Comparison of buffer and red blood cell perfusion of guinea pig heart oxygenation

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Schenkman, Kenneth A., Daniel A. Beard, Wayne A. Ciesielski, and Eric O. Feigl. Comparison of buffer and red blood cell perfusion of guinea pig heart oxygenation. Am J Physiol Heart Circ Physiol 285: H1819–H1825, 2003.—Myocardial mean myoglobin oxygen saturation was determined spectroscopically from isolated guinea pig hearts perfused with red blood cells during increasing hypoxia. These experiments were undertaken to compare intracellular myoglobin oxygen saturation in isolated hearts perfused with a modest concentration of red blood cells (5% hematocrit) with intracellular myoglobin saturation previously reported from traditional buffer-perfused hearts. Studies were performed at 37°C with hearts paced at 240 beats/min and a constant perfusion pressure of 80 cmH2O. It was found that during perfusion with a hematocrit of 5%, baseline mean myoglobin saturation was 93% compared with 72% during buffer perfusion. Mean myoglobin saturation, ventricular function, and oxygen consumption remained fairly constant for arterial perfusate oxygen tensions above 100 mmHg and then decreased precipitously below 100 mmHg. In contrast, mean myoglobin saturation, ventricular function, and oxygen consumption began to decrease even at high oxygen tension with buffer perfusion. The present results demonstrate that perfusion with 5% red blood cells in the perfusate increases the baseline mean myoglobin saturation and better preserves cardiac function at low oxygen tension relative to buffer perfusion. These results suggest that caution should be used in extrapolating intracellular oxygen dynamics from buffer-perfused to blood-perfused hearts.

myocardial oxygen consumption; myoglobin oxygen saturation; optical spectroscopy; Langendorff-perfused heart

THE LANGENDORFF METHOD isolated perfused rodent heart has long been used as an experimental model in which to study many aspects of cardiac physiology. Hundreds of reports have been published using the Langendorff preparation to understand cardiac function. However, there are limitations with the use of the traditional buffer-perfused heart as a model for the in vivo state. Several recent studies (8, 9, 16) have compared cardiac function in crystalloid versus blood-perfused hearts, and a recent review (22) has discussed the different perfusion techniques. Intracellular oxygen availability has not been evaluated in these studies, raising the question of how differences in perfusate affect intracellular oxygenation.

The relationship between intracellular oxygen tension and cardiac function in the crystalloid buffer-perfused guinea pig heart has been previously reported by this laboratory (17). Those experiments led to the conclusion that the energetic status of the buffer-perfused heart is precarious in that any reduction in perfusate oxygenation led to a direct decrease in cardiac function by a number of indexes studied. The present study demonstrates that the addition of even a modest concentration of red blood cells to the perfusate improves the function of the perfused heart, increases the initial intracellular myoglobin saturation, and better preserves cardiac function at low oxygen tension relative to that found in the crystalloid-perfused heart.

The reason for the difference in functional response to hypoxia between buffer- and red blood cell-perfused hearts at the same mean intracellular myoglobin saturation is not obvious. Because myoglobin saturation is related to intracellular oxygen tension, one might think that hearts with equivalent mean myoglobin saturation would function the same regardless of the perfusate carrying oxygen to the myocytes. However, the observations reported here do not support this idea. In an accompanying study (5), a mathematical model was developed that offers an explanation for this intriguing finding. This model suggests that an explanation is that great heterogeneity exists in the distribution of oxygen in the myocardium, and thus a substantial proportion (~20%) of the left ventricular myocardium is hypoxic in the Langendorff buffer-perfused heart. The addition of red blood cells to the buffer prevents the large degree of hypoxia seen in the buffer-perfused myocardium.

METHODS

Heart preparation. All experiments were performed in accordance with University of Washington Animal Care Committee regulations. Adult guinea pigs (750–1,000 g) of either sex were injected intraperitoneally with 1,000 units of heparin and 1 h later anesthetized by intraperitoneal injection of pentobarbital (100–125 mg/kg). Hearts were rapidly excised and immersed in ice-cold buffer, and the aorta was...
cannulated for perfusion in the Langendorff manner. A modified Krebs-Henseleit buffer containing albumin (7, 13) and washed sheep red blood cells was used for perfusion. The pulmonary artery was cannulated to obtain coronary venous measurements.

