The Effects of Acidosis and Bicarbonate on Action Potential Repolarization in Canine Cardiac Purkinje Fibers

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ABSTRACT Studies were performed on canine cardiac Purkinje fibers to evaluate the effects of acidosis and bicarbonate (HCO₃⁻) on action potential repolarization. Extracellular pH (pHₑ) was reduced from 7.4 to 6.8 by increasing carbon dioxide (CO₂) concentration from 4 to 15% in a HCO₃⁻-buffered solution or by NaOH titration in a Hepes-buffered solution. Both types of acidosis produced a slowing of the rate of terminal repolarization (i.e., period of repolarization starting at about -60 mV and ending at the maximum diastolic potential) with an attendant increase in action potential duration of 10-20 ms. This was accompanied by a reduction in the maximum diastolic potential of 2-8 mV. In contrast, if the same pH change was made by keeping CO₂ concentration constant and lowering extracellular HCO₃⁻ from 23.7 to 6.0 mM, in addition to the slowing of terminal repolarization, the plateau was markedly prolonged resulting in an additional 50- to 80-ms increase in action potential duration. If pHₑ was held constant at 7.4 and HCO₃⁻ reduced from 23.7 mM to 0 (Hepes-buffered solution), the changes in repolarization were nearly identical to those seen in 6.0 mM HCO₃⁻ except that terminal repolarization was unchanged. This response was unaltered by doubling the concentration of Hepes. Reducing HCO₃⁻ to 12.0 mM produced changes in repolarization of about one-half the magnitude of those in 6.0 mM HCO₃⁻. These findings suggest that in Purkinje fibers, HCO₃⁻ either acts as a current that slows repolarization or modulates the ionic currents responsible for repolarization.

INTRODUCTION

Action potential repolarization in heart cells results from the complex interaction of a large number of ionic events. The extent to which these events are influenced or otherwise modulated by the acid-base state of the extracellular fluid remains largely a matter of conjecture. The present study was undertaken to determine the individual actions of hydrogen and bicarbonate ions on action potential repolarization under experimental conditions simulating those of in vivo acidosis, i.e., increased CO₂ tension (respiratory acidosis) or decreased extracellular bicarbonate concentration (metabolic acidosis).

Previous studies of the effects of acidosis on the formation of cardiac action potentials have yielded conflicting results. For example, Poole-Wilson and...
Langer (1975) reported that the action potential duration for rabbit ventricular cells increased when \( \text{PCO}_2 \) was increased and extracellular pH (pHe) fell to 6.8. A similar reduction in pHe produced by lowering extracellular bicarbonate concentration had no effect. In contrast, Jóhannsson and Nilsson (1975) studying the same tissue found that neither respiratory nor metabolic acidosis (HCO\(_3\)-free solution buffered with histidine) had any effect on action potential duration. These investigators did, however, report that an elevation in extracellular bicarbonate per se did shorten action potential duration and suggested that bicarbonate may influence potassium conductance. The recognition of a separate bicarbonate action, independent of changes in pH, bears importantly on the interpretation of the direct effects of acidosis on membrane electrical activity.

For cardiac Purkinje fibers it has been reported (Hecht and Hutter, 1965; Coraboeuf et al., 1976) that acidosis lengthens action potential duration but nothing has been described for the action of extracellular bicarbonate on these cells.

Based on these considerations the present report focuses on changes in membrane potential in response to various forms of pH and HCO\(_3\) manipulation. Specifically, the objectives are to describe the response of the repolarization process in the Purkinje fiber to (a) acidotic stress to a reasonable physiologic level (pHe = 6.8) and (b) changes in extracellular bicarbonate. It will be shown that both have unique effects on repolarization and that the effects of lowered bicarbonate occur independently of changes in pH. A preliminary report of these findings has been published previously (Spitzer and Hogan, 1977).

**METHODS**

**Solutions**

The effects of acidosis were studied by reducing pHe from 7.35 to 6.80, the lowest arterial pH compatible with life in man (Woodbury, 1966). Solution pH was reduced by (a) increasing the CO\(_2\) concentration from 4 to 15% (respiratory acidosis), (b) decreasing the sodium bicarbonate concentration from 23.7 to 6.0 mM (metabolic acidosis), and (c) using an organic buffer system, Hepes (N-2-hydroxyethylpiperazine-n'-2-ethanesulfonic acid), in which pH was adjusted by titration with NaOH (metabolic acidosis). Hepes was used instead of Tris because of the undesirable side effects of Tris (Good et al., 1966; Gillespie and McKnight, 1976).

The composition of the Tyrode solutions used for these experiments is shown in Table I and their pH and PCO\(_2\) values in Table II. Note that the MgCl\(_2\), KCl, and dextrose concentrations were the same for all solutions. The osmolality of each solution was determined from freezing point depression (model 2007 osmometer, Precision Systems, Inc., Sudbury, Mass.).

Normal Tyrode and 6 mM HCO\(_3\) Tyrode were gas equilibrated with 4% CO\(_2\)-96% O\(_2\) so that each had the same PCO\(_2\) (40 ± 3 torr) but different pHs. Furthermore, the bicarbonate-buffered solutions were gassed for 15-20 min before adding CaCl\(_2\). In aqueous solutions containing calcium and bicarbonate, various carbonate complexes may form, thus reducing calcium activity (Schaer, 1974). This was particularly important in the present experiments because the level of ionized calcium has such wide-ranging effects on the heart. Furthermore, the complexing of calcium is pH labile. Thus, any attempt to evaluate the influence of pH on heart cells by varying bicarbonate or CO\(_2\)
concentration may be complicated by attendant changes in calcium activity. To avoid these complications an analysis of the calcium activity in control and test solutions was made using an Orion (92-20) calcium electrode (Orion Research Inc., Cambridge, Mass.) (Ross, 1967, 1969).

