Intracellular pH of Canine Subendocardial Purkinje Cells Surviving in 1-Day-Old Myocardial Infarcts

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A large reduction of intracellular potassium activity in depolarized subendocardial Purkinje fibers 24 hours after coronary artery ligation is accompanied by a much smaller increase in intracellular sodium activity. Similar intracellular ionic changes also occur during acute ischemia in ventricular muscle and are consistent with mechanisms based on intracellular acidification, which is known to occur in acutely ischemic muscle. To determine if canine subendocardial Purkinje cells 24 hours after myocardial infarction are also acidic, their intracellular pH, surface pH, and maximum diastolic potential (MDP) were measured with double-barrel pH-sensitive microelectrodes and compared with control fibers in noninfarcted hearts. In 12 mM bicarbonate Tyrode’s solution (5% CO₂-95% O₂), the average intracellular pH was not significantly different (p>0.25) for normal tissue (6.83±0.08, SD, MDP= -83.5±3.2 mV), for depolarized Purkinje fibers in infarct preparations during the first hour of superfusion (6.88±0.11, MDP= -47.8±11.8 mV), and for partially recovered Purkinje fibers in infarcts averaged over the third to sixth hours of superfusion (6.85±0.12, MDP= -74.5±9.6 mV). In 24 mM bicarbonate Tyrode’s solution, infarct intracellular pH during both the first hour of superfusion (7.08±0.13, MDP= -57.6±15.7 mV) and during the third to sixth hours of superfusion (7.06±0.15, MDP= -76.5±9.6 mV) was significantly alkaline (p<0.0005) compared with average control pH (6.92±0.12, MDP= 82.1±3.7 mV). In 24 mM bicarbonate Tyrode’s solution, the intracellular pH did vary with MDP (0.0032 pH units/mV). During superfusion of normal Purkinje fibers with hypoxic Tyrode’s solution, intracellular pH acidified by 0.22 pH units as they depolarized. Therefore, intracellular acidification does not seem to be a cause of the depolarization of subendocardial Purkinje cells 24 hours after myocardial infarction. (Circulation Research 1989;65:554-565)
whether the acidity could account for the K+ loss in the absence of equivalent Na+ gain.

It has been previously shown that acidic superfusate (pH 6.0) can depolarize free-running Purkinje fibers to levels comparable to those we measured in Purkinje cells from 1-day-old infarcts. Acidic pH is also known to alter membrane currents. In our previous study, we found that the reduction in potassium equilibrium potential accounted for, on average, only half of the membrane depolarization of the cells. Thus, pH acidification, if present, could explain a component of membrane depolarization in addition to the mechanism of K+ loss. By using double-barrel pH-selective microelectrodes in this study, we were able to correlate pHi with membrane depolarization on a cell-to-cell basis.

We also measured the pH just outside the cell membrane (e.g., the surface acidity [pHi]) of the Purkinje cells. Surface acidity is due to the accumulation of acid equivalents in a restricted extra-cellular space or unstirred layer within 200–300 µm of the cell membrane. It is a routine finding even in normal well-superfused tissue and is due to the inability of diffusion and extracellular buffers to control pH in the presence of this continuing efflux of acid equivalents. It is known to vary directly with the size of the preparation and the metabolic rate and to vary inversely with the external pH buffer capacity. The measurement is useful in that changes in pHi can indicate changes in the cell metabolism. However, it is also a required measurement for the calculation of the true transmembrane H+ gradient, which cannot be determined from the difference in pH of the bath and the bath pH (pHb) alone.

Materials and Methods

Surgical Production of Myocardial Infarction

Myocardial infarction was produced in 11 adult mongrel dogs weighing 9–15 kg by a two-stage ligation of the left anterior descending coronary artery. Twenty-three to 26 hours after ligation, dogs were reanesthetized with sodium pentobarbital (15–30 mg/kg i.v.), and the hearts were rapidly removed and immersed in 23°C Tyrode’s solution. Small variations in the percent CO2 content of the gas (±2%) caused variations in the pH of the Tyrode’s solution. It was therefore important to keep the pH constant. The pH was maintained at 7.40±0.05 with 5% CO2-95% O2.

The preparation was pinned at its edges (endocardium-up) to the Sylgard bottom of a Plexiglas tissue chamber (1 cm deep, 2 cm wide, and 5 cm long) and continuously superfused at a rate of 20 ml/min with Tyrode’s solution prewarmed to 37.5°C±0.5°C and gassed with 5% CO2-95% O2. Small variations in the percent CO2 content of the gas (±2%) caused variations in the pH of the Tyrode’s solution in the bath among experiments at any given HCO3− concentration since we did not attempt to titrate bath pH to any predetermined value. Tissue superfusion was started 10–15 minutes after dissection of the heart was begun. Spontaneous activity at rates of 30–120 per minute was evident in the infarct preparations immediately after they were mounted in the tissue chamber and continued for several hours with gradually diminishing rate. When the spontaneous rate decreased below 15 per minute, the preparations were stimulated at an 800-msec cycle length with 3–5-msec square pulses at twice diastolic threshold via Teflon-coated bipolar silver electrodes attached to a stimulus isolator. Noninfarcted preparations were stimulated at a cycle length of 800 msec for the entire experiment. The bath was grounded via a 2 M KCl/2% agar bridge.