A dual perfusion system was used for these experiments and has been described in detail previously (17). Briefly, a constant coronary perfusion pressure of 80 cmH2O and temperature at 37°C were maintained. The dual gas-exchange perfusion system allowed for a linear transition from perfusion with 95% oxygen- to 95% nitrogen-equilibrated buffer. Both nitrogen and oxygen gases contained 5% carbon dioxide to achieve a stable pH. Arterial oxygen tension was continuously measured just proximal to the aorta with a Clark oxygen electrode (model 203, Instech Dual Oxygen Analyzer). A second oxygen electrode was used to measure the oxygen tension in coronary venous buffer from the cannulated pulmonary artery.

**Physiological monitoring.** Hearts were paced at 240 beats/min with a pacing wire placed in the right ventricular cavity. Left ventricular pressure was continuously measured with a 5.5-Fr catheter-tipped Millar pressure transducer (model SPR-783) inside a balloon placed in the left ventricle, and the signal was amplified with a Hewlett-Packard pressure amplifier (model 8805B). The volume of the balloon was adjusted via the catheter lumen at the beginning of each experiment to give a left ventricular diastolic pressure of 8–10 mmHg. Left ventricular systolic and diastolic pressures were obtained for each heartbeat when an optical spectrum was acquired. Ventricular function was determined by the left ventricular developed pressure (systolic – diastolic) and the maximal first derivative of left ventricular pressure (dP/dtmax) computed by a standard routine in LabView with averaging over each second.

Coronary flow was measured with an ultrasonic flow probe (Transonics) in the aortic inflow tubing just above the coronary arteries. The flowmeter was calibrated by timed volume collections. Myocardial temperature was measured by a Mon-therm myocardial temperature sensor placed in the right ventricle (Malinckrodt Medical) using a thermocouple-to-analog converter (model TAC808-T Omega). All physiological data were acquired by a 12-bit analog-to-digital converter at a sampling rate of 1 kHz (model AT-MIO-16E-10, National Instruments) and displayed on a desktop personal computer (Gateway 2000 P133) using software developed in LabView. Mean values were determined by averaging over 1-s intervals and recorded for the time period corresponding to each acquired optical spectrum.

**Perfusate preparation.** A modified Krebs-Henseleit buffer of the following composition was prepared (in mM): 118 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 25 NaHCO3, 11 glucose, 1.75 CaCl2, and 2.0 pyruvate and 5 U/l insulin. The perfusate preparation was further modified to include glucose, 1.75 CaCl2, and 2.0 pyruvate and 5 U/l insulin. The perfusate was prepared with a perfusion system that allowed for a linear transition from perfusion with 95% oxygen- to 95% nitrogen-equilibrated buffer. Both nitrogen and oxygen gases contained 5% carbon dioxide to achieve a stable pH. Arterial oxygen tension was continuously measured just proximal to the aorta with a Clark oxygen electrode (model 203, Instech Dual Oxygen Analyzer). A second oxygen electrode was used to measure the oxygen tension in coronary venous buffer from the cannulated pulmonary artery.

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**Mean intracellular myoglobin saturation determination.** Mean left ventricular free wall intracellular myoglobin saturation was determined by an optical spectroscopic approach using partial least-squares analysis as previously described (17, 19, 20). A custom-built fiber-optic probe with a 1.25-mm separation between transmitting and receiving fibers was used to assure a 0.6-mm average depth of light penetration into the myocardium (6). To determine quantitative mean myoglobin saturation estimates, maximal oxygenated and deoxygenated end points were acquired from each heart. Mean myoglobin oxygen saturation was produced experimentally in each heart by perfusion with oxygenated buffer and by infusing adenosine to maximally vasodilate the coronary arteries and potassium chloride to arrest the heart and thus lower myocardial oxygen consumption. Maximal deoxygenation was produced by infusion of sodium dithionite (Na2S2O4) at the end of each experiment.

