Interaction of Acidosis and Increased Extracellular Potassium on Action Potential Characteristics and Conduction in Guinea Pig Ventricular Muscle

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SUMMARY. We studied the individual and combined effects of extracellular acidosis and increases in extracellular potassium on action potential characteristics and conduction in order to gain a better understanding of the effects of acute ischemia. At each level of potassium between 2.7 and 17 mm, acidosis induced by increasing Pco2 (respiratory acidosis) and by decreasing HCO3- (metabolic acidosis) decreased resting membrane potential, the maximum rate of rise of the action potential upstroke (Vmax), and slowed conduction. Metabolic acidosis consistently and significantly lengthened the steady state action potential duration whereas respiratory acidosis did not. Respiratory acidosis caused changes in resting membrane potential, Vmax, and conduction velocity, which occurred more rapidly and were of greater magnitude than the changes induced by metabolic acidosis. The changes in Vmax induced by both types of acidosis were due to a change in the resting membrane potential-Vmax relationship as well as to the changes in resting membrane potential. The conduction slowing induced by acidosis was greater when potassium was 9 and 13 mm than when potassium was 5.4 mm. Our results suggest that acidosis causes important changes in the electrophysiological properties of ventricular fibers and that many of the known electrophysiological effects of acute ischemia can be mimicked by the combined effects of extracellular acidosis and an increase in extracellular potassium. (Circ Res 51: 614–623, 1982)

THE precise cause of the slowing of conduction which occurs within the first few minutes following the acute reduction of coronary flow is not well understood. Harris et al. (1954) considered the increase in extracellular potassium to be the major factor responsible for these changes and, thus, to be a major determinant of the ventricular arrhythmias, particularly ventricular fibrillation, which occur following acute coronary ligation (Harris and Rojas, 1943; Kaplinsky et al., 1979). More recent studies have clearly shown that the conduction slowing induced by acute ischemia cannot be attributed solely to the changes in extracellular potassium (Downar et al., 1976; Hill and Gettes, 1980; Morena et al., 1980). There is increasing evidence to implicate a variety of other factors in the genesis of the conduction slowing. These include hypoxia and glucose lack, acidosis, and possibly the accumulation of lysophosphoglycerides (Vaughan Williams and Whyte, 1967; Corr et al., 1970; Marrannes et al., 1979; Wojtczak, 1979; DeMello, 1980; Morena et al., 1980). Until very recently, it has been difficult to model precisely the ionic changes associated with acute ischemia because the precise values for potassium and pH were not known. The values derived using ion-selective electrodes (Gebert et al., 1971; Wiegand, 1979; Hill and Gettes, 1980; Cobbe and Poole-Wilson, 1980; Horn et al., 1980; Hill et al., 1981) now permit the assessment of the individual and combined contribution of the changes in potassium and pH to the electrophysiological abnormalities induced by acute ischemia. The purpose of our investigation was to study the effects of the combined changes in potassium and pH on conduction velocity and action potential characteristics in the isolated superfused guinea pig papillary muscle in order to gain greater insight into the cause of the electrophysiological changes induced by acute ischemia.

Methods

Guinea pigs weighing 200–300 g were killed by cervical fracture. The hearts were rapidly removed and papillary muscles 3–5 mm long and 0.5–1.0 mm in diameter were mounted in a single compartment bath (3-ml capacity) and superfused at a rate of 6 ml/min. The temperature was maintained at 36.5–37.0°C. The composition of the control superfusion solution was (mm): NaCl, 125; KCl, 5.4; CaCl2, 1.8; MgCl2, 1.05; NaHCO3, 24; NaH2PO4, 0.42; and glucose, 5. The control solution was equilibrated for at least 30 minutes with a humidified gas mixture containing 5% CO2 and 95% O2. pH was lowered in steps from 7.4 to 6.0 by decreasing NaHCO3 (metabolic acidosis), or by increasing the CO2 in the equilibrating gas to levels as high as 40% (respiratory acidosis). In all solutions, the total sodium concentration was kept constant (Table 1). The potassium concentration in the bathing solution was changed in steps from 2.7 to 17 mm by subtracting or adding KCl to the control solution. The values of pH and potassium were chosen to lie within the range which has been reported to occur in the extracellular space in the early stage following acute coronary occlusion (Gebert et al., 1971; Wiegand et al., 1979; Cobbe and Poole-Wilson, 1980; Hill and Gettes, 1980; Horn et al., 1980; Hill et al., 1981).