Construction and Calibration of Double-Barrel pH-Sensitive Microelectrodes

We used double-barrel pH-sensitive microelectrodes to compare the maximum diastolic potential (MDP) and pH, for each cell that was impaled, as well as discriminate between Purkinje cells and the few ventricular
muscle cells that are sometimes found below the superficial Purkinje layers in the infarct preparation. 10,11 Double-barrel micropipettes were made from Corning 7740 borosilicate, theta style I, double-barrel glass tubing with an outer diameter of 2.5±0.2 mm (William Dehn-R&D Optical, Bethesda, Maryland). The properties of these micropipettes have been previously described in detail. 9 One barrel of the double-barrel micropipette was briefly exposed to triethylene glycolborate at 23°C (Eastman Kodak, Rochester, New York) and then baked (120°C for 4 hours). A drop of H+ neutral carrier oil containing tri-n-dodecylamine (Fluka 82500, Fluka Chem. Corp., Hauppauge, New York) was placed in the silanized barrel using a 50-μl Hamilton syringe to make a 2–4-mm column at the tip. 21,22 After air bubbles in the column were dissolved by gentle back pressure, the oil column was backfilled with aqueous solution containing buffer (50 mM sodium phosphate) with a pH of 7.00 (Fisher Chemicals). A slowly tapering micropipette was also filled with this solution, and its tip was broken to a 3–8-μm diameter. The first centimeter of this micropipette was broken off, placed in the opened end of the H+-selective barrel, and partially inserted into the oil column. 23 This brought aqueous backfill to within 0.2–0.7 mm of the ISE tip and reduced the ISE’s resistance, thereby speeding up its electrical response time. 23

The reference barrel was filled with 1 M KCl. This allowed us to reduce somewhat the high osmolality in the reference barrel, which might have altered the ionic milieu at the nearby tip of the H-ISE barrel. In previous experiments, we had assessed the effect of using 1 M KCl backfill by employing double-barrel microelectrodes that had one barrel filled with 1 M KCl and the other with 3 M KCl. We determined that the MDP was underestimated by the 1 M KCl–filled barrel compared with the 3 M KCl–filled barrel by an average of 1.5 mV. 9 To correct for this error, the average MDP was made more negative, and the average ISE differential potential (difference between barrel potentials) was increased by 1.5 mV. This differential potential, V_{diff}, is the change in potential caused by hydrogen ion activity at the electrode tip.

Three different pH solutions with a pH of 6.00, 7.00, and 7.40±0.01 were used to determine the slope of the response to the H-ISEs (V_{H} change/pH unit) at 23°C. We corrected the slope value for a bath temperature of 37.5°C. We added 110 mmoles KCl/liter to the National Bureau of Standards pH buffers (certified±0.01 at 25°C, Fisher Chemical), which contained 50 mM sodium phosphate or sodium phthalate. The pH of each 0.16 M ionic strength pH buffer was then adjusted to within 0.01 units of the desired pH at 23°C using a macro glass-combination pH electrode and digital pH meter (Fisher Accumet model 630). H-ISE errors due to known intracellular and extracellular ion interference were likely to be insignificant because the pH-sensitive oil is highly H+–selective: H+–K+ =6×109, H+–Na+ =2×1010, and H+–Ca2+ =1011. 2 Therefore, between pH 6–8 the intracellular pH was calculated from the Nernst equation:

\[ p\text{H} = \frac{(V_{H+VR} - V_{H})/S + pH_b}{1} \]

where p\text{H} is the intracellular pH (pH defined as log H+ activity), V_{H+VR} is the value of V_{H} in the bath, V_{H} is the value of V_{H} in the cell, S is the slope of the H-ISE at 37.5°C (unit change of pH (pH range 6.0–7.4). S averaged 58 mV (range 48–63 mV) similar to previously reported values. 24 pH_b is the established bulk-phase bath pH, which is independently determined.

**Experimental Procedure**

Data were obtained from as many subendocardial Purkinje cells as possible for a period of up to 8 hours after the tissue superfusion was initiated. Three potentials were recorded simultaneously during all experiments with the double-barrel pH-sensitive microelectrode (Figure 1; also see Dresdner et al): 25 the reference barrel potential (V_{R}), the pH-sensitive barrel potential (V_{H}+V_{H}), and V_{H}. The change in V_{H} relative to its value in the bath (where pH was known) was used to calculate measured pH at the H-ISE tip. The reference barrel potential followed the membrane and action potentials like a conventional microelectrode, except for some attenuation in the rate of rise of the upstroke and the value of the overshoot. Thus, the V_{R} output allowed us to distinguish cell type as well as measure MDP.

That the data we present is from Purkinje fibers and not ventricular muscle cells is based on the following factors: 1) Our previous ultrastructural studies have shown that the intact surviving cells in 1-day-old infarcts are Purkinje and only rarely muscle, particularly in the apical region. 20 This is in agreement with electrophysiological studies on equilibrated preparations (superfused for more than 1 hour) in which action potentials were recorded as the microelectrode was advanced downward through the subendocardium. Mostly Purkinje fibers survive in the region of transmural infarction as indicated by the action potential shape. 10 2) The shape of the transmembrane action potential recorded with the reference barrel of the H-ISE in our experiments could be identified as Purkinje-like in cells that had MDPs greater than -70 mV.