Both the calcium electrode and the reference electrode (90-01, Orion) were mounted inside a temperature-controlled, Plexiglas box containing a gas-tight, 1.5-ml chamber constructed to fit over the end of both electrodes. The output of the electrode pair was measured to the nearest 0.1 mV with a digital pH/mV meter (Orion 801A). All solutions were gas equilibrated in separatory funnels at 25 ± 0.5°C, and the calcium electrode system was held at 37 ± 0.5°C. The gas-tight chamber was thoroughly flushed with a solution sample at a flow rate of 1.5 ml min⁻¹ before making readings under stop-flow conditions. This system responded to CaCl₂ in double-distilled water at 37°C with a slope of 27 mV per 10-fold change in calcium concentration over the range of 1.35 to 8.1 mM calcium. Because the purpose of this analysis was to determine the calcium activity of 15% CO₂ Tyrode and 6 mM HCO₃⁻ Tyrode with respect to normal Tyrode, the calibrating solution was normal Tyrode containing varying CaCl₂ concentrations. The calculations were similar to those described previously by Moore (1969). Calibration was done immediately before and after the analysis of each test solution. Because one of the calibrating solutions contained the same calcium concentration as the test solution (2.7 mM), any difference in the millivolt readings between the two solutions represented a difference in calcium activity. For 15% CO₂ Tyrode, calcium activity was greater than that in normal Tyrode to a level equivalent to a calcium concentration of 2.93 ± 0.02 mM (n = 10). For 6 mM HCO₃⁻ Tyrode, calcium activity was equivalent to a calcium concentration of 3.57 ± 0.01 (n = 6). The difference in pH between the calibrating and test solutions (Table II) had no effect on the electrode output, in accordance with previous reports (Ross, 1969). Thus for the two test solutions to have the same calcium

| TABLE I |
| COMPOSITION OF TYRODE SOLUTIONS |

| Control pH | Low pH |
|------------|-------|
|            |       |
| **High Heps** |       |
| NaCl       | 137.0  |
| Dextrose   | 5.5    |
| NaHCO₃     | 23.7   |
| KCl        | 2.7    |
| MgCl₂      | 0.5    |
| CaCl₂      | 2.7    |
| Heps       | 0.0    |
| [Cl⁻]Total | 146.1  |
| [Na⁺]Total | 160.7  |
| Osmolarity, mOsm | 307  |

| **12 mM** | **15% CO₂** | **6 mM HCO₃⁻** |
|-----------|-------------|----------------|
| NaCl      | 148.7       | 154.7          | 157.7          |
| Dextrose  | 12.0        | 23.7           | 6.0            |
| NaHCO₃    | 5.5         | 5.5            | 5.5            |
| KCl       | 2.7         | 2.7            | 2.7            |
| MgCl₂     | 0.5         | 0.5            | 0.5            |
| CaCl₂     | 2.7         | 2.7 or 2.7     | 2.7 or 2.7     |
| Heps      | 12.0        | 146.5*         | 162.5*         |
| [Cl⁻]Total| 163.2       | 146.1          | 165.8 or 166.8 |
| [Na⁺]Total| 160.7‡      | 148.4‡         | 160.7‡         |
| Osmolarity, mOsm | 312  |

* Compensated calcium.
‡ Includes sodium from NaOH titration.

of 27 mV per 10-fold change in calcium concentration over the range of 1.35 to 8.1 mM calcium.
activity as normal Tyrode, the CaCl₂ added in preparation had to be reduced, i.e., compensated, to 2.48 mM for 15% CO₂ Tyrode and 2.94 mM for 6 mM HCO₃⁻ Tyrode. These values were verified experimentally. Changes in calcium activity in Hepes-buffered Tyrode were not measured but are reported to be negligible in the range of pH studied (Good et al., 1966).

**Apparatus**

Solutions were pumped (Masterflex roller pump, Cole Parmer Instrument Co., Chicago, Ill.) at a rate of 6 ml·min⁻¹ from glass reservoirs via glass tubing to a circular Plexiglas tissue bath (vol = 8 ml). The all-glass plumbing network was necessary to prevent gas leaks. The reservoirs were fully immersed in a temperature-controlled water bath (25°C) where the solutions were equilibrated with the appropriate gas mixture. This procedure minimized variations in solution gas tensions throughout the experimental series. 15 min were required to achieve a stable pH, PCO₂, and temperature in each reservoir. The change to a new solution in the tissue bath was complete within 5 min. To maintain a constant PCO₂ and pH and to reduce temperature fluctuations, a clear glass lid was used to cover the tissue bath. A glass standpipe in the center of the cover served as the entry point for the recording microelectrode. The solution temperature was raised to 37°C before reaching the tissue bath and was held constant at 37 ± 0.05°C by an electronic feedback circuit utilizing a thermistor probe in the bath and a pair of Peltier thermoelectric plates.

Solution samples were aspirated directly from the tissue bath into pH (E5021) and PCO₂ (D616) electrodes of the pH meter (PHM 27, Radiometer Co., Copenhagen, Denmark) which was equilibrated at the same temperature as the bath. Two precision buffers (S-1510, 7.383 ± 0.005; S-1500, 6.841 ± 0.005 at 37°C, Radiometer Co.) were used for pH electrode calibration, and the PCO₂ electrode was calibrated using two gases (2 and 9% CO₂, balance O₂) humidified with distilled water and previously analyzed for CO₂ concentration with a Scholander microgas analyzer.

