Low-O₂ affinity erythrocytes improve performance of ischemic myocardium

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Received 26 February 2001; accepted in final form 14 November 2001

The role of O₂ diffusion in O₂ delivery remains a controversial and difficult area. It is well known that O₂ flux from erythrocytes to cells of an organ depends on diffusion. Because the O₂ pressure in cells, including cardiac myocytes, is only a few Torr (15), the O₂ diffusion gradient depends heavily on the O₂ pressure in the microvasculature at the point of its release from hemoglobin, a variable determined in part by the position and shape of the blood O₂ dissociation curve (ODC). That changes in ODC position might enhance or limit O₂ flow to cells in certain settings seems intuitively evident, given the existence of the Bohr phenomenon, the relationship between blood O₂ affinity and hemoglobin concentration in mutant hemoglobins, the presence of higher O₂ affinity in fetuses, the relative left ODC shift of animals native to high altitude, and the rise in 2,3-bisphosphoglycerate (BPG) and P₅₀ (Pₒ₂ required to half-saturate hemoglobin) in anemia and low cardiac output states (7, 49). These observations are also consistent with the notion that the O₂ pressure head is regulated in a range that does not greatly exceed what is needed for O₂ flux. Indeed, in the case of myocardium, the fact that blood flow varies inversely with P₅₀ (47) provides further support for this idea, as does the tight relationship between cardiac work and coronary flow. Nevertheless, experiments that provide unambiguous evidence of modulation of in vivo O₂ off-loading by ODC shifts are comparatively sparse, and many experiments have shown only modest or no effect. Apart from its physiological significance, this is a matter of some importance in clinical medicine, given the changes in ODC position that are known to occur with cardiac disease, storage of red blood cells (RBCs), disturbances of acid-base balance and the like (40), as well as the possibility of therapeutic manipulation of the ODC (46).

A specific setting in which the O₂ diffusion gradient could be of considerable importance is myocardial O₂ delivery (50). Myocardial blood flow is characterized by a major degree of microheterogeneity, with flow rates in millimeter-range tissue volumes varying 6- to 10-fold under basal conditions (3, 5, 11, 12, 24, 41). This heterogeneity rises as tissue volume falls (12) and is greatest as the tissue volume analyzed approaches the domain of a single capillary (27). Local myocardial substrate uptake and O₂ consumption (VO₂) are also heterogeneous (24) and only somewhat matched to flow. When a major coronary vessel is constricted, downstream local flow also decreases but is initially random with respect to original local flow (9, 24). Anaerobic metabolism appears in fact with the greatest relative reductions in flow (24), a phenomenon believed to account for the patchiness of myocardial infarction after insults that reduce cardiac perfusion (3). Given that basal myocardial O₂ extraction is normally high and locally variable (43), this could be simply because limited O₂ extraction reserve caps VO₂ sooner in local...
areas with higher extraction. Alternatively, if O₂ diffusion between capillary units is of importance (50), one might expect dysxia in loci that are most dependent on diffusion from adjacent regions. Accordingly, induced shifts of the ODC with other O₂ transport variables held constant furnish a useful method to test the importance of local O₂ diffusion in myocardial ischemia.

Several recent studies of ODC shifts on oxygenation of the heart and other tissues have been performed with the compound 2-[4-[(3,5-dimethylanilino)carbonyl]-methyl]phenoxyl]-2-methylproprionic acid (RSR13). This molecule crosses the RBC membrane and interacts reversibly with hemoglobin, producing appreciable reductions in blood-O₂ affinity (1). Results indicate that this drug may improve oxygenation when flow is blood decreased, particularly in models of ischemic heart disease and stroke (21, 29, 44, 45), implying that an increase in the O₂ diffusion gradient may increase O₂ flux. However, there is at least some evidence that RSR13 has effects on vascular tone other than those expected from the rightward ODC shift (Ref. 32 and Woodson, unpublished observations), although other observations have shown no such effect (29, 44). This could be a confounding variable, especially because vascular tone appears to mediate the microheterogeneity of blood flow (3, 4). In any case, it would be desirable to establish whether comparable effects of ODC shifts can be demonstrated when the ODC is shifted in other ways, particularly given the paucity of positive results in the literature.