The earliest impalements with the double-barrel H-ISEs were made within 15–20 minutes after the heart was removed. The H-ISE was oriented perpendicular to the endocardial surface and advanced into the cells of the first or second subendocardial Purkinje cell layer. The location and time at which each impalement was made was noted to permit us to separate time-dependent and regional variations in MDP and p\text{H}. To define the region in which the impalement was obtained, the long axis of the preparation was divided into three zones of roughly equivalent area, which we called the apical, middle, and basal zones. The apical zone always consisted...
of transmural infarct with surviving subendocardial Purkinje cells on the subendocardial surface. In this region, action potentials could only be recorded from one to three layers of excitable cells below which there was electrical quiescence. This was the region of most severe ischemic damage as we have previously documented; the reduction of MDP is the greatest (as much as 30 mV during the first hour of superfusion). There is also marked spontaneous diastolic depolarization. The middle zone consisted of intermingling transmural and nontransmural infarct and had less severe reductions in MDP. The basal zone was not infarcted (see prior description of histology). We report here only data from impalements in the most ischemic apical zone.

The characteristics of the recording had to meet several criteria to be included in the data (Figure 1). First, the output of both barrels had to undergo a crisp, rapid, and continuous transition to a stable maximum deflection as the cell impalement was obtained. Second, the potential changes recorded through both barrels during and after sequential beats had to be identical, indicating a mechanically stable ISE impalement. Third, we required an essentially constant (less than 1 mV change) $V_H$ trace in late diastole. To further test for adequate common mode rejection in the differential trace, we often prolonged diastole by omitting several stimulated beats (Figure 1). Fourth, we required that $V_H$ and $V_R$ return to within 2 mV of their initial values when the ISE was removed from the cell and returned to the bath. If these criteria were met, then the late diastolic values of $V_H$ were used to calculate pH. The MDP was obtained from the $V_R$ trace just after the action potential.

**Statistical Analysis of Data**

Data are expressed as mean±SD. The MDP and pH were subjected to analysis of variance. Scheffe's test was used to test for differences between means for multiple groups. For simpler comparisons, the appropriate t test was used. Simple linear regressions were calculated as the sum of least squares and the degree of the association between the variables was measured by correlation coefficient. When comparing the slopes and intercepts of two simple linear regressions, a t test was used. Differences with $p<0.05$ were considered significant.

**Results**

Intracellular pH of Subendocardial Purkinje Cells in Regions of Myocardial Infarction: Comparison With Normal Purkinje Fibers

We measured pH in Purkinje fibers surviving in the apical third of the preparations from infarcted...
TABLE 1. pH Values for Control and Infarct Purkinje Fibers Exposed to 12 mM Bicarbonate Tyrode’s Superfusate

<table>
<thead>
<tr>
<th></th>
<th>Infarct PF (t&lt;1 hour)</th>
<th>Infarct PF (t&gt;1 hour)</th>
<th>Control PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$, n</td>
<td>5,24</td>
<td>6,70</td>
<td>3,40</td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>-47.8±11.8*</td>
<td>-74.5±9.6*</td>
<td>-83.7±3.2</td>
</tr>
<tr>
<td>pH$_b$</td>
<td>7.02±0.09†</td>
<td>7.04±0.04</td>
<td>7.05±0.05</td>
</tr>
<tr>
<td>pH$_i$</td>
<td>6.88±0.11</td>
<td>6.85±0.12</td>
<td>6.83±0.08</td>
</tr>
<tr>
<td>pH$_b$-pH$_i$</td>
<td>0.14</td>
<td>0.19</td>
<td>0.22</td>
</tr>
<tr>
<td>pH$_b$</td>
<td>6.93±0.10‡</td>
<td>6.94±0.09§</td>
<td>7.00±0.05</td>
</tr>
<tr>
<td>pH$_i$-pH$_b$</td>
<td>0.05±0.09*</td>
<td>0.09±0.11§</td>
<td>0.17±0.07</td>
</tr>
<tr>
<td>pH$_b$-pH$_i$</td>
<td>0.09</td>
<td>0.10</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Values are mean±SD. PF, Purkinje fibers; $N$, number of tissue preparations; $n$, number of cell measurements; MDP, maximum diastolic potential of the cells; pH$_b$, bath pH; pH$_i$, intracellular pH; pH$_i$, extracellular surface pH; t<1, less than 1 hour superfusion time; t>1, more than 1 hour superfusion time.

*p<0.0005 compared with control.

†The lack of significant statistical difference between infarct pH$_i$ and control pH$_i$ takes into account the fact that 0.03 pH units of the difference was due to the slightly more acidic bath pH (see Figure 2). The remaining corrected pH difference of 0.04 was not statistically different.

§p<0.01 compared with control.

hearts (the most ischemic area) and compared the values with pH$_i$ measured in randomly located subendocardial Purkinje fibers in noninfarcted preparations. Measurements were made in the infarcts during the first hour of superfusion (Tables 1 and 2) since the effects of the prior period of prolonged ischemia in situ are greatest at this time, as indicated by the large depolarization of the MDP (Tables 1 and 2). Experiments were done in both 12 and 24 mM HCO$_3^-$-containing Tyrode’s solution and thus at different values of pH$_b$ (Tables 1 and 2).