Microelectrodes made from Pyrex capillary tubing (Corning Glass Works, Corning, N.Y.) and filled with 3 M KCl gave resistances of < 20 Mohm and tip potentials of < 10 mV. The reference electrode was a Ag-AgCl wire recessed in the wall of the bath. This electrode system was unresponsive to the pH changes used in this study which is in accord with previously reported findings that tip potentials of Pyrex microelectrodes with resistances < 100 Mohm are unchanged by pH (Lavallée and Szabo, 1969). The microelectrode was connected to the input of a high impedance, capacitance neutralized amplifier (M 4A electrometer, W-P Instruments, Inc., New Haven, Conn.) whose output was displayed on a dual-beam oscilloscope (RM 565, Tektronix Inc., Beaverton, Ore.)

### Table II

|          | Control pH | Low pH          |
|----------|------------|-----------------|
|          | Normal     | HEPES* | High Hepes* | 12 mM HCO₃⁻ | 15% CO₂ | 6 mM HCO₃⁻ | Hepes* |
| pH       | 7.34       | 7.37   | 7.37        | 7.05       | 6.80   | 6.81       | 6.81   |
| PCO₂, torr | 40         | 0      | 0           | 41          | 149    | 37         | 0      |
| HCO₃⁻, mM | 23.7       | 0      | 0           | 12.0        | 23.7   | 6.0        | 0      |

* Gas equilibrated with 100% O₂.
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and photographed with a 35-mm kymographic camera (C4, Grass Instrument Co., Quincy, Mass.).

Isolated constant current stimuli (1-2 ms, 0.1 mA) were delivered to the tissue through a chlorided silver wire inside a Tyrode-filled glass tube. Unless otherwise stated, the stimulation frequency was 90 pulses·min⁻¹.

**Tissue Preparation**

Mongrel dogs weighing 13-18 kg, 1-2 yr old, of either sex were anesthetized with sodium pentobarbital (30 mg·kg⁻¹, i.v.) and the hearts removed via a left lateral thoracotomy. Within 3 min after opening the chest, both ventricles were thoroughly flushed with normal Tyrode, and all usable Purkinje strands with small pieces of attached ventricular muscle were excised and placed in normal Tyrode solution gas equilibrated at room temperature. To eliminate the possibility of electrotonic influences, Purkinje strands from two hearts were dissected free of any ventricular muscle. The response of these preparations was the same as that of all other preparations. 20-50 min after removal from the animal the tissue preparation was pinned to a Sylgard (Dow Corning Corp., Midland, Mich.) block in the bottom of the tissue bath and superfused with either normal Tyrode or Heps Tyrode (pH 7.37) for at least 1 h before switching to a test solution.

**Procedures**

Only surface cells of free-running Purkinje strands were studied. The procedure followed was to establish a 5- to 10-min stable impalement of a cell in control solution, then switch to the test solution while maintaining the impalement. At least 15 min were allowed to reach a steady state in the new solution before test data were taken. Thus, for the purpose of analysis, each cell served as its own control. The criteria for an acceptable impalement were that (a) the electrode enter the cell easily with little or no visible "dimpling" of the bundle surface, (b) upon withdrawal of the electrode the oscilloscope beam rezero to within ± 2.0 mV, and the electrode resistance remain within 5 Mohm of its original value. Most impalements rezeroed to within ± 1 mV. (c) Cells were rejected if under control conditions $V_{max}$ was < 400 V·s⁻¹ or the maximum diastolic potential was less negative than −90 mV. Using these criteria about 20% of all cells impaled were rejected.

Changes in action potential configuration were quantified by measuring various time and voltage characteristics of the waveform. The following standard designations of the Purkinje fiber action potential were used: $V_{min}$ = minimum diastolic potential or take-off potential (millivolts); $V_{max}$ = maximum diastolic potential (millivolts); APD₂₀, APD₆₀, APD₉₀ = action potential duration when repolarization has reached −20, −60, and −90 mV, respectively (ms); phase 1 = period of rapid repolarization immediately following the peak of the overshoot; terminal repolarization = period of repolarization beginning at about −60 mV and ending at the maximum diastolic potential. Action potential records in the figures have been carefully retraced from the original film records in order that direct comparisons of control and experimental events can be made.

To determine if the action potential response to acidosis or bicarbonate was influenced by changes in stimulation frequency, the following procedure was used: In control solution the cell was stimulated for 1-min periods at frequencies of 30, 60, 90, and 120 pulses·min⁻¹, with no pause between each frequency. Action potential records were photographed in the last 5 s of each 1-min stimulation interval. At the end of this 4-min sequence, frequency was returned to 90 pulses·min⁻¹. After at least 5 additional min, the test solution was started and the stimulation sequence repeated when action potential changes were stable.

The significance of the difference for paired and unpaired data were determined
using Student’s t test. Differences were considered significant if P < 0.05. Values are reported as the mean ± SEM.

The bicarbonate effects on repolarization were simulated using the mathematical model for the Purkinje fiber action potential described by McAllister (1970) and McAllister et al. (1975). Calculations were made on a Univac 1108 computer system (Sperry Univac, Sperry Rand Corp., Blue Bell, Pa.) at the University of Utah Computer Center, Salt Lake City. Action potentials, both control and test, were initiated by setting the initial membrane potential to −50 mV and all kinetic variables to their steady-state values at −80 mV. Approximately 30 s were required to compute an action potential. Results were plotted by the computer and carefully retraced for photographic reproduction.

RESULTS

Effects of 15% CO₂ and Hepes Acidosis

15% CO₂ ACIDOSIS Fig. 1 illustrates changes in action potential repolarization associated with 15% CO₂ acidosis. Typical changes included a slight reduction in V_max and a shortening of action potential duration in the voltage range of −20 to −60 mV. The rate of terminal repolarization was slowed leading to an increase in APD_{90}. There were no changes in the rate of phase 1 repolarization or the voltage level of the plateau. Occasionally, as shown here, the decrease in action potential duration was partially reversed with time, possibly reflecting a compensatory change in intracellular pH (pHi) as described by Ellis and Thomas (1976).