The partial least-squares analysis uses reference spectra obtained from in vitro solutions of hemoglobin, myoglobin, and cytochrome c in scattering media as described in earlier work (17, 19, 20). Briefly, the near-infrared wavelength region from 600 to 850 nm was used for these analyses. Spectra were preprocessed by taking second derivatives with respect to wavelength to reduce baseline offsets. Partial least-squares analysis is based on the interpretation of unknown spectra using spectra obtained under controlled conditions (calibration spectra). Partial least-squares analysis can be used to accurately predict concentrations of an analyte of interest (in this case, the saturation of myoglobin) from complex spectra taken from solutions or tissue in which multiple absorbing species with overlapping spectral features exist. The accuracy of the prediction depends, in part, on how well the calibration spectra represent the sample of interest. Previous studies have demonstrated successful prediction of myoglobin saturation using this method (17, 19–21).

**Myocardial oxygen consumption.** Myocardial oxygen consumption (MV02) was calculated for each optical spectrum obtained from simultaneous determinations of arterial and venous oxygen tension measurements and from the coronary flow. Venous oxygen tension values were corrected for the transit time delay from the arterial to venous Clark oxygen electrodes. Oxygen consumption was calculated using the relationships

\[ C_T(P) = \alpha_1 \left( P_{O_2} - P_{O_2}^* \right) + \alpha_2 P_{H_2} + C_{MNB} S_{MO}(P) \]

where \( C_T(P) \) is the arterial or venous oxygen content at partial pressure \( P \), \( \alpha_1 \) is the oxygen solubility in buffer and is \( 1.30 \times 10^{-6} \text{ M/mmHg} \), \( H \) is the volume fraction of red blood cells in the perfusate (0 for buffer perfusion and 0.05 for red blood cell perfusion), \( \alpha_1 \) is the oxygen solubility in the red blood cells and is \( 1.53 \times 10^{-6} \text{ M/mmHg} \), and \( C_{MNB} \) is the red blood cell oxygen binding capacity and is 0.0204 M. The oxygen saturation of hemoglobin (Smb) is described by a modified Hill equation

\[ S_{MO}(P) = \frac{P_{O_2}}{P_{O_2}^* + P_{O_2}^*} \]

where \( P_{O_2}^* \) is the half-saturation partial pressure of hemoglobin and is 43.5 mmHg (10) and \( n_H \) is the Hill exponent and is 2.6 (1). Thus MV02 is

\[ MV02 = f(C_T(P_A) - C_T(P_V)) \]

where \( f \) is the coronary flow (in ml·g⁻¹·min⁻¹) and \( P_A \) and \( P_V \) are the arterial and venous oxygen partial pressures (in mmHg). MV02 can then be expressed as microliters per
minute per gram using a conversion factor of 25.43 l/mol for an ideal gas at 37°C (4).

Experimental protocol. All hearts were perfused only with red blood cell-containing buffer. After hearts had reached a functional steady state (~20 min), baseline physiological variables were recorded. Hearts demonstrating a baseline systolic pressure of ≥80 mmHg and a dP/dt\text{max} of ≥1.0 mHg/s were deemed acceptable preparations. A steady linear decrease in perfusate oxygen content was begun by simultaneous ramping up the flow of deoxygenated perfusate and ramping down the flow of oxygenated perfusate using a pump-control routine written in LabView. A constant perfusion pressure was maintained by ensuring that the total flow from the two pumps exceeded the coronary flow at all times with excess flow overflowing from the upper reservoir. Thus each heart was exposed to a steady linear increase in hypoxia over 20 min, followed by reoxygenation. All experiments were completed in ~1 h after hearts were first perfused. Baseline values for each variable were determined by averaging the values corresponding to the 10 spectra acquired just before the desaturation begun.

Statistics and curve plotting. Physiological values were determined from the waveforms recorded by the LabView program for the same time points that the optical spectra were acquired. Values for each variable were averaged over 1-s time periods. These physiological values were compared with previously reported values obtained from buffer-perfused hearts (17). For clarity of illustration, mean myoglobin fractional saturation, dP/dt\text{max}, and myocardial oxygen consumption were plotted against arterial and venous oxygen tension for interval changes in oxygen tension. All measured values for each variable were averaged for each 50-mmHg interval for values above 100 mmHg and for each 20 mmHg between oxygen tensions of 0 and 100 mmHg. Mean values of red blood cell-perfused hearts (n = 9) with SEs for all experiments are shown in Figs. 1 and 2, along with the crystalloid buffer-perfused heart data (n = 7). In a similar manner, dP/dt\text{max} and MVO₂ (means ± SE) are shown as functions of mean myoglobin saturation (Fig. 3) for each 5% myoglobin saturation interval.