A comparison of the mean values of pH$_i$ and pH$_b$ for the control Purkinje fibers in 12 and 24 mM HCO$_3^-$ Tyrode’s superfusate, included in Tables 1 and 2, shows that the mean pH$_i$ of normal subendocardial Purkinje fibers was slightly acidic relative to the pH$_b$. A linear regression of pH$_i$ versus pH$_b$ was obtained for all control experiments in 12, 18, and 24 mM HCO$_3^-$ Tyrode’s and is plotted in Figure 2A. It is significant ($r=0.57$, $p<0.0005$; $n=79$) with a slope of pH$_i$/pH$_b$=0.48, similar to the previously published value of 0.40 for free-running Purkinje fibers. These data show that the acidic relation of pH$_i$ to pH$_b$ extended over the entire range of pH$_b$ values (6.9-7.4) that occurred in each of the experiments. When the regression is extrapolated to a pH$_b$ of 7.4, a frequently used value in the literature, our calculated pH$_i$ was 7.0, similar to the values reported by others in free-running Purkinje fibers.

pH$_i$ of the depolarized Purkinje cells in the infarcts (t<1 hour) was also acidic compared with pH$_i$ (Tables 1 and 2). However, it was not acidic compared with control values, as predicted, but was alkaline when the mean values for pH$_i$ were compared in the 12 and 24 mM HCO$_3^-$-containing superfusates (Tables 1 and 2). This relative alkalinity of ischemic Purkinje fibers attained statistical significance ($p<0.0005$) only in the 24 mM HCO$_3^-$ solution, where there was a difference of 0.16 pH units with controls. The relative alkaline pH$_i$ was not a result of any differences in pH$_b$ among the infarct and control experiments. As in the controls, the pH$_b$ varied among experiments on the infarct.

TABLE 2. pH Values of Control and Infarct Purkinje Fibers Exposed to 24 mM Bicarbonate Tyrode’s Superfusate

<table>
<thead>
<tr>
<th></th>
<th>Infarct PF (t&lt;1 hour)</th>
<th>Infarct PF (t&gt;1 hour)</th>
<th>Control PF</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$, n</td>
<td>4,26</td>
<td>4,33</td>
<td>5,39</td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>-57.6±15.7*</td>
<td>-76.5±9.6‡</td>
<td>-82.1±3.7</td>
</tr>
<tr>
<td>pH$_b$</td>
<td>7.34±0.04</td>
<td>7.34±0.04</td>
<td>7.30±0.09</td>
</tr>
<tr>
<td>pH$_i$</td>
<td>7.08±0.13*</td>
<td>7.06±0.15*</td>
<td>6.92±0.12</td>
</tr>
<tr>
<td>pH$_b$-pH$_i$</td>
<td>0.26</td>
<td>0.28</td>
<td>0.38</td>
</tr>
<tr>
<td>pH$_b$</td>
<td>7.24±0.06</td>
<td>7.23±0.09</td>
<td>7.20±0.08</td>
</tr>
<tr>
<td>pH$_i$-pH$_b$</td>
<td>0.16±0.11†</td>
<td>0.17±0.12†</td>
<td>0.28±0.11</td>
</tr>
<tr>
<td>pH$_b$-pH$_i$</td>
<td>0.10</td>
<td>0.11</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Values are mean±SD. PF, Purkinje fibers; $N$, number of tissue preparations; $n$, number of cell measurements; MDP, maximum diastolic potential of the cells; pH$_b$, bath pH; pH$_i$, intracellular pH; pH$_i$, extracellular surface pH; t<1, less than 1 hour superfusion time; t>1, more than 1 hour superfusion time.

*p<0.0005 compared with control.

†p<0.01 compared with control.
preparations, and $pHi$ correlated significantly with $pHe$ ($r=0.79, p<0.001; n=50$; solid circles and upper regression line, Figure 2A). The slope of the line ($pHi/pHe=0.68$) is not significantly different ($p>0.07$) from that of the control line, but the intercept on the $pHe$ axis is different ($p<0.001$). Consistent with these findings, the regression line for the infarct preparations was alkaline to the line for normals over this range of bath $pHe$ values.

**Extracellular pH of Subendocardial Purkinje Cells in Regions of Myocardial Infarction: Comparison With Normal Purkinje Cells**