The changes in APD_{90}, APD_{60}, APD_{50}, and V_max were stable after 15-20 min and fully reversible upon return to normal Tyrode. It was important in these experiments to determine whether a rise in calcium activity in the 15% CO₂ Tyrode solution was responsible for the decreases in action potential duration and rate of terminal repolarization because an increase in extracellular calcium is known to have such effects on canine Purkinje fibers (Temte and Davis, 1967).
To resolve this question, 5 of 12 experiments in 15% CO₂ were performed with the test solution compensated for calcium, i.e., constant calcium activity. In the compensated solution, decreases in APD₂₀ and APD₆₀ of 6.3 ± 1.6 and 4.0 ± 0.6 ms, respectively, were not significantly different from those in the uncompensated solution of 3.5 ± 1.3 and 2.1 ± 1.5 ms. Also there was no difference in terminal repolarization between the two solutions. It is noteworthy to point out, however, that although not significant the action potential did tend to shorten more when calcium activity was constant. It would thus appear that the observed changes in repolarization were not the result of an increase in calcium activity.

These findings differ from the results of Poole-Wilson and Langer (1975, Fig. 15) which showed that 15% CO₂ caused a prolongation of action potential duration in the rabbit ventricular myocardial cell. To rule out differences in technique as a possible cause for this difference, we have repeated their study.

For this series of experiments, the Tyrode solution contained the following concentrations (mM): NaCl 130, KCl 4.0, MgCl₂ 2.0, CaCl₂ 2.7, and NaHCO₃ 23.7. The bath temperature was 29°C, the pH of the control solution was 7.41, and the PCO₂ was 34 torr. The acidotic solution was equilibrated with 15% CO₂ to give a pH of 6.86 and PCO₂ of 117 torr (calcium uncompensated). Action potentials were recorded from rabbit papillary muscles from the right ventricle stimulated at 60 pulses·min⁻¹. Fig. 2 shows the results from one such experiment. Just as in the Poole-Wilson and Langer (1975) experiments, the major response of these cells to 15% CO₂ Tyrode was an increase in action potential duration at all voltage levels throughout repolarization. This same result was observed in six of six other experiments. Thus, there appears to be a cell type and/or species difference in the action potential response to respiratory acidosis.

**HEPES ACIDOSIS** For these experiments the control solution was 12 mM Hepes Tyrode, pH 7.37, and the test solution was 12 mM Hepes Tyrode, pH 6.81. Both solutions were gas equilibrated with 100% O₂. Hepes acidosis altered action potential repolarization in much the same manner as described above for
15% CO₂ acidosis. As shown in Fig. 3 (top panel), the most prominent changes were a fall in \( V_{\text{max}} \) and a slowing of the rate of terminal repolarization with an attendant increase in \( \text{APD}_{90} \). In this experiment, as in most others, changes were complete within 15 min. There was little or no change in \( \text{APD}_{20} \) or \( \text{APD}_{60} \) and the level of the plateau was either unchanged or shifted upward by 1-2 mV. There were no obvious changes in phase 1 repolarization. The response to acidosis was no different after 30-180 min incubation in control Hepes solution. In this experiment the cell had been incubated for almost 3 h before decreasing pile. The changes in repolarization were fully reversible upon return to control solution as shown in Fig. 3 (lower panel).

![Figure 3](http://rupress.org/jgp/article-pdf/73/2/199/1247044/199.pdf)

**Figure 3.** Effects of Hepes acidosis on action potential repolarization. All tracings were recorded from the same cell during a continuous impalement. The upper panel shows the effects of changing from control solution (Hepes Tyrode, pH 7.37) to test solution (Hepes Tyrode, pH 6.81). The lower panel shows recovery tracings after the return to control solution. Elapsed time in minutes is indicated for each action potential. The records taken just before the solution changes are labeled \( T = 0 \) for the upper panel and \( T = 22 \) for the lower panel. Calibration marks on the right are: horizontal = 100 ms, vertical = 100 mV.

The effect of altering stimulation frequency on the action potential response to Hepes acidosis is shown in Fig. 4. The two longest action potentials occurred at a frequency of 30 pulses·min⁻¹ and the two shortest at 120 pulses·min⁻¹. The dashed lines represent the action potentials recorded after 15 min superfusion
in low pH Hepes Tyrode. The control records (solid lines) show the typical shortening of action potential duration and disappearance of the notch with increased frequency. At both frequencies Hepes acidosis slowed the rate of terminal repolarization and reduced $V_{\text{max}}$. The important point of this figure, however, is the absence of an acidic effect on phase 1 repolarization and the notch response to increased frequency, suggesting that slow inward current and transient outward current were unchanged. The same response to frequency changes was found with 15% CO$_2$ acidosis.