These considerations prompted us to examine the role of O₂ diffusion in cardiac ischemia, in which we tested the hypothesis that a shift in the ODC due to the presence of intraerythrocytic inositol hexaphosphate (IHP) would improve O₂ diffusion and VO₂ when the latter is limited by reduced O₂ transport. We employed an isolated, isometrically contracting rabbit heart in these studies, a preparation widely used in studies of cardiac physiology and metabolism. The rabbit heart is known to display the same microheterogeneity of blood flow and metabolism observed in larger animals and humans (27, 35). This model allowed us to evaluate myocardial function and VO₂ at a normal coronary flow rate and during ischemia when the erythrocyte (RBC) P₅₀ was increased from a subnormal value to a supranormal one. Although other investigators have studied effects of altered RBC O₂ affinity in the isolated heart, they employed quite different models and/or did not examine effects of altering P₅₀ during ischemia.

MATERIALS AND METHODS

Preparation of RBCs

Krebs-Henseleit buffer. Krebs-Henseleit buffer (KHB) was prepared as follows. The basic solution (in mM: 118 NaCl, 4.7 KCl, 2.75 CaCl₂, 1.2 MgSO₄, 1.2 KH₂PO₄, 0.52 Na₂EDTA, 25 NaHCO₃, and 11 dextrose and 1,000 U sodium heparin per liter) was equilibrated by bubbling with 95% O₂-5% CO₂ at room temperature. Bovine serum albumin (1.5%) was then added, and the solution was filtered (0.22 μm).

High-affinity RBCs (control cells). Human packed RBCs stored for 6–14 days in standard CPDA-1 solution (citrate-phosphate-dextrose-adenine) were washed three times in an isotonic saline solution (1,350 g, 5 min); the supernatant and the buffy coat were carefully removed. RBCs were then diluted with KHB. At this stage, the RBC solution was stored in a refrigerator overnight at a hematocrit of 30–40%. The following day, the RBCs were further washed twice in saline containing 10 mM CaCl₂, 10 mM MgCl₂, and 2 mM glucose. Base excess was corrected to ~0 meq/l (pH of 7.4 at PCO₂ of 40 Torr) with addition of NaHCO₃. The cells were diluted with KHB to give a hematocrit of 25%. The diluted RBC suspension was passed through a leukocyte removal filter (PALL RC100).

Low-affinity RBCs (IHP-loaded cells). Packed RBC units were stored for 6–14 days at 4°C. The cells were washed once in isotonic saline and then passed through a leukocyte removal filter (PALL RC100). After two more washes in isotonic saline, IHP was incorporated into the cells by the continuous-flow hypotonic dialysis technique, similar to that described by Teisseire et al. (38). The method was modified by reducing the flow rate of the RBCs through the hemodialyzer (Lundia 1C plate dialyzer) to 10 ml/min and by diluting the IHP solution with 0.15 M NaCl (1:1 vol/vol for the first 5 experiments and 1:1.5 for the subsequent experiments) to reduce the degree of P₅₀ shift. After they were resuspended, the cells were washed once in isotonic saline, once in hypotonic saline (240 mosmol/kgH₂O) to lyse the most fragile cells, and twice in isotonic saline containing 10 mM CaCl₂, 10 mM MgCl₂, and 2 mM glucose. The RBCs were then diluted with KHB containing albumin (1.5%) and stored in a refrigerator overnight. On the day of perfusion, the cells were washed once in isotonic saline, once in hypotonic saline, and finally three times in saline with CaCl₂, MgCl₂, and glucose. The cells were then diluted with KHB with 1.5% albumin and NaHCO₃ to achieve a hematocrit of 25% and pH 7.4. The IHP incorporation resulted in a P₅₀ of 25–42 Torr (mean shift of 18.0 ± 5.1 Torr, range of 9–28 Torr). Mean recovery of RBCs was 61%. Supernatant hemoglobin concentration during perfusion was consistently below 0.1 g/dl, and the concentrations of ionized calcium, sodium, and potassium were within the normal range.