Perfusate pH was measured using a microcombination pH probe (Microelectrodes, Inc.) connected to an Orion...
The effect of increases in extracellular calcium on conduction was assessed by increasing the calcium chloride content of the perfusion to achieve a calcium concentration of 1.8 mM Ca++ , but less than those described by Spitzer and Hogan (1979), who used a solution containing 2.7 mM Ca++ . The effect of increases in extracellular calcium on conduction was assessed by increasing the calcium chloride content of the perfusion to achieve a calcium concentration of 2.05 ± 0.05 mM (n=6), i.e., a 14% increase. When pH was lowered to 6.0 by lowering HCO3~ to 1%, calcium activity was equivalent to a calcium concentration of 2.21 ± 0.1 mM (n=6), a 23% increase. These changes are similar to those reported by Frye and Poole-Wilson (1981), who used a solution containing 1.8 mM Ca++ , but less than those described by Spitzer and Hogan (1979), who used a solution containing 2.7 mM Ca++ . The effect of increases in extracellular calcium on conduction was assessed by increasing the calcium chloride content of the perfusion to achieve a calcium concentration of 3.6 mM (2 times calcium) and 7.2 mM (4 times calcium). This increase did not alter the pH. Calcium activity increased in proportion to the increase in concentration.

The potassium concentration of the perfusing solution was monitored by means of a miniature K+-sensitive electrode designed in our laboratory (Hill et al., 1978). Both electrodes were positioned as closely as possible to the surface of the papillary muscle. Steady state values of pH and potassium in the bath were achieved within 20 seconds of changing the perfusate.

The muscles were stimulated at the cut end at a rate of 0.5/sec by pulses 2.5 msec in duration and 1.2-2.0 times diastolic threshold strength using bipolar silver/silver chloride surface electrodes. This rate of stimulation was chosen to limit the possibility of use-dependent effects. The stimulus strength was adjusted to maintain a constant latency.

Transmembrane potentials were recorded with glass microelectrodes filled with 3 M KCl coupled to a Transdyne model MPA 6 DC amplifier via Ag/AgCl wires. The maximum rate of rise of the action potential upstroke (Vmax) was determined by electronic differentiation as previously described (Gettes and Reuter, 1974). Action potential duration was determined at 90% repolarization. Conduction velocity was determined as the time between peaks of the differentiated upstroke signals recorded simultaneously from microelectrodes placed 1.5-2.0 mm apart in line with the stimulation site along the long axis of the fiber divided into the interelectrode distance determined by a micrometer eyepiece in a Nikon dissection microscope. The common extracellular reference electrode was positioned as close as possible to the midpoint of the line between the intracellular electrodes. The action potentials and the differentiated upstrokes were displayed on a Tektronix model 565 dual beam oscilloscope and photographed on Polaroid film.

The muscles were superfused with control solution for at least 60 minutes before changing to a test solution. The steady state data were recorded 20 minutes after changing solutions because initial experiments indicated that approximately 15 minutes were required for all the action potential characteristics to reach a steady state. The records were accepted for analysis when resting membrane potentials were more negative than —80 mV and Vmax was more than 200 V/sec in control solution. In all reported experiments, the microelectrodes were maintained in the same cells throughout the entire protocol. The results are expressed as mean values ± sd. Statistical evaluation was performed using Student’s t-test for paired and unpaired observations.