We also measured the extracellular pH at the surface of the Purkinje cells ($pHs$) in both the normal and infarct preparations since $pHe$, to a certain extent, reflects properties of cellular metabolism (see "Discussion"). Surface acidification relative to bath pH has been reported for sheep Purkinje fibers and ventricular muscle and is measured just before penetration of the cell membrane by the microelectrode (within 200 μm of the tissue surface) (Figure 1). We observed this acidification at the surface of Purkinje cells in both normal and infarct preparations in both the 12 and 24 mM HCO$_3^-$ Tyrode’s (Tables 1 and 2). The values for noninfarcted and infarcted preparations were not significantly different in 24 mM HCO$_3^-$ Tyrode’s (Table 2). The $pHe$ also varied with $pHe$ in the individual experiments on normals and infarcts and was significantly correlated to it. Therefore, $pHe$ was also significantly correlated to $pHi$ for both normals and infarcts (Figure 2B). The slopes of the two linear regression lines for $pHi$ versus $pHe$ were not significantly different ($p>0.50$) than those for $pHi$ versus $pHe$ for both types of tissue. By quantifying $pHe$, we were then able to determine the pH gradient across the cell membrane ($pHe–pHi$). The pH gradient is useful for understanding the mechanisms underlying cellular control of $pHi$ and whether these mechanisms differ in normal fibers and fibers from infarcted hearts. At both bicarbonate concentrations and all values of $pHe$, the transmembrane pH gradient of the infarct Purkinje cells during the first superfusion hour was significantly reduced compared with normal cells (Tables 1 and 2).

**Relation of $pHi$ to Maximum Diastolic Potential in Subendocardial Purkinje Cells in Infarcts**

One of the goals of our study was to determine if any of the depolarization of the MDP of Purkinje cells in infarcts could be related to changes in $pHi$, since the depolarization is more than can be explained by the decrease in the potassium equilibrium potential that we previously measured. We did this by plotting the relation between MDP and $pHi$ for all impalements of infarct Purkinje fibers both during the early period of study ($t<1$ hour) and at later times ($t>1$ hour). The MDP of the Purkinje cells surviving in infarcts gradually increased over time during tissue bath superfusion in both 12 and 24 mM HCO$_3^-$ (Figure 3) although it remained less than normal after 6 hours of superfusion (Tables 1 and 2). We obtained data during this entire period, giving us a wide range of membrane potentials at which $pHi$ was measured (approximately -40 to -80 mV). If $pHe$ in some way influenced the level of MDP, it would be expected to change as well during superfusion. Conversely, if MDP changed $pHe$, we would also expect to see a correlation. As a baseline, we compared MDP and $pHi$ for all impalements of control Purkinje fibers and found no significant correlations over a range of membrane potentials of -77 to -93 mV ($r=0.20; p>0.20$).

In 12 mM bicarbonate Tyrode’s, the correlation between MDP and $pHe$ in infarct Purkinje cells over the wide range of membrane potentials was not significant ($r=0.071, p=0.50$; the linear regression
with MDP as the dependent variable and pH, as the independent variable had a slope that was not significantly different from zero (Figure 4A). Likewise, the mean pH, measured for all cells after 1 hour of superfusion in 12 mM HCO₃⁻ was not significantly different than the mean pH, at less than 1 hour despite a large hyperpolarization of the membrane potential (Table 1). It was also not different from control cells. The pH, also did not change after the first hour of superfusion and was still slightly acidic compared with control (Table 1).

For the experiments in 24 mM bicarbonate Tyrode's, there was a significant correlation between MDP and pH, with pH, more alkaline in the more depolarized cells (r = −0.37, p < 0.005 for regression equation pH, = 0.0032 MDP [mV] + 7.28; n = 57; see Figure 4B). Despite this correlation, the mean pH, during the first hour of superfusion was not different than the mean pH, for later times (it remained alkaline compared with control) although MDP did hyperpolarize with time (Table 2). The pH, did not change after the first hour of superfusion and continued to be no different than the control pH, (Table 2). The transmembrane pH gradient (pH,−pH,) of the infarct Purkinje cells after the first hour of superfusion was not significantly different than the average value during the first hour of superfusion and was still less than the control gradient (Table 2).

Effects of Acute Hypoxia on pH, of Normal Subendocardial Purkinje Cells

We also did experiments subjecting subendocardial Purkinje cells in noninfarcted preparations to hypoxia to determine the pH, changes that occur in acutely hypoxic cells. Hypoxia was established by
Canine Purkinje Cell pH After Myocardial Infarction

Figure 4. Relation between intracellular pH (pHi) and maximum diastolic potential (MDP) measured during individual cell impalements for apical Purkinje fibers surviving in infarcted hearts. The open circles are impalements during the first hour of superfusion and the filled circles are for later times. The points for 12 mM HCO₃⁻ Tyrode's (panel A) and 24 mM HCO₃⁻ Tyrode's (panel B) are plotted separately along with the regression lines. In 24 mM HCO₃⁻ Tyrode's, pHi tended to be more alkaline in the more depolarized cells. Its regression equation was pHi = 0.0032 MDP (mV) + 7.28 (r = -0.37, p < 0.005; n = 57). The 12 mM HCO₃⁻ regression line was not significantly different than zero (p = 0.5; slope = 0.0005 pHi/MDP [mV]). In the experiments done in 24 mM HCO₃⁻ Tyrode's, the pHb averaged 7.34 for cells from infarcts versus 7.30 for controls. Using the regression line in Figure 2A, the difference is expected to increase infarct pHi by 0.03 units. Correcting for this value still does not alter the finding that infarct pHi is alkaline to control.

Figure 5. Effects of hypoxic 18 mM bicarbonate Tyrode's superfusate (gassed with 95% N₂-5% CO₂) on intracellular pH (pHi) of two different normal subendocardial Purkinje cells. pH is plotted on the y axis versus time in minutes on the x axis. The period of exposure to the hypoxic Tyrode's (solid line on top of graph) was followed by a return to oxygenated Tyrode's solution.