Isolated Heart Preparation

Experimental procedures were approved by the Animal Care Committee of the University of Wisconsin and were conducted in accord with the Guiding Principles in the Care and Use of Animals of the American Physiological Society and the Guide for the Care and Use of Laboratory Animals (DHSS Publication No. (NIH) 85-23). Our method paralleled those used in other laboratories (25, 42). Male New Zealand White rabbits weighing between 1.5 and 2 kg were anesthetized with an 8:1 mixture of ketamine-xylazine administered intramuscularly and then were given 1,000 U of sodium heparin intravenously. The heart was quickly removed after an intravenous bolus injection of pentobarbital sodium (25–30 mg/kg). The heart was placed in a heated cabinet, the ascending aorta was immediately cannulated, and retrograde perfusion was started at once with either KHB solution (series A) or human RBCs suspended in KHB solution (series B). The time from sternal incision to cardiac perfusion was well under 1 min. A drain was created in the apex of the left ventricle (LV) by puncture with an 18-gauge needle to allow egress of blood from the Thebesian vessels. A cannulated, fluid-filled balloon connected to a pressure transducer was placed in the LV via a left atriotomy for measurement of LV pressure during isovolumic contraction. A second catheter
was placed in the pulmonary artery to collect myocardial venous effluent. Aortic pressure was monitored by a pressure transducer connected to a stopcock inserted into the line just above the aortic cannula.

**Perfusion Setup**

A schematic diagram of the perfusion setup is shown in Fig. 1. The suspended RBCs were brought to physiological blood-gas concentration and temperature in a primary circuit. From a continuously stirred, covered reservoir, suspended RBCs were pumped at a relatively high rate (about 25 ml/l) through a membrane oxygenator (SciMed Life Systems, Minneapolis, MN) and a transfusion filter (PALL UltraTorr) to a second similar overflow reservoir, from which they returned by gravity to the main reservoir. Red blood cells were then propelled by a second pump at the desired flow rate from the overflow reservoir, which also served as a bubble trap, to the heart cannula. Perfusion temperature was recorded by a needle probe in the aortic line just above the heart. Blood passing through the heart was not recirculated, which avoided influence of metabolites. The reservoirs were water-jacketed to maintain a perfusate temperature close to 37°C, and the entire apparatus was enclosed in a thermostated cabinet. The system was designed so as to avoid settling of RBCs, with the possibility of altered perfusate hematocrit, at any point in the circuit.

**Series A.** In this series, we used normal stored human RBCs (“control cells”) to evaluate the reproducibility and sensitivity of the isolated heart model and to study the effects of ischemia on LV physiological parameters. Hearts (n = 9) were paced at a rate of 160–180/min (4–8 V, 10-ms pulse duration). They were initially perfused with KHB by gravity at a constant aortic pressure of ~90 mmHg. The intraventricular balloon volume was set to produce an end-diastolic pressure of 10 mmHg (2). The balloon volume was held constant during the experiment so that developed LV pressure [peak LV systolic pressure minus peak LV diastolic pressure (LVS-LVD)] reflected the contractile state of the myocardium. Hearts were allowed to stabilize for ~15 min under these conditions. Hearts that did not generate an LVS pressure of at least 60 mmHg or whose function declined during the stabilization period were discarded (2). About 20% of hearts were rejected for these reasons.

Perfusion by pump was then started with oxygenated RBCs at a constant flow rate of 9 ml/min. This corresponds to a perfusion rate of 2.1 ± 0.2 ml·min⁻¹·g ventricular wet weight⁻¹ (mean ± SD), which is similar to the rate used by others in RBC-perfused isolated hearts (2, 20, 25) and close to the means reported for awake rabbits (28) and anesthetized, open-chest rabbits (17, 43). This flow rate produced a mean aortic pressure of 95 ± 22 mmHg. Ischemia was then induced by reducing the flow rate to 3.5 ml/min and then to 2 ml/min for at least 5 min. Hearts were allowed to recover for at least 5 min at a flow rate of 9 ml/min after each level of ischemia. Finally, flow was interrupted completely for 2 min (total ischemia), after which the flow rate was returned to 9 ml/min.

**Series B.** In this series, hearts (n = 12) were perfused with suspended control RBCs immediately after isolation at a flow rate of 9 ml/min, paced (130–180/min), and allowed to stabilize for ~15 min. Each heart was then perfused with control (high-affinity) and with IHP-loaded (low-affinity) RBCs at flow rates of 9.0 ml/min, 3.5 ml/min, and back to 9.0 ml/min. Arterial and venous samples were obtained in duplicate after at least 5 min of perfusion, and the results were averaged. The order of perfusion with control and IHP-loaded RBCs was randomly varied such that the order for half of the hearts was C9-IHP9-IHP3.5-C3.5-C9-IHP9, whereas the order for the other half was IHP9-C9-C3.5-IHP3.5-IHP9-C9, where C indicates perfusion with control cells, numbers indicate rates of perfusion (in ml/min), and IHP indicates perfusion with IHP-loaded cells. In most experiments, hearts were then exposed to total ischemia (no perfusion) for 2 min once (n = 10) or twice (n = 3), after which the flow rate was returned to 9.0 ml/min. Total experimental time including the stabilization period was 60–90 min.