## Results

### Steady State Effects

The effects of respiratory and metabolic acidosis on conduction velocity and action potential characteristics were examined at potassium concentrations of 5.4, 9, and 13 mM. In eight experiments, pH was lowered from 7.4 to 6.5 by both methods. A typical experiment is shown in Figure 1. Increasing potassium from 5.4 to 9 at pH 7.4 caused an 8% increase in conduction velocity, a 12 mV decrease in resting membrane potential, and a 6.5% decrease in Vmax. The further increase in potassium to 13 mM caused a further 10-mV decrease in resting membrane potential, a greater decrease in Vmax, and a decrease in conduction velocity. At each level of potassium, both types of acidosis decreased resting potential, decreased Vmax, and slowed conduction. In the experiment shown in Figure 1, both types of acidosis also prolonged action potential duration. However, the prolongation induced by metabolic acidosis was greater than that induced by respiratory acidosis. The compiled results of the eight experiments are shown in Table 2. The effects of respiratory acidosis on resting membrane potential, Vmax, and conduction velocity were slightly but significantly greater than those induced by metabolic acidosis except for the effect on Vmax at K+ = 5.4. Action potential duration was significantly prolonged by metabolic acidosis. However, respiratory acidosis did not cause consistent or significant steady state changes in the action potential duration.

The magnitude of decrease in conduction velocity induced by each type of acidosis at potassium levels of 5.4, 9, and 13 mM is plotted in Figure 2. The conduction slowing induced by respiratory acidosis at each level of potassium was significantly greater than that induced by metabolic acidosis. The figure also shows that the magnitude of the decrease in conduction velocity induced by both types of acidosis at potassium levels of 9 and 13 mM was significantly greater than that induced at a potassium level of 5.4 mM. No statistically significant difference was observed between the decrease in conduction induced by acidosis at K+ = 9 and K+ = 13 mM.

To determine whether the maximum conduction speeding effect of increasing potassium occurred at the same potassium concentration before and after acidosis and to determine the effect of acidosis on the relationship between conduction velocity and Vmax, we performed three experiments in which potassium...
**FIGURE 1.** Effects of extracellular metabolic (M) and respiratory (R) acidosis (Acid) on conduction velocity and action potential characteristics at three levels of extracellular potassium concentrations. θ = conduction velocity; V_{max} = maximum rate of rise of the action potential upstroke; RMP = resting membrane potential; and APD = action potential duration. In each panel, the action potential closest to the stimulating electrode is recorded at the slower sweep speed. The more distal action potential and the differentiated upstrokes are recorded at the more rapid sweep speed. Thus, only the upstroke of this action potential is displayed. The arrows indicate V_{max}. At each potassium level, both types of acidosis decrease θ, V_{max}, and RMP, and lengthen APD. Note that respiratory acidosis causes more marked changes in θ, V_{max}, and RMP and less marked changes in APD than does metabolic acidosis.

<table>
<thead>
<tr>
<th>K mM</th>
<th>Control (pH=7.4)</th>
<th>M.Acid. (pH=6.5)</th>
<th>R.Acid. (pH=6.5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.4</td>
<td>θ (m/sec) 0.92</td>
<td>θ (m/sec) 0.87</td>
<td>θ (m/sec) 0.85</td>
</tr>
<tr>
<td></td>
<td>V_{max}(V/sec) 225</td>
<td>V_{max}(V/sec) 203</td>
<td>V_{max}(V/sec) 198</td>
</tr>
<tr>
<td></td>
<td>RMP(-mV) 88</td>
<td>RMP(-mV) 86</td>
<td>RMP(-mV) 84</td>
</tr>
<tr>
<td></td>
<td>APD(msec) 168</td>
<td>APD(msec) 186</td>
<td>APD(msec) 180</td>
</tr>
<tr>
<td>9</td>
<td>θ (m/sec) 0.99</td>
<td>θ (m/sec) 0.92</td>
<td>θ (m/sec) 0.90</td>
</tr>
<tr>
<td></td>
<td>V_{max}(V/sec) 210</td>
<td>V_{max}(V/sec) 188</td>
<td>V_{max}(V/sec) 184</td>
</tr>
<tr>
<td></td>
<td>RMP(-mV) 76</td>
<td>RMP(-mV) 74</td>
<td>RMP(-mV) 73</td>
</tr>
<tr>
<td></td>
<td>APD(msec) 123</td>
<td>APD(msec) 182</td>
<td>APD(msec) 148</td>
</tr>
<tr>
<td>13</td>
<td>θ (m/sec) 0.91</td>
<td>θ (m/sec) 0.84</td>
<td>θ (m/sec) 0.81</td>
</tr>
<tr>
<td></td>
<td>V_{max}(V/sec) 156</td>
<td>V_{max}(V/sec) 145</td>
<td>V_{max}(V/sec) 138</td>
</tr>
<tr>
<td></td>
<td>RMP(-mV) 66</td>
<td>RMP(-mV) 65</td>
<td>RMP(-mV) 64</td>
</tr>
<tr>
<td></td>
<td>APD(msec) 102</td>
<td>APD(msec) 160</td>
<td>APD(msec) 110</td>
</tr>
</tbody>
</table>