**Acidosis Produced by Lowering Bicarbonate**

In contrast to the above findings, if pH$_e$ was reduced to the same extent by lowering HCO$_3^-$ from 23.7 to 6.0 mM, dramatic changes in repolarization occurred as shown in Fig. 5. In this example the control action potential was recorded in normal Tyrode and test action potentials were recorded after 5 and 15 min of superfusion with 6 mM HCO$_3^-$ Tyrode (top panel). ADP$_{20}$, APD$_{90}$, and APD$_{90}$ were all increased, and the plateau was elevated markedly. However, there was little or no change in the point of initiation of the plateau, i.e., immediately following phase 1. In addition to these changes the rate of terminal repolarization slowed and $V_{\text{max}}$ fell in a manner similar to that seen in CO$_2$ and Hepes acidosis. This pattern was stable after 15 min superfusion and was fully reversible, as illustrated in Fig. 5 (lower panel). In 8 of 13 experiments the calcium activity of the low HCO$_3^-$ solution was compensated. Although calcium compensation had no effect on the aforementioned changes in repolarization to $-60$ mV, it did modify the response of terminal repolarization and APD$_{90}$. In 2 of 13 experiments low bicarbonate acidosis reduced $V_{\text{max}}$ to a value less negative than $-90$ mV, thus precluding the measurement of APD$_{90}$. Of the remaining 11, 4 were done with uncompensated calcium to give a mean increase in APD$_{90}$ of $92.4 \pm 5.3$ ms which differed significantly from the increase seen in the
compensated solution of 70.8 ± 5.3 ms. This difference may be explained by the action of calcium itself to decrease the rate of terminal repolarization (Temte and Davis, 1967).

In four experiments in which HCO₃⁻ concentration was lowered to 12 mM (calcium uncompensated), changes in action potential duration were about half those observed in 6 mM HCO₃⁻ Tyrode.

To summarize, decreasing pH₄ from 7.36 to 6.80 by either raising Pco₂ or by titrating an organic buffer in zero bicarbonate solution produced similar changes in repolarization, the major change being a slowing of the rate of terminal repolarization. In contrast, if the same pH change was made by keeping Pco₂ constant and lowering bicarbonate from 23.7 to 6.0 mM, in addition to a slowing of terminal repolarization, the action potential was markedly prolonged throughout repolarization.
Effects of Decreasing Bicarbonate at Constant pH_e

The findings thus far suggest that not only acidosis but also extracellular bicarbonate per se can influence the repolarization process. One way to further test this hypothesis is to determine the changes in repolarization that accompany a decrease in bicarbonate concentration while keeping pH_e constant. In this experiment the control solution was normal Tyrode, pH 7.34, and the test solution was 12 mM Hepes Tyrode, pH 7.37. As shown in Table I the two solutions contained the same concentration of Na⁺, K⁺, dextrose, Mg²⁺, and Ca²⁺. They differed only slightly in osmolarity and chloride concentration.

The changes in action potential repolarization seen upon changing from normal Tyrode to Hepes Tyrode (Fig. 6, upper panel) were very similar to those produced by 6 mM HCO₃⁻. Within 7 min APD₀ increased by about 40 ms with no change in terminal repolarization and only a slight fall in V_max. All changes in repolarization were complete by 15 min and remained unchanged for cells monitored continuously for 30 min in Hepes Tyrode. Longer continuous impalements were not made but records from multiple impalements after 1-3 h in Hepes were the same as those after 15-20 min. These effects were fully

![Figure 6](http://rupress.org/jgp/article-pdf/73/2/199/1247044/199.pdf)

**Figure 6.** Effects of lowering HCO₃⁻ on action potential repolarization. The record in the upper panel labeled T = 0 was taken in normal Tyrode solution (pH 7.34, HCO₃⁻ = 23.7 mM) just before changing to Hepes Tyrode (pH 7.37, HCO₃⁻ = 0). The record labeled T = 35 was taken from another cell just before returning to normal Tyrode. The remaining records were taken at the indicated elapsed time (minutes). All records were taken from the same cell during continuous impalement. Calibration marks are: horizontal = 100 ms, vertical = 100 mV.
reversible upon return to normal Tyrode as shown in Fig. 6 (lower panel). Just as in the 6 mM HCO₃⁻ experiments, going from normal Tyrode to Hepes Tyrode elevated the plateau but failed to alter its point of initiation. Furthermore, fast sweep recordings revealed that the rate of phase 1 repolarization was unchanged.

The increase in action potential duration observed in low HCO₃⁻ solutions tended to be smaller at higher stimulation frequencies. For example, mean increases (n = 6) in APD₆₀ after a change from normal to Hepes Tyrode (pH 7.37) were 53, 51, 42, and 34 ms at 50, 60, 90, and 120 pulses·min⁻¹, respectively. Expressed as percent of control values, the increases were 19, 19, 17, and 16%, respectively.

It is noteworthy that the low bicarbonate effects on repolarization as shown in Figs. 5 and 6 were very similar to those produced by reduced stimulation frequency (Fig. 4, control record). Except for the notch, a decrease in stimulation frequency elevates the plateau and lengthens the action potential in much the same manner as does lowering extracellular bicarbonate, suggesting that similar mechanisms are involved.

Supporting Experiments
Although the experiments described thus far suggest a role for HCO₃⁻ in action potential repolarization, the possibility that 12 mM Hepes per se somehow mimics the effects produced by 6 mM HCO₃⁻ cannot be ruled out. Furthermore, the difference in chloride concentration and osmolarity between normal and Hepes Tyrode must be considered. The influence of these factors was examined by comparing the changes in repolarization caused by 12 mM Hepes (pH 7.37) with those resulting from superfusion with 24 mM Hepes (pH 7.37) solutions (Table I, A and B). Note that solution A contains the same sodium concentration as normal Tyrode and 12 mM Hepes Tyrode but has a higher chloride concentration and osmolarity than normal Tyrode. The other solution, B, has the same chloride concentration as normal Tyrode but a lower osmolarity and sodium concentration than either normal Tyrode or 12 mM Hepes Tyrode. In spite of these differences, in the eight cells examined, 24 mM Hepes Tyrode produced the same changes in \( V_{\text{max}} \) and action potential duration as previously described for 12 mM Hepes. Furthermore, a change from 12 to 24 mM Hepes Tyrode had no effect on repolarization. These findings indicate that neither Hepes itself nor the differences in osmolarity and chloride concentration were responsible for the changes in repolarization. Moreover, they support the hypothesis that the changes were primarily the result of a decrease in extracellular bicarbonate concentration.