Discussion

Intracellular pH in Subendocardial Purkinje Fibers From Normal and Infarcted Hearts

The data we have presented show that, over a range of extracellular pHb values from 7.0 to 7.4, the pHi of canine subendocardial Purkinje cells surviving in 1-day-old infarcts is not acidic in vitro when compared with normal Purkinje cells from noninfarcted hearts but actually is slightly alkaline. This rules out the possibility that the marked depolarization of the fibers recently removed from infarcted hearts is due to intracellular acidification.

Surface pH in Subendocardial Purkinje Cells From Normal and Infarcted Hearts

The extracellular pHs, measured just before impalement, was acidic to the bulk superfusate for fibers from both normal and infarcted hearts. This surface acidity phenomenon has been described in some detail for free-running sheep Purkinje fibers and is attributed to cellular efflux of acid equivalents. It is detectable at a distance of about 200 μm from the surface of the fibers and increases to a maximum at the cell surface. We found a similar H⁺ gradient with maximum acidity of 0.05–0.1 pH units, comparable to values reported in sheep.
Since both normal and infarct preparations were superfused at the same flow rates and H\(^+\) buffer strengths, the similarity in surface pH gradients implies that the steady-state rate of production of acid equivalents in the two tissue types was similar.\(^{18}\) A complicating factor is that in the normal preparation there is a live mass of ventricular muscle beneath the subendocardial Purkinje fibers. This muscle, which is not present in the preparation from infarcted hearts,\(^{9-12}\) may be an additional source of surface acidity. The H\(^+\) leaving the ventricular muscle cells, which is not bound by extracellular buffers, will diffuse toward the surface of the preparation\(^{34}\) and augment the surface acidity.

When Purkinje fibers are made acutely hypoxic, there is a clear increase in the rate of generation of acid equivalents, which causes a decrease in both pH\(_i\) and pH\(_j\).\(^{18,24}\) (also, authors’ unpublished results). Neither response is seen for subendocardial Purkinje fibers from 1-day-old infarcts.

**Significance of the Transmembrane pH Gradient**

**\((pH_i - pH_j)\) in Subendocardial Purkinje Cells From Normal and Infarcted Heart**

A significant difference between the Purkinje fibers in infarcts and normal Purkinje fibers was that the transmembrane pH gradient (the gradient between pH\(_i\) and pH\(_j\)) was reduced in the infarcts. The importance of the transmembrane pH gradient is that it determines the H\(^+\) equilibrium potential (E\(_{H}\)), where according to the Nernst equation $E_{H} = RT/F \ln (aH^+/aH^+)$, where $aH^+$ and $aH^+_i$ are the surface and intracellular activities for the hydrogen ion, respectively. Since pH is defined as $-\log (aH^+)$, $E_{H}$ can be readily shown to be $(2.303RT/F)(pH_i - pH_j)$. Whether $E_{H}$ is calculated for the transmembrane H\(^+\) gradient or the intracellular-to-bath pH gradient, its values are generally within the range of 0 to −20 mV.

Changes in $E_{H}$ cause changes in both the passive H\(^+\) influx\(^{35}\) and the activity of the Na-H exchanger\(^{26}\) as well as reflecting changes in the flux caused by both processes. The passive H\(^+\) influx is dependent on the driving force for H\(^+\), which is the difference between the transmembrane potential ($V_m$) and $E_{H}$. Using our measured values for membrane potential, the driving force for H\(^+\) is calculated to be more negative for normal fibers than fibers from infarcts during the first hour of superfusion, by 28.5 mV (−73.3 vs. −44.8) for 12 mM bicarbonate Tyrode’s and by 17.3 mV (−65.3 vs. −48.0) for 24 mM bicarbonate Tyrode’s. Thus, for the same membrane H\(^+\) permeability, one would expect a larger passive H\(^+\) influx for normal fibers. This would contribute to a more acidic pH\(_i\) in normal cells. However, only Purkinje fibers from infarcts superfused in 24 mM bicarbonate Tyrode’s showed any correlation between pH\(_i\) values and MDP (0.0052 pH units/mV), where the MDP is a major determinant of H\(^+\) driving force. Increases in H\(^+\) influx make $E_{H}$ larger and more negative since H\(^+\) influx depletes surface H\(^+\) (pH\(_i\) becomes more basic) while increasing cellular H\(^+\) (more acidic). This will in general reduce the inward driving forces on H\(^+\) since $V_m$ is nearly always more negative than $E_{H}$ at rest (while during the action potential the H\(^+\) efflux driving force is increased).

There is also interaction between exchangers and the transmembrane pH gradient.\(^{26}\) The Na-H exchanger would be at equilibrium if the equilib-rium potentials for Na\(^+\) and H\(^+\) were equal. Using $aNa_i$ values reported for normal subendocardial Purkinje fibers and Purkinje fibers surviving in 1-day-old infarcts,\(^9\) we calculate sodium equilibrium potential ($E_{Na}$) values of +66.2 and +52.6 mV for normals and infarcts, respectively. The Na-H exchanger is, therefore, predicted to be at equilibrium for these $E_{Na}$ values when pH\(_i\) values are 1.07 and 0.85 units alkaline to the bath. If the exchanger equilibrium were to determine pH\(_j\), the infarct pH\(_j\) values would be 0.23 units more acidic than control values. However, previously we have determined that $aNa_i$ in the infarct preparation\(^9\) falls to near normal levels during 6 hours of tissue bath superfusion, while the present study found that pH\(_i\) remains constant or becomes slightly acidic depending on the Tyrode’s buffer. Thus, pH\(_i\) does not appear to be influenced by changes in the displacement of Na-H exchange from equilibrium.