was increased in progressive 20-minute steps from 2.7 to 5.4, 7, 9, 11, 13, 15, and/or 17 mM at pH 7.4 and 6.5. In these experiments, acidosis was induced by increasing P_{CO_2}. Figure 3 illustrates the results of one of these experiments. In all potassium concentrations at any level of V_{max}, conduction velocity was slower at pH 6.5 than at pH 7.4. The figure also shows that the relationship between V_{max} and conduction velocity was similar at both pH levels. That is: (1) maximal speeding occurred at K^+ = 9 mM, (2) conduction velocity decreased to a value less than that observed at K^+ = 5.4 when K^+ was between 11 and 13 mM, and (3) decreasing K^+ to 2.7 mM caused a decrease in conduction velocity with no change in V_{max}. Similar results were obtained in the other two experiments.

To characterize further the interaction between changes in potassium and pH on V_{max} and conduction velocity, we performed three experiments in which pH was lowered in steps from 7.4 to 6.9, 6.5, and 6.0 by decreasing HCO_3^- at potassium levels of 5.4, 9, and 13 mM. The results of one experiment are shown in Figure 4. At each level of potassium, V_{max} and conduction velocity decreased progressively as pH was lowered. As a result, the speeding in conduction
TABLE 2
Compiled Mean Results ± 1 SD from the Eight Experiments in which Both Types of Acidosis (pH = 6.5) were Induced at the Three Levels of Extracellular Potassium

<table>
<thead>
<tr>
<th></th>
<th>K = 5.4</th>
<th></th>
<th>K = 9</th>
<th></th>
<th>K = 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP (mV)</td>
<td>C</td>
<td>86.4</td>
<td>±1.4</td>
<td>72.9*</td>
<td>65.0</td>
</tr>
<tr>
<td></td>
<td>MA</td>
<td>83.8*</td>
<td>±1.8</td>
<td>71.6*</td>
<td>63.3*</td>
</tr>
<tr>
<td></td>
<td>RA</td>
<td>81.5</td>
<td>±1.9†</td>
<td>71.6*</td>
<td>61.7*</td>
</tr>
<tr>
<td>APD (ms)</td>
<td>C</td>
<td>163</td>
<td>±1.1</td>
<td>134</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td>MA</td>
<td>191*</td>
<td>±0.9</td>
<td>176*</td>
<td>145*</td>
</tr>
<tr>
<td></td>
<td>RA</td>
<td>167</td>
<td>±0.8†</td>
<td>136</td>
<td>107</td>
</tr>
<tr>
<td>V max (V/sec)</td>
<td>C</td>
<td>±36</td>
<td>±0.27</td>
<td>±36†</td>
<td>±25</td>
</tr>
<tr>
<td></td>
<td>MA</td>
<td>±37</td>
<td>±0.27</td>
<td>±36†</td>
<td>±25</td>
</tr>
<tr>
<td></td>
<td>RA</td>
<td>±37†</td>
<td>±0.27</td>
<td>±36†</td>
<td>±25</td>
</tr>
<tr>
<td>θ (m/sec)</td>
<td>C</td>
<td>0.794</td>
<td>±0.12</td>
<td>0.718*</td>
<td>0.781</td>
</tr>
<tr>
<td></td>
<td>MA</td>
<td>0.733*</td>
<td>±0.12</td>
<td>0.704*</td>
<td>0.761</td>
</tr>
<tr>
<td></td>
<td>RA</td>
<td>0.718*</td>
<td>±0.12</td>
<td>0.704*</td>
<td>0.761</td>
</tr>
</tbody>
</table>

C = control, MA = metabolic acidosis, RA = respiratory acidosis. Definitions of RMP, APD, V max, and θ are in Figure 1.