**Measurements**

Heart rate and LV and aortic pressures were recorded continuously (Gould 481 strip-chart recorder). Duplicate arterial (oxygenated blood in the reservoir) and venous (pulmonary artery catheter) blood samples were taken after ~5 min at each flow rate for measurement of pH, PO₂, PCO₂ (Radiometer ABL 30, Copenhagen, Denmark), O₂ content and saturation, and hemoglobin concentration (CO-oximeter, model 282, Instrumentation Laboratory, Lexington, MA). O₂ content was determined from O₂ saturation and hemoglobin concentration with allowance for dissolved O₂. O₂ extraction was expressed as follows: arterial O₂ content - venous O₂ content/arterial O₂ content. VO₂ was calculated as the product of perfusion rate (calibrated) and arteriovenous O₂ content difference. LV-developed pressure was expressed as LVS-LVD. Cardiac work was expressed as the double product (LVS-LVD) × heart rate. ODCs were determined with either a Hemoxy Analyzer (TCS Medical Products) or with a Hem-O-Scan (Amino) at 37°C and expressed at pH 7.4.

**Histology**

Three hearts from series A were examined histologically after perfusion with control RBCs. Muscle fiber structure
was intact with normal striations and no visible edema at 1.5 h, the maximal time of any experiment. Compared with normal hearts, perfused hearts showed minimal, spotty hemorrhage in the LV myocardium, with a tendency of more hemorrhage with increasing perfusion time. These hemorrhages involved <5% of the myocardium. Only occasional punctate hemorrhages could be seen grossly. These changes are not surprising in light of absence of platelets and coagulation proteins in the perfusate and compare favorably with what others have observed grossly (M. Vogel, personal communication, and Ref. 42). By contrast, there was considerably more hemorrhage in the right ventricular wall. Because our study dealt only with LV function, we believe this did not affect our conclusions. The behavior and gross appearance of experimental hearts were similar to those of the histologically examined hearts. We found no other studies in which histopathology in this preparation was described.

Statistics

Duplicate values obtained for each parameter during each perfusion condition were first averaged. Differences in parameters with changes in flow rate at constant P50, and with changes in O2 affinity at constant flow rate, were examined by paired t-test. Differences as a function of P50 in series B following total ischemia were examined by unpaired t-test.

Table 1. Arterial blood gas parameters and temperature

<table>
<thead>
<tr>
<th>Series</th>
<th>Temperature, °C</th>
<th>P02, Torr</th>
<th>O2 Saturation, %</th>
<th>Pco2, Torr</th>
<th>pH</th>
<th>P50 (7.4), Torr</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>35.2 ± 1.1</td>
<td>120 ± 22</td>
<td>97 ± 1</td>
<td>31 ± 4</td>
<td>7.45 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Control</td>
<td>35.8 ± 0.7</td>
<td>130 ± 20</td>
<td>97 ± 1</td>
<td>34 ± 6</td>
<td>7.41 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>IHP loaded</td>
<td>35.6 ± 0.7</td>
<td>139 ± 9</td>
<td>90 ± 2</td>
<td>34 ± 2</td>
<td>7.41 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SD. IHP, inositol hexaphosphate; P50 (7.4), Po2 required to half-saturate hemoglobin at pH 7.4.

RESULTS

Series A

Table 1 shows that arterial blood gases were close to the physiological range. Temperature averaged 35.2 ± 1.1°C. Figure 2 displays the relationship of LV-developed pressure, positive change in pressure over time (+dP/dt), and LV work as a function of flow rate (n = 9). At a normal flow rate of 9 ml/min (2.1 ± 0.2 ml·min⁻¹·g ventricular wet wt⁻¹), mean value ± SD for LVS-LVD averaged 58 ± 6 mmHg and LV work was 9,463 ± 1,027 mmHg·beats·min⁻¹. These values are in agreement with those of Apstein et al. (2), whose methodology closely paralleled ours. When total O2 transport was decreased to simulate ischemia by the reduction of flow rate to ~40% of the initial value (3.5 ml/min = 0.8 ± 0.1 ml·min⁻¹·g⁻¹), to 20% (2 ml/min = 0.4 ml·min⁻¹·g⁻¹), and to 0% (total ischemia), there were progressive decreases in LV function. Thus LVS-LVD decreased from the starting value by 39, 53, and 77% with the three flow decrements, respectively (Fig. 2A; P < 0.01 or better for each decrement by paired t-test). Peak +dP/dt decreased by similar amounts (Fig. 2B; P < 0.01) or better, with changes in LV
relaxation rate, as judged by negative peak dP/dt, paralleling changes in +dP/dt. Cardiac work decreased by 39, 52, and 77%, respectively (Fig. 2C; \( P < 0.01 \) or better). Myocardial O₂ extraction increased by 73 and 163% at 40 and 20% of the initial perfusion rate, respectively (Fig. 2D; \( P < 0.001 \)), whereas Vo₂ decreased by 37 and 46% (\( P < 0.001 \)).