In summary, therefore, the direct effects related to membrane potential depolarization would act to make the Purkinje fibers from infarcts more alkaline; the effects due to the reduced transmembrane Na\(^+\) gradient would act through Na-H exchange to make the fibers more acidic. The outcome would depend also on the affinities of the Na-H exchanger binding sites for H\(^+\) and Na\(^+\), the H\(^+\) affinities of the intracellular H\(^+\) buffers, the maximum velocity of the Na-H exchanger, and the cell membrane’s H\(^+\) permeability. The fact that the pH\(_i\) is not that different in the normal and infarct preparations implies either a fortuitous similarity in balance between the passive and active H\(^+\) transport, or a set point due to a steep dependence of an exchanger on pH. The set point is presumably mediated through the binding affinity of the intracellular H\(^+\) site of the Na-H exchanger, which we assume to have a steep dependence on pH.\(^{36}\) The rate of metabolic generation of acid equivalents does not seem to play a major role here based on the lack of significant acidification of pH, as discussed previously.

**Comparison of Intracellular pH in Acute Versus Chronic Ischemia**

Our results indicate that a fundamental difference exists between the subendocardial Purkinje cells surviving in infarcts and acutely ischemic and hypoxic ventricular muscle and Purkinje cells. We have shown here that pH\(_i\), of acutely hypoxic subendocardial Purkinje cells is markedly acidic, which is similar to results found using H-ISEs in free-running sheep Purkinje fibers.\(^{24}\) In perfused rat heart, nuclear magnetic resonance measurements
show that pH decreases from 7.05 to 6.20 during global myocardial ischemia. Even though pH is not a good quantitative measure of production of acid equivalents due to intracellular buffering and variations in extrusion rates, there is a marked qualitative difference in the response to acute ischemia and/or hypoxia (acidification) versus the response to survival in infarcted hearts (alkalinization). The extracellular pH responses are also suggestive of increased generation of acid equivalents in the former case. Extracellular acidification in cardiac muscle during acute ischemia has been well documented.

There are various sources of proton production during hypoxic as well as normoxic metabolism. The rate of generation of acid equivalents increases during hypoxia and ischemia accounting for the decrease in pH. Although ischemia and hypoxia may differ in their production of H+ and lactate due to variation in the availability of substrate or feedback of accumulated by-products on anaerobic glycolysis, the pH response in both cases is much greater in magnitude and of opposite polarity to the pHj response in Purkinje fibers surviving in 24-hour-old infarcts. It is clear that these metabolic alterations either 1) do not occur in subendocardial Purkinje fibers after a coronary occlusion, 2) do occur acutely but do not persist in situ for 24 hours after coronary occlusion (they may reverse after the acute ischemic period), or 3) if they do persist in situ, they rapidly reverse during in vitro superfusion with oxygenated Tyrode’s. With regards to 1), subendocardial Purkinje fibers may not become ischemic as ventricular muscle after coronary occlusion since they are separated from normal oxygenated cavity blood only by a thin endothelial sheath and are at most one to three cell layers away from the endocardial surface. Alternatively, the effects of ischemia may occur gradually, judging by the 3–6 hours that are necessary for the appearance of lipid droplets after coronary occlusion. However, the Purkinje fibers are in contact by extracellular diffusion with a large volume of infarcting ventricular muscle. Thus, even if they are not truly ischemic nor hypoxic, they still cannot avoid exposure to a wide range of substances released over many hours from the adjacent ischemic tissue. With regards to 2), if pHj acidifies in vivo earlier and then normalizes, a mechanism must be described whereby the reduced aK does persist after the normalization (see below). With regards to 3), if metabolic alterations do reverse during superfusion, the reversal must be considerably faster than the reversal of the aK, reduction, aNa, elevation, or membrane potential depolarization, since all these perturbations could be measured in vitro, and slowly recovered during up to 6 hours of tissue bath superfusion. Comparable displacements of aK, and aNa with reversible experimental interventions in normal hearts are known to recover within less than an hour (see also Chapman). Experimental acidification of pHj during tissue bath hypoxia in normal Purkinje and muscle fibers recovers 15–20 minutes after reoxygenation (see Figure 5). Recovery from acid loading due to abrupt changes in perfusate CO2 and bicarbonate concentration is complete within 15–40 minutes in sheep Purkinje fibers and proceeds with similar time constants (3–5 minutes) in canine subendocardial Purkinje fibers from normal and infarcted hearts using ammonium chloride acid loading techniques (authors’ unpublished observations).