*P < 0.001: control vs. metabolic and respiratory acidosis
†P < 0.001: metabolic vs. respiratory acidosis
‡P < 0.05: metabolic vs. respiratory acidosis

which occurred when the potassium concentration was changed from 5.4 to 9 mM at pH 7.4 was eliminated when the potassium concentration was changed from 5.4 mM at pH 7.4 to 9 mM at pH 6.5 (see Fig. 1 and Table 2).

As indicated above, acidosis caused a consistent decrease in resting potential and V max. However, the results suggested that the changes in V max were not due solely to the change in resting potential, since the effect of acidosis on V max at K* = 5.4 mM was greater than that induced by increasing the potassium concentration to 9 mM, although the effect of acidosis on resting potential was less than that induced by increasing the potassium concentration. This observation suggests an acidosis-induced effect on V max which was independent of changes in membrane potential. Figure 5 illustrates the results of an experiment performed to assess the effects of acidosis on the relationship between resting potential and V max at pH 7.4 and 6.5 as the potassium concentration was changed in 20-minute steps from 2.7 to 17.0 mM when pH was 7.4 and from 2.7 to 15 mM when pH was 6.5. In this experiment, acidosis was induced by increasing PCO2. At resting potentials between —100 and —70 mV (Fig. 5A), V max was less at pH 6.5 than at pH 7.4. However, at resting potentials less negative than —70 mV, V max was greater at pH 6.5 than at pH 7.4. This results in a shift of the normalized curve (Fig. 5B) along the
Because of the similarity of the acidosis-induced shift in the membrane potential-V_{max} relationship to that induced by increasing extracellular calcium concentration (Weidmann, 1955; Gettes and Reuter, 1974; Windisch and Tritthart, 1981) and the known increase in calcium activity which occurs when pH is lowered by decreasing bicarbonate and by increasing P_{CO2} (Spitzer and Hogan, 1979; Frye and Poole-Wilson, 1981), we analyzed the effect of increasing extracellular calcium on the V_{max} conduction velocity relationship. Figure 6 illustrates the results of an experiment in which calcium was varied between 1.8 mM and 7.6 mM at pH of 7.4, normalized to the results achieved when calcium equals 1.8 mM. The figure also displays the results illustrated in Figure 4 when pH was lowered from 7.4 to 6.0 at K+=5.4 mM. These results are also normalized to the control value at K+=5.4 mM, Ca^{++}=1.8 mM, and pH=7.4. The figure shows that a 2-fold increase in extracellular calcium concentration to 3.6 mM causes a 6% decrease in conduction velocity. This decrease is only slightly less than that associated with a change in pH from 7.4 to 6.5 induced by lowering bicarbonate. As mentioned in Methods, this degree of bicarbonate-induced pH change resulted in a change in calcium activity equivalent to a concentration of 2.05 mM. The change in conduction associated with a 4-fold increase in extracellular calcium to 7.6 mM is similar to the change in conduction which occurs when pH is lowered to 6.0 by decreasing bicarbonate. As indicated in Methods, this degree of bicarbonate-induced pH change resulted in a change in calcium activity equivalent to a concentration of 2.21. Thus, the results of this experiment indicate that the changes in conduction induced by acidosis cannot be attributed solely to the increase in extracellular calcium activity.