Hearts recovered completely with restoration of perfusion to the basal level after the two levels of partial ischemia; there were no statistically significant changes postischemia in any parameter from basal values. With restoration of perfusion to the basal level after total ischemia, which occurred at the end of the protocol, there was a 15% decrease from starting value in mean LVS-LVD (\( P < 0.05 \)) and a 24% decrease in work (\( P < 0.02 \)); arteriovenous O₂ extraction and Vo₂ were unchanged.

### Series B

Because this model responded to stepwise decreases in total O₂ transport with parallel decrements in function, we evaluated the effect on function of ODC shifts in combination with changes in total O₂ transport. Table 1 shows that arterial blood gases and temperature series in the circuits with control and IHP-loaded cells were virtually identical and close to the physiological range. Although an arterial Po₂ slightly above the physiological level was employed, saturation of IHP-loaded cells, as expected, averaged 90 ± 2% (mean ± SD). P₅₀ averaged 17 ± 1 and 33 ± 5 Torr, respectively (\( P < 0.001 \)).

When perfused at 9 ml/min with control cells, LVS-LVD averaged 84 ± 21 mmHg, work (double product) was 13,173 ± 3,047 mmHg·beats·min⁻¹, mean aortic

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**Fig. 3. Interaction of hemoglobin-O₂ affinity and flow rate on LV-developed pressure (A), peak positive change in pressure over time (+dP/dt) (B), LV work (C), arteriovenous (A-V) O₂ extraction (D), O₂ consumption (Vo₂; E), and coronary vascular resistance (CVR; F) during control red blood cell perfusion (open bars) and high P₅₀ [inositol hexaphosphate (IHP)-loaded] RBC perfusion (solid bars) at various coronary perfusion rates. Differences between control and high P₅₀ perfusion at the same flow rate are as follows: *\( P < 0.05 \) or better, **\( P < 0.02 \) or better, ***P < 0.002 or better.**
pressure was 82 ± 28 mmHg, coronary vascular resistance was 42 ± 16 mmHg·ml⁻¹·min⁻¹·g⁻¹, O₂ extraction was 3.1 ± 0.9 ml O₂/dl, and VO₂ was 0.062 ± 0.022 ml·min⁻¹·g⁻¹. These values are shown as 100% in Fig. 3. When perfused with IHP-loaded cells, there were small but significant increases in LVS-LVD (P < 0.05), peak +dP/dt (P < 0.05), work (P < 0.02), O₂ extraction (P < 0.002), and VO₂ (P < 0.002). There were no significant changes in mean aortic pressure or coronary vascular resistance.

The flow rate was reduced to 3.5 ml/min, simulating ischemia, mean LVS-LVD, +dP/dt, work, aortic pressure, VO₂, and coronary vascular resistance decreased significantly, as in series A, whereas O₂ extraction increased; this was true for control and for IHP-loaded cells in relation to their respective controls. Importantly, parameters of function and O₂ delivery improved significantly (P < 0.01 or better) during simulated ischemia with IHP-loaded vs. control cells (Fig. 3). The increase in LV work and VO₂ was sufficient to restore 17 and 20%, respectively, of the decrements due to this degree of ischemia.

Complete ischemia caused further significant decreases in functional parameters. Function during complete ischemia was independent of the type of RBC perfusion (control vs. IHP loaded) preceding the period of ischemia. Upon reperfusion after ischemia, function and VO₂ improved significantly. These parameters were somewhat better when reperfusion was carried out with IHP-loaded cells, but the differences from reperfusion with control cells did not attain statistical significance.