Data are presented in the figures as means with SE bars. The critical value of a difference in means is 3.03 times the SE, based on an unpaired two-sided t-test with eight observations/group, equal SEs, and α = 0.05. If the SE bars in the figures do not overlap, the difference between two points is already >2SE. Thus, as an approximation, the reader may judge a difference between the means in the figures to be significant if the white space between SE bars exceeds the average length of the two SEs shown.

### RESULTS

Mean baseline values for the red blood cell-perfused hearts compared with previously reported values (17) for buffer-perfused hearts are given in Table 1. Baseline mean myoglobin oxygen saturation was 92.6 (±1.9)% before the start of the perfusate desaturation. The data from the present red blood cell-perfused hearts (n = 9) are presented together with the data from the previous buffer-perfused study (n = 7) (17). Except for the addition of red blood cells, the experimental protocol with progressive hypoxia was the same in both studies. Although the buffer perfusion data have been previously published, the graphical relation between variables has not been previously presented except for parts of Figs. 1A and 2A. The data are repeated here to facilitate the comparison between buffer- and red blood cell-perfused experiments.

For the red blood cell-perfused hearts, mean myoglobin saturation was initially nearly constant as the perfusate oxygen tension decreased, until the perfusate oxygen tension dropped below ~100 mmHg. Below 100 mmHg, the mean myoglobin saturation dropped precipitously, as shown in Fig. 1A. In contrast, the mean myoglobin saturation in the buffer-perfused heart began to decrease even when the oxygen tension of the perfusate was ~600 mmHg. Similarly, ventricular function, as demonstrated by left ventricular developed pressure and dP/dt\text{max}, remained fairly constant during red blood cell perfusion while the perfusate oxygen tension remained above 100 mmHg, as shown in Fig. 1, C and D. However, with buffer perfusion, ventricular function began to decline when the buffer oxygen tension dropped below ~450 mmHg. Myocardial oxygen consumption also remained high with the perfusate oxygen tension above 100 mmHg during red blood cell perfusion but dropped steadily from an oxygen tension of 600 mmHg in the buffer-perfused hearts, as shown in Fig. 1E. Coronary flow at baseline was higher in the red blood cell-perfused hearts (12.9 ml·min⁻¹·g⁻¹) compared with the buffer-perfused hearts (7.4 ml·min⁻¹·g⁻¹), as shown in Fig. 1B.

The relationship between mean myoglobin oxygen saturation and venous oxygen tension also differs for