Dynamic Effects

The dynamic nature of the effects of both types of acidosis was investigated at control and elevated potassium levels. Potassium was altered either separately or simultaneously with the change in pH. As indicated in the methods, the new steady state values of potas-
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Conduction Velocity

**Figure 6.** Comparison of effects of increasing extracellular calcium concentration (closed circles) and metabolic acidosis (open circles) on $V_{\text{max}}$ and conduction velocity. The pH data are the same as illustrated in Figure 4 for $K^+ = 5.4$ mM. The values are normalized to those obtained at $K^+ = 5.4$ mM, $Ca^{2+} = 1.8$ mM, and pH = 7.4. Note that a decrease in pH to between 6.9 and 6.5 causes conduction slowing similar to that associated with a 2-fold increase in calcium, and that a decrease in pH to 6.0 causes conduction slowing which is only slightly less than that associated with a 4-fold increase in extracellular calcium concentration.

Conduction Velocity

**Figure 7.** Time course of change in conduction velocity (above) and action potential duration (below) induced by metabolic and respiratory acidosis at two steady state potassium levels. Arrows indicate onset and termination of acidotic perfusate. The pH of the bathing solution reached a steady state within 20 seconds of solution change; however, the dynamic changes in conduction velocity and action potential duration followed a slower time course. These changes occurred more rapidly with respiratory than with metabolic acidosis. In addition, the changes in APD induced by respiratory acidosis were diphasic.

Discussion

Our experiments were performed to determine the interaction between acidosis and increases in extracellular potassium concentration on conduction velocity and action potential characteristics. We hoped that by so doing we would gain greater insight into the causes of the changes in conduction and other electrophysiological parameters associated with ischemia than was available from previous studies (Downar et al., 1976; Marrannes et al., 1979; Senges et al., 1979; Gilmour and Zipes, 1980; Morena et al., 1980). The effects of increasing potassium on conduction and action potential characteristics have been studied previously (Han et al., 1967; Domingues and Fozzard, 1970; Holland and Brooks, 1976; Arnsdorf et
whereas respiratory acidosis did not cause consistent potential duration at all studied potassium levels, by acidosis was more marked at the higher K+ levels.

The decrease in conduction velocity induced by acidosis was more marked than that of metabolic acidosis. Vaughan Williams and Whyte (1967), Brown and Noble (1978a), Spitzer and Hogan (1979), Marrannes et al. (1979), and Nattel et al. (1981) also reported a decrease in resting potential associated with acidosis. However, Johansson and Nilsson (1975), Frye and Poole-Wilson (1979, 1981), and Grant et al. (1980) were able to document a significant decrease in Vmax after the induction of acidosis. The reasons for this difference is unclear although differences in pH levels or fiber types may be partially responsible. Two mechanisms can be postulated to explain the decrease in Vmax which we observed: (1) a decrease in resting potential with a secondary effect on Vmax; and (2) a direct effect of acidosis on the resting potential-Vmax relationship.

Our results indicate that decreasing pH to 6.5 was associated with a 2- to 5-mV decrease in resting potential at all levels of potassium tested in this study, and that the effect of respiratory acidosis was more marked than that of metabolic acidosis. Vaughan Williams and Whyte (1967), Brown and Noble (1978a), Spitzer and Hogan (1979), Marrannes et al. (1979), and Nattel et al. (1981) also reported a decrease in resting potential associated with acidosis. However, Johansson and Nilsson (1975), Frye and Poole-Wilson (1979, 1981), and Grant et al. (1980) did not detect a significant change in resting potential. Again, the reasons for this difference are not obvious. The change in resting potential may be due to intracellular
acidosis inducing a loss of intracellular potassium (Skinner and Kunze, 1976). We would attribute the greater effect of respiratory acidosis than metabolic acidosis to a greater degree of intracellular acidosis (Poole-Wilson and Cameron, 1975).