Summary
Tables III and IV provide statistical summaries of the experimental results just described. Only data from paired experiments where continuous impalements were maintained were analyzed. Several points may be drawn from these summaries.

Firstly, acidosis under conditions where extracellular bicarbonate concentration was either constant or zero (Table III, groups 1 and 5) produced only slight
TABLE III
EFFECTS OF THE BATHING SOLUTIONS ON APD<sub>20</sub>, APD<sub>60</sub>, AND APD<sub>90</sub>

| Group no. | Control       | Test                      | APD<sub>20</sub> | Mean difference | P  | APD<sub>60</sub> | Mean difference | P  | APD<sub>90</sub> | Mean difference | P  |
|-----------|---------------|---------------------------|-------------------|-----------------|----|-----------------|-----------------|----|-----------------|-----------------|----|
| 1         | Normal Tyrode | vs. 15% CO<sub>2</sub>   | -4.7±1.0          | (12)            | 0.001 | -2.9±0.9       | (12)            | 0.02 | 12.2±2.8        | (12)            | 0.01 |
| 2         | Normal Tyrode | vs. 12 mM HCO<sub>3</sub> | 18.2±3.1          | (4)             | 0.02 | 23.0±2.2       | (4)             | 0.01 | 47.6±3.5        | (3)             | 0.02 |
| 3         | Normal Tyrode | vs. 6 mM HCO<sub>3</sub>  | 31.8±1.6          | (13)            | 0.001 | 41.6±1.7       | (13)            | 0.001| 70.8±5.3        | (7)             | 0.001|
| 4         | Normal Tyrode | vs. Hepes (pH 7.37)       | 35.9±1.9          | (9)             | 0.001 | 41.5±2.5       | (9)             | 0.001| 55.8±3.3        | (8)             | 0.001|
| 5         | Hepes (pH 7.37)| vs. Hepes (pH 6.81)    | -0.1±1.5          | (12)            | 0.5   | 2.6±1.8        | (12)            | 0.2  | 27.1±4.3        | (12)            | 0.001|

APD values were obtained after 14-20 min equilibration in the test solutions. Values are reported as means ± SEM. The P values were determined from paired difference analysis. The number of cells studied is given in parentheses. Absolute values for APD<sub>20</sub>, APD<sub>60</sub>, APD<sub>90</sub> (milliseconds, mean, ± SEM) in control solutions were 199.0 ± 2.4, 253.5 ± 2.9, and 296.1 ± 3.5 in normal Tyrode (n = 41), and 242.6 ± 5.7, 300.5 ± 5.7, and 348 ± 5.3 in 12 mM Hepes Tyrode (n = 13).

TABLE IV
EFFECTS OF THE BATHING SOLUTIONS ON V<sub>max</sub>

| Group no. | Control       | Test                      | Mean difference | P  |
|-----------|---------------|---------------------------|-----------------|----|
| 1         | Normal Tyrode | vs. 15% CO<sub>2</sub>   | -1.8±0.5        | (21) | 0.01 |
| 2         | Normal Tyrode | vs. 12 mM HCO<sub>3</sub>| -4.5±0.9        | (4)  | 0.02 |
| 3         | Normal Tyrode | vs. 6 mM HCO<sub>3</sub> | -7.8±0.5        | (12) | 0.001|
| 4         | Normal Tyrode | vs. Hepes (pH 7.37)       | -3.1±0.5        | (9)  | 0.001|
| 5         | Hepes (pH 7.37)| vs. Hepes (pH 6.8)       | -3.7±0.5        | (12) | 0.001|

V<sub>max</sub> values were obtained after 14-20 min equilibration in the test solutions. Values are reported in means ±SEM. P values were determined from paired difference analysis. The number of cells studied is given in parentheses. The absolute value of V<sub>max</sub> (millivolts, mean ± SEM) in the control solutions was -97.8 ± 0.5 in normal Tyrode (N = 41) and -98.0 ± 0.7 in 12 mM Hepes Tyrode (N = 13).
changes in APD$_{20}$ and APD$_{90}$ with the only significant change being a small decrease during 15% CO$_2$ acidosis.

Secondly, decreases in HCO$_3^-$ always produced significant increases in APD$_{20}$ and APD$_{90}$, as well as elevations in plateau height. These effects occurred with (groups 2 and 3) or without (group 4) an accompanying decrease in pH. Because ADP$_{90}$ was relatively unaffected by acidosis, changes in APD$_{90}$ under conditions where HCO$_3^-$ concentration varied most likely reflect the effect of bicarbonate per se. On the basis of this assumption we have constructed a concentration-response curve for the relationship between extracellular HCO$_3^-$ concentration and APD$_{90}$. The data for the curve, shown in Fig. 7, were taken from Table III.

Thirdly, APD$_{90}$ increased significantly under all test conditions. The increase, however, varied and depended on two distinct responses, one to acidosis and the other to decreased HCO$_3^-$. In all cases where acidosis occurred (groups 1-3 and 5), the rate of terminal repolarization slowed, leading to a prolonged APD$_{90}$. In those cases where HCO$_3^-$ decreased (groups 2-4), the increase in APD$_{90}$ resulted primarily from the lengthening of the plateau phase. Note that the greatest lengthening of APD$_{90}$ occurred when both effects were present (group 3) and that the least degree of lengthening occurred when only acidosis was present (group 1). When a decrease in HCO$_3^-$ was the only condition present (group 4), the response was between the two extremes.

Finally, Table IV indicates that $V_{\text{max}}$ was reduced significantly under all test conditions with the greatest fall occurring when both pH$_e$ and bicarbonate concentration were reduced (group 3).

