Effect of modifying O₂ diffusivity and delivery on glomerular and tubular function in hypoxic perfused kidney

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Departments of Laboratory Medicine and Pathobiology, and Chemistry, University of Toronto, Toronto M5G 1L5; and Hemosol, Etobicoke, Ontario, Canada M9W 4Z4

Baines, A. D., G. Adamson, P. Wojciechowski, D. Pliura, P. Ho, and R. Kluger. Effect of modifying O₂ diffusivity and delivery on glomerular and tubular function in hypoxic perfused kidney. Am. J. Physiol. 274 (Renal Physiol. 43): F744–F752, 1998.—Is O₂ diffusivity within renal capillaries rate limiting for O₂ delivery to hypoxic renal tubules? Equations based on diffusion theory and developed here predict that soluble hemoglobin (Hb) increases O₂ diffusivity by a factor of 1 + [442 HB%/(P50 + PO₂)], where P50 is the partial pressure of O₂ at which the Hb is half saturated. To examine the effect of P50 and Hb concentrations on renal function, we perfused isolated rat kidneys with Hb-P35 (P50 = 35 mmHg) and Hb-P11 (P50 = 11 mmHg). Venous PO₂ was lower with Hb-P11 (10 ± 1 vs. 16 ± 1 mmHg with arterial PO₂ = 35 mmHg and 28 ± 2 vs. 40 ± 2 mmHg with arterial PO₂ = 140 mmHg; P < 0.001). Perfusate P50 did not influence vascular resistance, glomerular filtration rate, O₂ consumption, Na reabsorption, protein excretion, or free water clearance. Percent glucose and phosphate excretion were lower with Hb-P11 than with Hb-P35 (P < 0.001). Urine glucose was 0.17 mmol/l with Hb-P11 and 0.77 mmol/l with Hb-P35 (P < 0.001). Hb-P35 (2%) doubled O₂ delivery and lowered glucose and phosphate excretion to the level obtained with 1% Hb-P11. Thus Hb-P11 delivered O₂ twice as effectively as Hb-P35 to high-affinity sodium glucose and phosphate cotransporters in the late proximal tubule (S3 segment). Hb-P11 may also have shunted O₂ from the outer cortex to the outer medulla and facilitated O₂ diffusion where PO₂ was low. We conclude that diffusivity is a limiting factor in delivery of O₂ to hypoxic tubules.

METHODS

Materials. Hemosol (Etobicoke, ON, Canada) provided the cross-linked hemoglobins, prepared in lactated Ringer solution. Tm-Hb, 82–82-Hb, and ethanolamine-Hb were prepared as previously described (15, 16, 18). Tm-Hb contains 33% αβ(Val1)-Tm-(Lys82)b and 67% αβ(Val1,Lys82)-Tm-(Lys82)b. Tm is the cross-linker trimers acid, 82–82-Hb contains 20%αβ(Lys82,Lys144)-Tm-(Lys82)b and 80%αβ(Lys82)-Tm-(Lys82)b. Ethanolamine-Hb contains 60% αβ(Lys82)-Tm(ethanolamine)-(Lys82)b. Ethanolamine is conjugated to the free trimers acid carboxylate group via an amide link (15). The remainder is composed mainly of αβ(Lys82,Lys144)-Tm-(Lys82)b and αβ(Lys82)-Tm-(Lys82)b. O₂ affinity was measured with a Hemox analyzer. P50 at 37°C, pH 7.4, was 11 mmHg for 82–82-Hb. The Hill coefficient was 1.8. Ethanolamine-Hb P50 was 12 mmHg, and the Hill coefficient was 2.0. 82–82-Hb and ethanolamine-Hb produced similar renal function and will be referred to hereafter as Hb-P11. For Tm-Hb, hereafter called Hb-P35, the P50 was 35 mmHg, and the Hill coefficient was 2.4. Solutions were adjusted to 22–24 mmHg onotic pressure by dilution with appropriately constituted salt solutions containing bovine serum albumin (BSA, low endotoxin; Pentex) dialyzed against a modified Krebs-Henseleit salt solution for 36 h (3). The final perfusate contained (mmol/l) 142 Na, 5 K, 25 HCO3, 2 Ca, 113 Cl, 14 lactate, 5 glucose, and a mixture of 20 amino acids totaling 6 mmol/l. The pH was 7.4 ± 0.1 at 37°C when equilibrated with 5% CO₂, [H]inulin was dialyzed against distilled water for 24 h, and a fresh batch was prepared every 4 wk.