The decrease in resting potential induced by acidosis would account, at least in part, for the decrease in V\text{max}. However, as shown in Figure 5, the decrease in V\text{max} could not be accounted for entirely by this mechanism. Rather, the change in V\text{max} represents a direct effect of acidosis on the relationship between membrane potential and V\text{max}. We found that acidosis depressed V\text{max} at membrane potentials more negative than \(-70\) mV but increased V\text{max} at less negative values, resulting in a shift of the curve describing the membrane potential- V\text{max} relationship along the voltage axis in the direction of more positive potentials. Nattel et al. (1981) showed no change in the membrane potential- V\text{max} relationship to canine Purkinje fiber using metabolic acidosis to a pH of 6.9. It is possible that this result reflects the lesser degree of acidosis. However, our results are supported by the observations of van Bogaert and Carmeleit (1972), who studied the effect of acidosis in cow Purkinje fibers. This shift is analogous to that induced by a 4-fold increase in extracellular calcium (Weidmann, 1955; Beeler and Reuter, 1970; Gettes and Reuter, 1974; Windisch and Tritthart, 1981). Similar shifts in the steady state voltage dependence of the sodium channel conductance variables by acidosis and by an increase in extracellular calcium have been reported in the node of Ranvier by Hille (1970). Brown and Noble (1978b) attribute these changes in conductance variables to the effects of hydrogen and calcium ions on fixed surface charges.

It is likely that a decrease in rapid inward current, as reflected by the decrease in V\text{max}, is one of the factors responsible for the acidosis-induced decrease in conduction velocity. However, in some situations, decreases in V\text{max} have been shown to be associated with speeding of conduction (Dominguez and Fozzard, 1970; Spear and Moore, 1974; Peon et al., 1978; Saito et al., 1978). An example of this is the increase in conduction velocity which occurs when the potassium concentration is raised from 5.4 to 9 mm. Moreover, the decrease in V\text{max} does not explain the greater effect of acidosis on conduction at potassium levels of 9 and 13 mM than at 5.4 mM. Of other factors which may have influenced conduction are those related to excitability (Dominguez and Fozzard, 1970; Spear and Moore, 1974; Peon et al., 1978). This would include threshold potential and the difference between resting and threshold potentials. Brown and Noble (1978b) reported that the threshold potential for the sodium current was shifted in a positive direction by extracellular acidosis. This effect may also be attributed, at least in part, to the increase in calcium activity, and might have contributed to the slowing of conduction. It is possible that the more marked slowing of conduction induced by acidosis at potassium levels of 9 and 13 mM than at a potassium of 5.4 mM reflects a greater shift in the threshold potentials at the higher potassium levels.

An increase in internal longitudinal resistance would also contribute to slowing of conduction. DeMello (1980) has recently reported that the intracellular injection of HCl caused a marked increase in intracellular longitudinal resistance in canine Purkinje fibers. His report also cites unpublished observations of Weingart and Reber showing similar results in Purkinje fibers exposed to high PCO2. The greater effect of respiratory than metabolic acidosis on conduction velocity which we have observed may reflect a greater increase in longitudinal resistance by respiratory acidosis. This could be related to the fact that CO2 crosses the cell membrane and induces intracellular acidosis more rapidly and to a greater degree than does a lowering of extracellular bicarbonate (Poole-Wilson and Cameron, 1975; Steenbergen et al., 1977). This effect might also be responsible for the shorter time required for the effects of respiratory acidosis to reach a steady state. Intracellular acidosis might have caused changes in intracellular calcium activity or changes in calcium binding that would also tend to uncouple cells (Williamson et al., 1975; Fabiato and Fabiato, 1978; DeMello, 1975). The intracellular acidosis might also be responsible for the shorter time required for the effects of respiratory acidosis to reach a steady state.

We would therefore attribute the effects of acidosis on conduction to both extracellularly and intracellularly mediated events. The shift in the curve relating resting potential to V\text{max} and the shift in threshold potential to more positive potentials (Brown and Noble, 1978b) may be attributed to the effects of extracellular acidosis. The intracellular mediated effects include the leakage of potassium from the intracellular to interstitial space (Skinner and Kunze, 1976) which would explain the reduction in resting potential and an increase in longitudinal resistance leading to cellular uncoupling (DeMello, 1980).

