<td>2.7</td>
<td>±11.2</td>
<td>±12.6</td>
</tr>
<tr>
<td>(12)</td>
<td>±13.7</td>
<td>±7.4</td>
</tr>
<tr>
<td>1.35</td>
<td>496.7 ±13.7</td>
<td>507.9 ±7.4</td>
</tr>
<tr>
<td>(6)</td>
<td>240.4 ±6.3</td>
<td>222.9 ±9.7</td>
</tr>
<tr>
<td>0.9</td>
<td>430.0 ±12.6</td>
<td>410.0 ±11.3</td>
</tr>
<tr>
<td>(6)</td>
<td>(12)</td>
<td>(12)</td>
</tr>
</tbody>
</table>

The control value of [Ca\(^{2+}\)]\(_o\) = 2.7 mM. Values expressed as mean ± SE. Numbers in parentheses shown for each determination.

\* P < 0.05; \(\dagger\) P < 0.01; \(\ddagger\) P < 0.001.
may modify APD through effects on the kinetics and/or magnitude of a time-varying dynamic conductance. The finding that exposure to lidocaine reverses the usual effects of either increasing or decreasing [Ca\(^{2+}\)]\(_o\) on APD suggests that, in a setting in which potassium conductance is increased, effects of calcium on action potential configuration are related more directly to its role as a carrier of inward transmembrane current. A recently published illustration presented in the context of studies of effects of Mn\(^{2+}\) on Purkinje fibers (Hogan and Spitzer, 1975) supports this hypothesis. Elevation of [Ca\(^{2+}\)]\(_o\) from 1.8 to 7.2 mM in Tyrode’s solution containing 21.6 mM K\(^+\) leads to marked prolongation of both APD\(_{e0}\) and APD\(_{io0}\) of Purkinje fibers.

Our observation that the effects of 9-AA on APD are reversed largely by addition of lidocaine to the superfusate further suggests that the two agents may be exerting opposite effects on similar or related membrane ionic permeabilities. It is evident that they provide significantly different settings for the modulation of Purkinje fiber action potential duration by calcium.

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