Isolated kidney perfusion. Male Wistar rats (Harlan Farms, 250–300 g) were fed Purina Laboratory Rodent Diet 5001 during acclimatization to the laboratory. They were starved overnight with free access to tap water prior to being anesthetized (Somnotol, 50 mg/kg ip) for isolated kidney perfusion, as we have previously described (1). After the right kidney and mesenteric artery were exposed through a mid-line incision, the right ureter was cannulated with PE-50 tubing, and 1 ml of 10% mannitol and 100 U heparin was injected intravenously. A double-lumen catheter was advanced through the mesenteric artery, the perfusion flow was started, and the catheter was inserted into the right renal artery and tied in place. The aorta and vena cava were rapidly cut to free the kidney. After several seconds to permit flushing of blood, the kidney was placed into the warmed cup of a recirculating
perfusion apparatus. The surface of the kidney was covered with Parafilm to reduce dehydration. The perfusion system recirculated 160–180 ml of perfusate through a Hollow Fiber dialyzer (Fresenius F4; Fresenius, Bad Homburg, Germany). The perfusion fluid was continuously dialyzed against 1.5 liters of protein-free salt solution. To maintain a constant perfusate volume and protein concentration, the fluid level in the venous reservoir was monitored with an electronic sensor that activated a pump to inject appropriate volumes of protein-free salt solution. In all experiments, the dialyzing fluid was initially equilibrated with 95% air-5% CO₂. After a 40-min perfusion in some experiments, the gas was switched to 5% O₂-5% CO₂-90% N₂. The circuit included in-line borosilicate glass prefiltration filters and cellulose ester filters with 8-μm pore size (Millipore, Bedford, MA). Renal arterial pressure was measured through a no. 30 needle, which passed into the center of the 18-gauge perfusion catheter. Perfusate flow was adjusted to maintain constant arterial pressure of 80 mmHg. As we have done previously (1), we weighed the unperfused left kidney and used it as a reference for perfusion flow rate and inulin clearance, to compensate for any change in the perfused kidney volume and weight that might occur during perfusion with different solutions. At the end of the perfusion, some kidneys were flushed with 50 ml of isotonic salt solution, followed by 10 ml of 10% buffered Formalin. The kidney was then cut in 1-mm-thick slices and fixed in Formalin, and paraffin sections were cut at 5–20 μm for staining with periodic-acid Schiff/1% alcian blue and Perl's Prussian blue method for iron. In some experiments, the kidney was perfused for 5 min with 300 units/ml collagenase (Clostridium histolyticum; Sigma Chemical) with 5% BSA in perfusate solution. The kidney was removed, sliced with a Stadie-Riggs microtome, and incubated for 30 min at 37°C with 300 units/ml collagenase in perfusate solution with BSA and equilibrated with 95% O₂-5% CO₂. The tubule fragments were washed through a sieve with BSA-free perfusate solution, washed with centrifugation three times, and suspended in 43% Percoll, as previously described (2). The Percoll suspension was centrifuged for 30 min at 12,000 g. The fourth layer, which contains >85% proximal tubules, was washed with centrifugation three times. One aliquot of tubules was dissolved in nitrilotriacetic acid and analyzed for Na and K with a flame atomic absorption in a Varian Spectra A300 Zeman Graphite Furnace. Another aliquot was analyzed by the Lowry method for protein content.

O₂ content of arterial perfusate was varied by using different concentrations of cross-linked Hb and either 5% O₂-5% CO₂-90% N₂ or 95% air-5% CO₂. After an equilibrium period of 20 min, urine samples were collected at 20-min intervals up to 120 min. Perfusate samples were collected at the midpoint of each urine collection. Samples (1 ml) were drawn into airtight syringes from the venous outflow and the arterial bypass tube for measurement of pH, PO₂, and PCO₂ in a blood gas analyzer, and O₂ saturation of Hb and methemoglobin was measured by spectrophotometry (Co-oximeter). Hb modified the PO₂ readings from O₂ electrodes; therefore, solutions with various concentrations of Hb were equilibrated with analyzed gas mixtures to produce calibration curves. PO₂ was also measured with a Yellow Springs Instruments micro-oxygen probe in temperature-regulated, flow-through cells at 37°C and recorded on a YSI model 5300 Biological Oxygen Monitor (Yellow Springs Instruments). The detectors were incorporated into the bypass from the arterial inflow and in a catheter that collected part of the venous outflow. Total O₂ content was measured with a LexO₂con-K (Lexington Instruments, Waltham, MA). With samples containing low Hb concentration and low PO₂, we used two to four times the recommended sample volume of 20 μl for analysis; readings were linearly related to sample volume.