The implication is that pHj acidification in vivo may normalize too quickly to be measured in vitro based on evidence from reversible interventions in normal fibers. However, the in situ changes in aK, and aNa, from infarcted hearts appear to normalize more slowly than they do following reversible Na-K pump blockade interventions in normal tissue. This indicates that normalization of a persistent pHj acidification in vitro is not the rate limiting step in the partial normalization of aK, and aNa. Furthermore, whatever cellular changes are allowing for normalization of aK and aNa are not also slowing recovery of pHj in the tissue bath if it were acidic initially in vivo.

Mechanisms of K+ Loss in Purkinje Fibers Surviving in Infarcts

The initial attractiveness of the hypothesis that K+ loss from Purkinje fibers in infarcts was due to cell acidity was that the gain of Na+ (as estimated from the increase in aNa) was only about 10% of the loss of K+ (estimated from the reduction in aK, and assuming no change in Na+ and K+ activity coefficients). Thus, equal and opposite movements of cations did not preserve electroneutrality. In fact, the total aK, plus aNa, activities of the cells fell from a value of 121 mM in normals (nearly equal to the value of 115 mM in the superfusate) to 77 mM in cells from 1-day-old infarcts during the first hour of superfusion. Assuming electroneutrality is preserved during the net loss of cations, the same number of milliequivalents of anions per liter cell cytoplasm would have to be lost from the cell. Larger losses of K+ and gains of Na+ have been seen using pharmacologically induced Na-K-ATPase pump blockade. However, in this case, the total cation activity was preserved.

The hypothesis based on cell acidity is essentially that intracellular electroneutrality is preserved by a balancing anion loss (equal to the K+ loss) rather than a cation gain. (This anion loss could occur passively through anion channels or any other anion transfer [exchanger, transporter] so long as such phenomena have an electrogenic component.) This hypothesis can still account for the loss of K+ in the subendocardial Purkinje fibers but not for the maintenance of lowered aK, once it is lost. A reconciliation of the acidification hypothesis with our findings might hold that cellular K+ loss occurred during acute ischemia and was accompanied by intracellular acidification; however, the acidification then subsided by 24 hours due to unspecified metabolic adjustment, leaving the intracellular aK,
fibers, which is 76% of the total aKj reduction seen at 24 hours.43

It is important to note that acidification per se is not required for an anion loss to balance the net monovalent cation loss. Acidification could potentiate the process by increasing the intracellular concentration of membrane-permeable, mobile anions. A smaller continually replenished pool of such anions could still sustain anion efflux and intracellular electroneutrality. Such a pool exists normally (e.g., Cl\(^-\), HCO\(_3\)-, ATP, and creatine phosphate). The neutralization of immobile proteins by intracellular acidification would have an additional effect on the distribution of osmotically active substances since mobile negatively charged molecules contribute to osmolality. Shifting negative charge from immobile to mobile molecules reduces the osmotic space available to monovalent cations.

It remains to hypothesize how membrane depolarization and aKj reduction could be sustained in vivo once cellular acidification had subsided. There are two basic types of mechanisms that we have considered: one relates to transmembrane flux and the other relates to control of cell volume. According to the first mechanism, after the acute phase of net cellular K\(^+\) loss, the resulting low aKj is sustained as a result of altered membrane conductance coupled with possible Na-K pump suppression.9 Altering membrane conductances other than K\(^+\) (Cl\(^-\), Na\(^+\), Ca\(^2+\)) could increase the driving force for K\(^+\) by depolarizing the resting (diastolic) membrane potential.9 This could increase K\(^+\) efflux. If Na-K pump maximum velocity or affinity for intracellular Na\(^+\) were reduced,9 this would decrease K\(^+\) uptake. Both effects would contribute to a reduced aKj. However, a steady state with reduced aKj and elevated aNa\(_i\) could be maintained due to the following: elevated aNa\(_i\) tends to stimulate Na-K pump function increasing K\(^+\) uptake, and reduced aKj can lead to decreased K\(^+\) currents through the inward rectifying potassium channel.44 Thus, the ionic activity changes due to altered fluxes may act to restore a steady state for aKj, albeit at a lower value for aKj, and with altered but balanced unidirectional K\(^+\) fluxes. Regardless of the mechanism by which K\(^+\) was lost, reduction in aKj could be continued due to a continued alteration in some membrane conductance and/or alteration in Na-K pump function.

The second mechanism relates to the requirement for osmotic equilibrium in addition to the compartmental requirement for electroneutrality (i.e., equality of lost positive and negative charge). Since the total of aNa\(_i\) and aKj is reduced by 44 mM (59 mM in units of concentration), a comparable milliequivalent loss of negative charge must occur for electroneutrality. However, to make up for the lost osmotic strength, which could be as large as 118 mosm (depending on average anion valence), the cell must increase its concentration of mobile anions or neutral osmotically active metabolites. One way to satisfy both requirements simultaneously would be to reduce the average valence of the mobile impermanent anions. Further complications may occur since cell volume changes can alter the concentration of impermanent anions, and membrane potential depolarization (which occurs in the Purkinje fibers we studied) can lead to increased intracellular activity of the membrane permeant anions. Thus, a strong interaction between electrical and osmotic forces is expected during the establishment of the ionic gradients after cellular acidification.45,46 Continued osmotic abnormalities in association with continuing metabolic abnormalities could help to reinforce the effects of the changes in conductance and membrane transport, which may function to maintain low aKj.

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