Figure 4 depicts in vivo ODCs obtained by plotting arterial and venous O₂ saturation and pressure on perfusate samples obtained under the various conditions described above. These curves redemonstrate the right shift measured in vitro for IHP-containing cells and show that the ODC is less steep. At 9 ml/min, mean venous O₂ saturation was appreciably lower and mean venous PO₂ appreciably higher with the IHP-loaded cells (Table 2, Fig. 4). This same pattern was observed at 3.5 ml/min. Accordingly, arteriovenous O₂ content difference (Fig. 3) was significantly greater during perfusion with IHP-loaded RBC under both conditions and accounted for the significantly greater VO₂ observed.

**DISCUSSION**

We employed a basal coronary flow rate (and resulting coronary perfusion pressure) to duplicate conditions of the in vivo rabbit heart (17, 28, 43). Our results for basal O₂ extraction and VO₂, as well as for LV-developed pressure and double product in this model, are virtually identical to those determined by Apstein et al. (2), whose conditions most closely paralleled ours, and are in good agreement with those of other workers (20, 25). In addition, our results clearly establish that this ex vivo heart preparation is sensitive to decreased O₂ delivery due to ischemia, as there were stepwise decrements in myocardial function, increases in O₂ extraction, and decreases in VO₂ with each decrement in coronary flow. Because our protocol could produce stunning, which is observed in isolated hearts with somewhat longer periods of ischemia (29), it was important to document recovery of contractile function after successive bouts of ischemia. Indeed, we observed complete recovery after all periods of low-flow ischemia in series A and B, a finding that allowed us to use each

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**Table 2. Venous PO₂ and O₂ saturation**

<table>
<thead>
<tr>
<th>Blood Flow</th>
<th>O₂ Saturation, %</th>
<th>PO₂, Torr</th>
<th>PCO₂, Torr</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.0 ml/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>71 ± 8</td>
<td>36 ± 6</td>
<td>39 ± 6</td>
<td>7.39 ± 0.05</td>
</tr>
<tr>
<td>IHP loaded</td>
<td>58 ± 8</td>
<td>53 ± 15</td>
<td>40 ± 3</td>
<td>7.39 ± 0.02</td>
</tr>
<tr>
<td>3.5 ml/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>54 ± 7</td>
<td>29 ± 4</td>
<td>43 ± 7</td>
<td>7.35 ± 0.05</td>
</tr>
<tr>
<td>IHP loaded</td>
<td>42 ± 8</td>
<td>35 ± 7</td>
<td>44 ± 5</td>
<td>7.35 ± 0.05</td>
</tr>
</tbody>
</table>

Values are means ± SD.
heart as its own control for comparing low and high $P_{50}$ RBCs. We did observe a modest reduction in contractile function after circulatory arrest. However, this was the last step of our protocol, after the data comparing effects of $P_{50}$ during low-flow ischemia had been collected. Importantly, we achieved excellent matching of hemoglobin concentration, $O_2$ saturation, and blood flow with low and high $P_{50}$ RBC perfusions, so that ODC-related changes in microvascular $P_{O2}$ was the only change in paired perfusions. Our protocol also avoided treatment-order bias because the order of perfusion with low and high $P_{50}$ cells was varied. The protocol by its very design does not, of course, address global changes in blood flow that may result from a $P_{50}$ change. It does, however, implicitly address any local changes in blood flow distribution that might result from a $P_{50}$ shift.

Of considerable interest was the clear effect of a $P_{50}$ increase of 16 Torr on cardiac function, $O_2$ extraction, and $V_{O2}$ when ischemia was present. This increase in $P_{50}$ was sufficient to reverse 20% of the decrease in work and 36% of the decrease in $V_{O2}$ due to ischemia. We believe this constitutes strong evidence that $O_2$ delivery in this model is diffusion limited during ischemia, the increase in microvascular $P_{O2}$ increasing the $O_2$ diffusion gradient and improving $O_2$ diffusion.