**Discussion**

The principal conclusions drawn from these experiments are: (a) acidosis (pH$_e$ 6.8) has little effect on Purkinje fiber repolarization other than to slow terminal
repolarization and cause a slight decrease in maximum diastolic potential, and (b) a reduction in extracellular bicarbonate has a marked effect on repolarization, causing an elevation of the plateau, an increase in action potential duration, and a reduction in $V_{\text{max}}$. These effects are independent of a change in extracellular pH.

**Effects of Acidosis**

Several previous studies have examined the effects of acidosis on repolarization. Hecht and Hutter (1965), for example, noted that reducing $pH_e$ from 8.9 to 5.9 in a Tris-buffered solution slowed all phases of repolarization in sheep Purkinje fibers, and the effect was more pronounced in elevated potassium (5.6 mM). Coraboeuf et al. (1976) published a record of a dog Purkinje fiber action potential showing that 20% CO$_2$ produced a slowing of terminal repolarization and a fall in maximum diastolic potential. Most other mammalian studies, done on rabbit atrial and ventricular myocardial cells, have reported that acidosis (respiratory or metabolic) either increased or did not change action potential duration (Vaughan Williams and Whyte, 1967; Jóhannsson and Nilsson, 1975; Poole-Wilson and Langer, 1975; Skinner and Kunze, 1976). Indirect evidence suggests that changes in potassium conductance may account for many of the action potential alterations seen during acidosis and alkalosis (Hecht and Hutter, 1965; van Bogaert and Carmeliet, 1973; Poole-Wilson and Langer, 1975).

In the present study, the major effect of acidosis was to slow the rate of terminal repolarization and reduce $V_{\text{max}}$. Because terminal repolarization extends from about $-60$ mV to $V_{\text{max}}$, it may be related to the $i_{\text{Ks}}$, the pacemaker current (Noble and Tsien, 1968). Diastolic depolarization, resulting mainly from the decline of $i_{\text{Ks}}$ (McAllister et al., 1975), is best seen in spontaneously firing cells or those stimulated at frequencies $<90$ pulses·min$^{-1}$. In the present experiments we did not examine directly the effect of acidosis on diastolic depolarization. However, both 15% CO$_2$ and Heps acidosis reduced $V_{\text{max}}$ more than $V_{\text{min}}$, indicating that diastolic depolarization was reduced which is in accord with the observations of Coraboeuf et al. (1976). Decreased diastolic depolarization might be explained by the action of acidosis to cause a slight negative shift in the $i_{\text{Ks}}$ activation curve (Brown and Noble, 1972) but such a shift cannot account for slowing of terminal repolarization. Thus it is difficult to reconcile the observed changes in terminal repolarization and $V_{\text{max}}$ with the decrease in diastolic depolarization based on a simple shift in the $i_{\text{Ks}}$ activation curve. Further experiments are needed to resolve this problem.

Acidosis is also reported to reduce slow inward current in mammalian and frog heart cells (Kohlhardt et al., 1976; Chesnais et al., 1975). This effect by itself would shorten action potential duration and depress the plateau. Judging from our action potential records there were no changes in phase 1, the notch, the plateau, or repolarization to about $-60$ mV that would suggest an effect of acidosis on either slow inward current or transient outward current.

**Effects of Bicarbonate**

The dramatic effect of reduced extracellular bicarbonate concentration $[\text{HCO}_3^-]_e$, on action potential repolarization suggests that this anion either
participates directly as a current during repolarization or acts to modulate other currents involved in repolarization. Anionic currents reported for Purkinje fibers include a small background chloride current and a large chloride current associated with phase 1 repolarization (Hutter and Noble, 1961; Dudel et al., 1967; McAllister et al., 1975). Recent evidence, however, raises questions concerning the role of chloride during phase 1 (Kenyon and Gibbons, 1977).

To postulate a bicarbonate current we have considered the simplest case in which bicarbonate ions cross the membrane as a linear function of membrane voltage, i.e.:

\[ i_{\text{HCO}_3^-} = g_{\text{HCO}_3^-} (V_m - V_{\text{HCO}_3^-}), \]  

where \( V_m \) is the instantaneous membrane potential (millivolts), \( V_{\text{HCO}_3^-} \) is the bicarbonate equilibrium potential (millivolts), and \( g_{\text{HCO}_3^-} \) is bicarbonate conductance. Under these circumstances the critical dependence of the putative bicarbonate current on the bicarbonate equilibrium potential may provide an explanation for our findings. Estimates of intracellular bicarbonate concentration \([\text{HCO}_3^-]_i\) vary from 10 to 17 mM (Lai et al., 1973a; Gonzalez and Clancy, 1975) placing \( V_{\text{HCO}_3^-} \) between \(-23 \) and \(-9 \) mV for an \([\text{HCO}_3^-]_e\) of 23.7 mM, assuming equal intracellular and extracellular activity coefficients. The outward flux of bicarbonate, expected whenever \( V_m \) is negative to \( V_{\text{HCO}_3^-} \), constitutes a depolarizing current that would prolong the action potential. Thus, for example, in the case where \( V_{\text{HCO}_3^-} \) is \(-23 \) mV \(([\text{HCO}_3^-]_e = 10 \) mM\), as repolarization proceeds beyond \(-23 \) mV outward flowing bicarbonate ions would reduce the net outward current associated with repolarization. A reduction in \([\text{HCO}_3^-]_e\) would increase bicarbonate efflux by causing a positive shift in \( V_{\text{HCO}_3^-} \). For example, a rapid change in \([\text{HCO}_3^-]_e\) from 23.7 to 6 mM would cause a shift in \( V_{\text{HCO}_3^-} \) from \(-23 \) to \(+13 \) mV. Under this circumstance \( V_m \) becomes negative to \( V_{\text{HCO}_3^-} \) earlier during repolarization, promoting even greater bicarbonate efflux and further prolongation of the action potential.