Both the extracellularly and intracellularly mediated effects of acidosis may involve changes in calcium activity. Our studies do not define the precise contribution of the changes in calcium activity to the changes induced by extracellular acidosis. A 4-fold increase in extracellular calcium concentration has been shown to cause a 4-mV shift in the curve relating V\text{max} to membrane potential (Weidmann, 1955; Beeler and Reuter, 1970; Gettes and Reuter, 1974; Windisch and Tritthart, 1981). Thus, it is doubtful that the shift induced by the change in pH from 7.4 to 6.5 which averaged 3.4 mV can be attributed entirely to this mechanism, since the increase in intracellular calcium activity associated with this pH change is less than that associated with a 4-fold increase in extracellular calcium concentration. Likewise, we cannot attribute the acidosis-induced changes in conduction to the associated change in calcium activity, since acidosis caused a smaller change in calcium activity than that required to cause a similar degree of conduction slowing. We cannot rule out the possibility that intra-
cellularly mediated events involving calcium may have participated in both phenomena.

The changes in pH induced by ischemia, which this study attempts to model, involve changes in calcium activity, since total extracellular calcium concentration does not change. For this reason, our results are applicable to an understanding of the effects of ischemia on conduction, even though they do not define precisely the contribution of the calcium activity increase to these changes. We have also not taken into consideration the possible changes in osmolality and chloride associated with the various perfusates. However, based on the results of others (Spitzer and Hogan, 1979; Kishida and Surawicz, 1979), it is unlikely that those changes contributed significantly to the changes in resting potential, Vmax, conduction velocity or action potential duration which we have obtained.

The steady state and dynamic changes in action potential duration which we observed with both types of acidosis are similar to those reported by others (Johannson and Nilsson, 1975; Spitzer and Hogan, 1979). Spitzer and Hogan (1979) showed that the prolongation of action potential duration induced by lowered bicarbonate acidosis was due to the lowering of bicarbonate rather than to the acidosis.

The nature of the acidosis induced by ischemia is complex (Williamson et al., 1976; Gevers, 1977; Poole-Wilson, 1978) and difficult to model. In acute ischemia, intracellular acidosis most likely precedes extracellular acidosis and both an increase in extracellular PCO2 (Khuri et al., 1975; Case et al., 1979) and lactate are known to accompany the fall in extracellular pH. We studied the effects of both respiratory and metabolic extracellular acidosis. In this way, our study differs somewhat from previous studies which attempt to model ischemia using metabolic acidosis (Morena et al., 1980; Gilmore and Zipes, 1980). Increasing PCO2 may be a more appropriate method for inducing acidosis than decreasing bicarbonate when seeking an explanation for the rapidly occurring changes in conduction velocity and action potential duration associated with ischemia. The dynamic changes which we recorded when potassium was increased to 9, and pH was simultaneously lowered to 6.5 by increasing PCO2 may account for the conduction speeding which has been observed to precede the conduction slowing effects of acute ischemia (Holland and Brooks, 1976). As shown in Figure 8, this can be attributed to the fact that the conduction speeding effects of the rising extracellular potassium concentration were more pronounced and not immediately balanced by the conduction slowing effects of increasing tissue PCO2.

Clearly, ischemia also results in accumulation of lactate in the extracellular space (Williamson et al., 1976). This effect may be most important in considering the more slowly developing changes in action potential duration (Hiraoka et al., 1981).

Our study also differs from others in that it provides an analysis of the various combinations of potassium and pH likely to be encountered during acute ischemia. In this way, our study permits an appreciation of the effects of inhomogeneities in extracellular potassium (Hill and Gettes, 1980) and pH (Hill et al., 1981) which have been reported to exist, not only between the center and lateral margins of the ischemic zone, but also within the center of the ischemic zone (Hill and Gettes, 1980). Our study suggests that conduction velocity may be simultaneously increased, decreased or unchanged at various locations within the ischemic zone, depending on the effect of the various combinations of potassium and pH and other factors, as well as the varying sensitivity of the fiber types to these factors (Han et al., 1967; Gilmore and Zipes, 1980). The inhomogeneities in conduction may be important in the creation of reentry circuits and the development of reentrant ventricular arrhythmia, particularly ventricular fibrillation known to be associated with acute ischemia.

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