A number of previous investigators have examined the effects of a shift of the ODC on the coronary circulation, cardiac function and $O_2$ uptake. In a small study, Ramo and colleagues (31) showed an effect of $P_{50}$ on cardiac function. We showed in awake rats that a drop in $P_{50}$ produced by exchange transfusion with low $P_{50}$ blood (change in $P_{50}$ of 21 Torr) caused coronary flow to increase by $\sim 100\%$ (47), a finding suggesting that myocardial perfusion is normally autoregulated in relation to microvascular $P_{O2}$. Stucker et al. (36) showed a similar $P_{50}$-coronary flow relationship in isolated, nonworking (Langendorff-type) rat hearts during perfusion with stored RBCs and resealed IHP-containing RBCs. In that study, coronary flow decreased by 36% (from 5.32 to 3.40 ml·min$^{-1}$·g wet wt$^{-1}$) when $P_{50}$ was raised from 19 to 47 Torr at constant perfusion pressure, arterial $O_2$ saturation, and hematocrit. Apstein et al. (2) also studied rabbit hearts contracting isometrically against a fluid-filled balloon during perfusion at fixed, constant flow, and arterial $O_2$ content. Their study differed from ours in that they utilized a different method of increasing $P_{50}$ (induction of BPG synthesis to supraphysiological levels), which would have yielded an ODC of a different shape (7, 38, 39, 49). Importantly, they studied the effect on $O_2$ uptake and function of raising $P_{50}$ during an isoproterenol infusion at fixed basal flow, whereas we studied hearts when flow was reduced well below basal levels. We are aware of no studies that address microheterogeneity of blood flow and its matching to metabolism under Apstein’s conditions. However, it is known that the distribution pattern of local flow is maintained during adrenergic stimulation (13) and becomes less heterogeneous with increased work (26), the two conditions employed by Apstein et al.; by contrast, local flow patterns are completely disrupted during acute ischemia (9, 24). Apstein et al. nevertheless showed that $O_2$ uptake and function improved with increased $P_{50}$ under their conditions. Because their and our results are similar, it seems likely that increased $O_2$ diffusion improves $O_2$ flux in both settings despite presumed differences in the degree of perfusion heterogeneity. This serves to emphasize further the importance of $O_2$ diffusion in myocardial oxygenation.

Also of note is our finding that significant changes in $O_2$ extraction, $V_{O2}$, and function resulted from the same $P_{50}$ shift when the heart was being perfused at the basal rate at a $P_{50}$ of 17 Torr, which suggests that $O_2$ flux from microvessels to myocytes under these conditions was somewhat diffusion limited at the start. This finding is perhaps not unexpected because 17 Torr is a relatively low value for the rabbit (19) and because coronary blood flow is known to rise in intact animals when $P_{50}$ falls (33). This finding is also consistent with data, albeit indirect, suggesting that $O_2$ diffusion between adjacent capillary units occurs under normal conditions (50). However, any increased perfusion heterogeneity or unknown abnormalities that are due to the ex vivo preparation (edema, sluggish microvascular flow, and the like) would also be expected to benefit from a larger $P_{O2}$ gradient.

Recently, the relationship between $P_{50}$ and function has been examined in other cardiac models with the compound RSR13. These studies differed from the above in that $P_{50}$ in all instances was raised from a normal level to an appreciably supranormal value. Thus Woods et al. (45) showed no effect of RSR13 (rightward $P_{50}$ shift of 14 Torr) on function or on phosphocreatine and ATP concentrations in isolated, potassium-arrested rat hearts at basal perfusion. When perfused, however, at very low flow rates, there was significantly improved function and better preservation of the high-energy intracellular metabolites. Pagel and colleagues (29) examined recovery of function after myocardial stunning in dogs caused by repeated bouts of left anterior descending (LAD) occlusion without and with RSR13. They found better preservation of function when RSR13 was given in a dose sufficient to raise $P_{50}$ from 33 to 46 Torr. Killgore et al. (21) showed improved function and morphology of dog hearts after cold cardioplegia when RSR13 is present. Weiss et al. (44) recently conducted studies in open-chest dogs given RSR13. When given before regional low-flow ischemia (accomplished by perfusing the LAD coronary artery at a constant pressure of 35 mmHg), an RSR13 dose sufficient to raise $P_{50}$ from 33 to 51 Torr attenuated the regional decrease in high-energy compounds and intracellular pH observed in controls. When given after onset of low-flow ischemia, the RSR13 resulted in significantly improved high-energy PCr-to-ATP ratios and contractility in the LAD bed. Our demonstration of improved function and $O_2$ uptake in the isolated heart with a $P_{50}$ shift due to IHP is in agreement with these $P_{50}$ results and furnishes strong evidence that the increase in $P_{50}$ per se is the likely explanation for the benefit observed with RSR13.
Wagner et al. (reviewed in Refs. 43 and 44) systematically examined numerous perturbations of O_2 delivery to working skeletal muscle, in both isolated canine muscle and normal human subjects, to clarify which factor or factors in the O_2 transport pathway limit maximal V_O2 (V_O2_max). In brief, they concluded that O_2 uptake is limited in various settings not only by altered total O_2 transport to the microvasculature but also by diffusion from the microvascular network to mitochondria. The most compelling data in support of the latter are from studies of isolated canine skeletal muscle during electrical stimulation that produces V_O2_max as defined in this model (18, 32). In these studies, a left ODC shift due to carbamylation decreased V_O2_max (18), whereas a right shift due to RSR13 increased V_O2_max (improvement observed in five of eight animals) (32). Meanwhile Kohzuki et al. (22) showed decreased O_2 uptake in perfused dog gracilis muscle with a left ODC shift due to carbamylation during submaximal work. Because total O_2 transport to the microvasculature is unchanged in these studies with altered P_50, these findings argue strongly that O_2 diffusion does limit O_2 uptake can be modulated by ODC shifts. This conclusion is supported by the recent demonstration of adaptations that would improve muscle O_2 diffusion in animals without myoglobin (16), a protein shown to facilitate O_2 diffusion (37).