<table>
<thead>
<tr>
<th>Variable</th>
<th>Red Blood Cell Perfusion</th>
<th>Buffer Perfusion</th>
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<tbody>
<tr>
<td>Arterial oxygen tension, mmHg</td>
<td>632 ± 13</td>
<td>643 ± 18</td>
</tr>
<tr>
<td>Venous oxygen tension, mmHg</td>
<td>328 ± 46</td>
<td>105 ± 40</td>
</tr>
<tr>
<td>Coronary flow, ml·min⁻¹·g⁻¹</td>
<td>12.9 ± 2.0</td>
<td>7.4 ± 1.4</td>
</tr>
<tr>
<td>Mean myoglobin oxygen saturation, %</td>
<td>92.6 ± 2.9</td>
<td>72 ± 7</td>
</tr>
<tr>
<td>Left ventricular developed pressure, mmHg</td>
<td>93.4 ± 19.1</td>
<td>77 ± 8</td>
</tr>
<tr>
<td>dP/dt\text{max}, mHg/s</td>
<td>1.51 ± 0.32</td>
<td>1.25 ± 0.15</td>
</tr>
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n = 9 red blood cell-perfused hearts and 7 previously reported (17) buffer-perfused experiments. dP/dt\text{max}, maximal first derivative of left ventricular pressure.
Fig. 1. Cardiac function as arterial oxygen tension is decreased. Mean myoglobin fractional saturation (A), coronary flow (B), maximal first derivative of left ventricular pressure (dP/dt max; C), left ventricular developed pressure (D), and myocardial oxygen consumption (MVO2; E) are shown as functions of arterial oxygen tension. Results from the red blood cell (RBC)-perfused hearts in the present study undergoing increasing hypoxia are depicted as open circles. Results from crystalloid-perfused hearts [previously published data (17)] are shown as filled circles (n = 7). Mean myoglobin saturation remained fairly constant as arterial oxygen tension decreased during buffer perfusion. Similarly, dP/dt max developed pressure, and oxygen consumption are maintained at values close to baseline for arterial oxygen tensions above 100 mmHg for the RBC-perfused hearts. In contrast, mean myoglobin saturation began decreasing almost immediately as arterial oxygen tension was decreased during buffer perfusion. Similarly, dP/dt max, developed pressure, and oxygen consumption are maintained at values close to baseline for arterial oxygen tensions above 100 mmHg for the RBC-perfused hearts, whereas these variables both begin decreasing at high oxygen tensions in the buffer-perfused case. Coronary flow was lower at high arterial oxygen tension in the buffer-perfused hearts compared with RBC-perfused hearts, but flow increased with hypoxia in the buffer-perfused hearts to that seen in the RBC-perfused case.

Ventricular function as a function of mean myoglobin fractional saturation for both perfusates is shown in Fig. 3A. Mean myoglobin saturation remained higher for the red blood cell-perfused hearts than for the buffer-perfused hearts as venous oxygen tension decreased. Ventricular function (developed pressure and dP/dt max) was higher at all venous oxygen tensions for the red blood cell-perfused hearts compared with the buffer-perfused hearts, as shown in Fig. 2, C and D. Ventricular function decreased with either perfusate as the venous oxygen tension dropped below ~50 mmHg. Similarly, MVO2 decreased with either perfusate as the venous oxygen tension decreased, as shown in Fig. 2E.

DISCUSSION

It has been previously shown that the isolated guinea pig heart perfused with buffer oxygenated with 95% oxygen and an aortic pressure of 80 cmH2O in the Langendorff manner has a precarious oxygen supply under baseline conditions at 37°C (17). The present study was undertaken to provide a comparison between the buffer-perfused heart and hearts perfused with buffer containing a modest concentration of red blood cells. A 5% hematocrit was chosen as an appropriate way to augment oxygen capacity while minimizing other changes in the perfusion fluid. The present experiments were completed using the same protocol as the buffer-perfused experiments and performed under the same experimental conditions. The only difference between the two sets of experiments was in the composition of the perfusion fluid. In the present experiments, the addition of red blood cells to the buffer and the addition of albumin for stabilization of the red blood cells differed from the prior experiments.
In the crystalloid buffer-perfused preparation, baseline mean myoglobin saturation was determined to be 72% saturated. In the present study, under baseline perfusion with a hematocrit of only 5%, myoglobin was found to be 92.6% saturated. Thus a modest increase in oxygen-carrying capacity contributed by the presence of red blood cells along with increased flow greatly improves the intracellular mean myoglobin saturation. Buffer containing red blood cells with a 5% hematocrit has approximately twice ($\sim 2.18$) the oxygen-carrying capacity of crystalloid buffer when saturated with 95% oxygen (oxygen tension $\sim 632$ mmHg in these experiments). In the normal in vivo blood-perfused heart, blood contains more than four times the oxygen as the perfusate used in the present experiments. Oxygen binding to hemoglobin in the perfusate contributes more to total oxygen content at low oxygen tension than it does at high oxygen content due to the hemoglobin-oxygen dissociation relationship. The purpose of adding red blood cells to the perfusate in the present experiments was to increase the oxygen delivery above that provided by crystalloid buffer perfusion.