This assumes of course that \([\text{HCO}_3^-]_i\) remains fairly constant. In fact, recent findings indicate that although \([\text{HCO}_3^-]_i\) of heart tissue does fall during low bicarbonate acidosis, the change is relatively less than the change in \([\text{HCO}_3^-]_e\) resulting in a decreased \([\text{HCO}_3^-]_i/[\text{HCO}_3^-]_e\) ratio. Gonzalez and Clancy (1975), for example, using perfused rabbit hearts found that a 30-min reduction in \([\text{HCO}_3^-]_e\) from 21.3 to 6.5 mM, reduced \([\text{HCO}_3^-]_i\) from 17.0 to 8.4 mM, thus shifting \( V_{\text{HCO}_3^-} \) from \(-6 \) to \(+7 \) mV. In sheep Purkinje fibers a reduction in \([\text{HCO}_3^-]_e\) from 20 to approximately 2 mM reduced pH\(_i\) from 7.0 to a stable level of 6.8 within 15-40 min (Ellis and Thomas, 1976). We have calculated that this would correspond to a reduction in \([\text{HCO}_3^-]_i\) from 10 to 6 mM (calculated assuming \( \text{Pco}_2 = 40 \) torr, \( \text{pK} = 6.1 \), \( \text{CO}_2 \) solubility coefficient = 0.032 mM·liter\(^{-1}\)·torr\(^{-1}\)) and a shift in \( V_{\text{HCO}_3^-} \) from \(-18 \) to \(+29 \) mV. These results indicate that even with the slight decrease in \([\text{HCO}_3^-]_i\) expected in our experiments, \( V_{\text{HCO}_3^-} \) would still shift in a positive direction in response to reduced \([\text{HCO}_3^-]_e\). The fact that the prolongation of the action potential was stable after 20 min in low \([\text{HCO}_3^-]_e\) is compatible with a small but "limited" reduction in \([\text{HCO}_3^-]_e\). A "limited" fall in \([\text{HCO}_3^-]_i\) is expected in light of recent evidence.
indicating that bicarbonate is not passively distributed (Lai et al., 1973a,b; Gonzalez and Clancy, 1975; Ellis and Thomas, 1976; Poole-Wilson, 1978).

Fig. 8 shows the results of including a bicarbonate current in the Purkinje fiber action potential model. For computational purposes \( g_{\text{HCO}_3} \) was assumed to be at least as low (0.01 mmho·cm\(^{-2}\)) as the background chloride conductance used by McAllister et al. (1975) to compute the "standard" action potential. No adjustment was made for a change in \([\text{HCO}_3^-]_e\). The record labeled A is the "standard" action potential described by McAllister et al. (1975). Records B and C include a background bicarbonate current as described by Eq. 1. Record B simulates the case when \([\text{HCO}_3^-]_e\) was 23.7 mM, giving a \( V_{\text{HCO}_3} \sim \) of −23 mV. Note that bicarbonate current generated under these conditions caused the predicted slight increase in the duration of the standard action potential at potentials negative to −23 mV. Record C shows the simulated response when \([\text{HCO}_3^-]_e\) was reduced to 6 mM with a shift in \( V_{\text{HCO}_3} \) to +13 mV. Under these conditions, \( \text{APD}_{50} \) was 46 ms longer than in record B, an increase remarkably similar to the average increase of 41.6 ms observed experimentally for the same test conditions (see Fig. 5, upper panel, and Table III). Note also the similarity between simulated and experimentally induced effects on plateau height when changing from 23.7 to 6 mM \([\text{HCO}_3^-]_e\). It would appear, judging from the comparison of such computed and experimental records, that a shift in \( V_{\text{HCO}_3} \), with attendant changes in a putative background bicarbonate current, may provide a plausible explanation for our major experimental findings.

The possibility that bicarbonate may modulate cation permeabilities must also be considered. The influence of extracellular anions on cationic currents is not without precedence because a reduction in extracellular chloride is reported to reduce potassium permeability in calf Purkinje fibers (Carmeliet and Verdonck, 1977). Similarly, a time-independent background sodium current has been

![Figure 8](http://rupress.org/jgp/article-pdf/73/2/199/1247044/199.pdf)
reported to increase in Purkinje fibers when extracellular chloride is reduced (Dudel et al., 1967; McAllister et al., 1975). The present experiments do not allow for differentiating among these various choices. However, it seems unlikely that changes in transient outward current or slow inward current are responsible because of the insensitivity of phase 1 and the notch to decreases in bicarbonate. Moreover, if the plateau were elevated by increasing slow inward current, the action potential might be expected to shorten due to greater activation of the outward repolarization current, \( i_{\text{K}} \) (Kass and Tsien, 1976).

Finally, it should be emphasized that the present experiments demonstrate that the low bicarbonate effect on repolarization occurred independently of changes in pH. Furthermore, it is unlikely that the effect was related to change in pH. This conclusion is based on the observation that the two methods for lowering \([\text{HCO}_3^-]\) (normal to 6 mM HCO\(_3^-\) Tyrode, normal to Hepes Tyrode) although producing the same change in repolarization, are known to have opposite effects on pH in Purkinje fibers (Ellis and Thomas, 1976). Our experiments also demonstrate that although pH and HCO\(_3^-\) have complex actions on Purkinje fiber electrogenesis, the magnitude of the changes produced by acidosis alone (pH 6.8) is fairly small, which is striking considering that pH 6.8 is considered the extreme lower limit of arterial pH compatible with life in man (Woodbury, 1966).

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