The above results with altered O_2 affinity in the heart and in working skeletal muscle are of note because they differ completely from results in resting skeletal muscle during ischemia and in whole animals with decreased cardiac output. Thus Ross and Hlastala (33) and Kohzuki et al. (23) found no effect of a left ODC shift on O_2 uptake in resting canine skeletal muscle at basal perfusion and no change in the relationship between O_2 delivery and V_O2, as perfusion was lowered to and below the critical point (defined as the point at which V_O2 begins to decrease), despite appreciable changes in venous P_O2. Curtis et al. (10) also recently examined the effect of a large right shift due to RSR13 in isolated resting skeletal muscle and found no change in the O_2 delivery-V_O2 relationship as O_2 delivery was reduced, despite higher muscle surface P_O2 and higher venous P_O2 in the high P_50 (RSR13) animals. Likewise, there appears to be no effect of a leftward ODC shift in whole animals on V_O2 or on the O_2 delivery-V_O2 relationship as cardiac output is lowered to and below the critical point (34). Such results have at times been broadly interpreted as indicating that changes in the microvascular-to-cell O_2 diffusion gradient do not ordinarily limit O_2 diffusion to cells, with the necessary implication that changes in P_50 are unlikely to be of much practical importance. However, the present results suggest that the heart, like brain (48) and working skeletal muscle, is critically dependent on O_2 diffusion. This is particularly so during cardiac ischemia and is consistent with our earlier observation that coronary flow in intact animals rises with an acute left shift of the ODC (47).

A detailed understanding of how improved diffusion counteracts the absolute reduction in total O_2 transport due to ischemia is not available, as measurements of O_2 pressure, flux density, and V_O2 throughout the microvasculature are not locally accessible. In general, however, O_2 diffusion is known to depend on path length and conductance as well as on the P_O2 gradient (30). Well-established or possible changes affecting these three parameters during low-flow ischemia include 1) longer residence time for RBCs within the microvasculature, thus allowing more complete O_2 extraction, 2) increased time for O_2 diffusive shunting from arterioles to venules, 3) increased perfusion heterogeneity (9, 24), and, at least with time, 4) tissue edema and other anatomic changes. A shift of the ODC to the right should have little impact if mechanism 1 is of major importance for O_2 delivery during ischemia (given the convergence of ODCs at low O_2 saturation) and might actually impair V_O2 if mechanism 2 is of major importance (given longer arteriolar residence times for RBCs). The fact that a right shift improves function and V_O2 thus suggests that mechanisms 3 and 4 are of importance in this model, inasmuch as both should increase the average path length and/or decrease conductance, which an increase in P_50 would tend to counteract.

These observations may have clinical significance, as changes in P_50 of this magnitude are observed in clinical medicine. For example, a P_50 of ∼17 Torr is normally observed after storage of human blood for more than 10 days (8, 40); a P_50 of 33 Torr is only ∼6 Torr higher than the human mean normal and well within the range observed in low cardiac output states in humans (49). Drugs and techniques that avoid such P_50 changes could be of benefit when organ function is threatened by ischemia, as could methods that increase P_50 above normal in a number of clinical conditions (1, 6, 14). However, the current results, obtained in isolated hearts subjected to global ischemia, although provocative, cannot be translated directly to clinical settings.

This work was supported in part by the US Army Medical Research and Development Command under Grant No DAMD17-98-1-9004. G. Berlin acknowledges a grant from the Swedish Medical Research Council (Project B90-19F-8947-01), and R. D. Woodson acknowledges a grant from the University Hospital of Linköping, Sweden and sabbatical support from the University of Wisconsin-Madison, Madison, WI.

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