MacDonald and Winslow (14), using a cell-free hemoglobin-based perfusion with a similar hemoglobin concentration as in the present study, demonstrated improved cardiac performance at low arterial oxygen tension. In their study, a hemoglobin concentration of 1.5 g/dl was used, which is comparable to the hematocrit of 5% used in the present study. MacDonald and co-workers demonstrated that myocardial oxygen uptake was better maintained with hemoglobin perfusion than with crystalloid perfusion. In that study, however, intracellular myoglobin saturation was not determined. The present study demonstrates a difference in functional response for a given myocardial intracellular mean myoglobin saturation based on the composition of the perfusate.

Previous studies have demonstrated that adding hemoglobin or red blood cells to the perfusate of Langendorff preparation hearts improves the mechanical (7, 8, 16) and electrical performance (9) of the heart. That the intracellular mean myoglobin saturation is increased with increased oxygen delivery is not surprising, but that ventricular performance is improved with
red blood cell perfusion at the same myocardial intracellular mean myoglobin saturation is unexpected (Fig. 3A). At first glance, this would suggest that the myocyte behaves differently depending on the composition of the perfusate outside the cell. A more likely explanation would be that the mean intracellular myoglobin saturation does not completely characterize intracellular oxygenation. Heterogeneity in the distribution of myoglobin saturation values throughout the tissue could result in different values of intracellular oxygenation even though mean myoglobin saturation estimates might be similar. Furthermore, the nonlinear relationship between oxygen tension and myoglobin saturation (18) distorts the estimation of mean intracellular oxygen tension from mean myoglobin saturation when heterogeneity is present (12).

The role of flow heterogeneity in myocardial oxygenation is extremely important but also difficult to measure. Flow heterogeneity has been traditionally measured with microspheres and more recently by regional NADH videofluorimetry and Pd-porphyrin phosphorescence (24). Heterogeneity as measured in the perfused rat heart by NADH fluorescence has been shown to increase during hypoxia and to persist during recovery (11). The discrepancy in myoglobin saturation between buffer-perfused and red blood cell-perfused hearts led to the development of a computer model described in the companion paper (5) to understand the present results.

It is possible that explanations other than tissue oxygen heterogeneity may contribute to the differences in myoglobin oxygenation measured between the two different perfusates. Differences in the degree of edema formation in the hearts with different perfusates could affect the degree of heterogeneity and thus the myoglobin saturation. Also, differences in edema in the hearts could alter oxygen delivery directly. Increased flow in the red blood cell-perfused hearts may be due to release of nucleotides or adenosine from red blood cells. Increased coronary flow certainly could account for some of the improved performance seen in the red blood cell-perfused hearts compared with buffer perfusion seen under conditions of maximal oxygenation. However, as shown in the companion paper (5), the computer model prediction is that increased flow will not explain the performance differences seen in these experiments with progressive hypoxia.

Spectroscopic measurements, both optical and magnetic resonance, result in a regional tissue oxygenation value that represents an average of local variation in oxygenation. Clearly within the myocyte, as well as between myocytes, there exist significant oxygen tension gradients. Thus determination of intracellular oxygen tension based on an average myoglobin saturation across a tissue region on the macroscopic scale (i.e., measured in mm) may result in a skewed estimate of the intracellular oxygen tension value.

Papadopoulos and co-workers (15) measured radial and longitudinal diffusion of myoglobin in cardiac cells and argued, based on their measurements of diffusivity, that myoglobin oxygen gradients are small within myocytes. In contrast to the studies of myoglobin diffusivity, Takahashi and co-workers (23) have demonstrated significant intracellular radial oxygen gradients based on the measurement of myoglobin saturation using high-resolution spatial spectrophotometry in isolated cardiac cells. These studies demonstrated a drop in oxygenation near the center of the myocyte, with highest oxygenation closest to the sarcolemma. Thus heterogeneity of oxygen tensions in the heart may be important to understanding cardiac function.

In conclusion, the present results demonstrate that a modest concentration of red blood cells present in the perfusate improves the function of the isolated perfused heart, increases the resting mean myoglobin saturation, and better preserves cardiac function at low oxygen tension relative to that found in the crystalloid buffer-perfused heart. These results thus suggest that caution should be used in extrapolating intracellular oxygen dynamics from the buffer-perfused heart.
heart to the in vivo, blood-perfused heart. In addition, mean myoglobin saturation estimates may not directly reflect average intracellular oxygen tension in perfused hearts.

DISCLOSURES

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