O₂ delivery was calculated as arterial perfusate O₂ (μmol/ml) multiplied by perfusate flow (ml/min). O₂ consumption was calculated as perfusate flow multiplied by the difference between arterial and venous O₂ content. Perfusate flow was measured with an electromagnetic flow transducer in the arterial line. Urine and perfusate samples were analyzed for Na, K, glucose, osmolality, protein, and [methoxy-H]inulin (NEN Products, Boston, MA). Inulin clearance was calculated as urine H excretion (dpm/min)/H in perfusate (dpm/ml). Perfusate oncotic pressure was measured at the beginning and end of each experiment (Refractometer and/or Weil Onometer System IL-186, Instrument Laboratories). BSA concentrations in perfusate and urine were measured using an high-performance liquid chromatography (HPLC) assay incorporating an anion exchange column (Pharmacia Mono-Q, 1-ml capacity), using a Beckman System Gold and a salt gradient. Total Hb was determined after HPLC by optical density at 280 and 414 nm. Percent excretion of Na, K, Pi, glucose, BSA, and Hb were calculated as (urine excretion/filtered load) × 100, with filtered load equal to perfusate concentration of the relevant solute multiplied by inulin clearance. There was no urea in the perfusate; therefore, most of the urine osmolality was due to Na and K salts, and we calculated free water clearance as CFW = C_inulin - 1 - ([urine Na/K]/[perfusate Na/K])/[urine inulin/([perfusate inulin]).

Statistical analysis was by paired and unpaired t-tests and one-way ANOVA with Student-Newman-Keuls method for multiple comparisons and by two-way ANOVA. For nonparametric data, Kruskal-Wallis ANOVA was used with Dunn's method for pair-wise multiple comparisons. The SigmaStat program was used for these analyses.

RESULTS

The O₂ binding characteristics of Hb-P₃₅ and Hb-P₁₁ before and during perfusion are compared in Fig. 1. To create the lines in Fig. 1, we measured O₂ saturation before perfusion with a spectrophotometer (Hemox analyzer at 37°C, pH 7.4). The points for O₂ saturation in arterial and venous samples obtained after 100 min of perfusion were calculated from the maximum O₂ binding capacity of hemoglobin-methemoglobin and the measured O₂ content of each sample. Calculated O₂ bound to Hb at 100% saturation (mmol/l) = Hb (mg/ml) × [(1 - %methemoglobin/100) × 0.062] (10). Methemoglobin concentration rose from 10% of the total Hb at the beginning of perfusion to 25 and 35% after 100 min. Total O₂ content was measured by galvanometry with the LexO₂con, and O₂ bound to Hb was calculated by subtracting dissolved O₂ (PO₂ [mmHg] × 1.257 × 10⁻³ [mmol/l]) (11). Perfusion for 100 min shifted P₅₀ to the left, with a more pronounced shift for Hb-P₃₅. The shift in P₅₀ is consistent with the known effect of methemoglobin to increase O₂ affinity (9).

The relationship between Hb concentration and renal function was explored with Hb-P₃₅. Arterial O₂ content was proportional to Hb concentration and almost twice as high when 95% air rather than 5% O₂ was used (Fig. 2). Per fusate flow increased during the first 30 min of perfusion; thereafter, flow and renal vascular resistance were similar in all experiments and were not related to the concentration of Hb, arterial O₂ content, or arterial PO₂. Glomerular filtration rate
(GFR) decreased and fractional Na excretion increased over the course of the 2-h experiments (data not shown). The data shown in the Figs. 1–7 and Tables 1–3 were obtained between 80 and 100 min. Filtration fraction, GFR, and total Na reabsorption ($T_{Na}$) increased with increasing O$_2$ delivery and plateaued above delivery rates $>16$ µmol·min$^{-1}$·g$^{-1}$. O$_2$ consumption increased with O$_2$ delivery up to 30 µmol·min$^{-1}$·g$^{-1}$, which was the highest delivery rate obtained (Fig. 2). Percent Na reabsorption was not significantly altered by changes in O$_2$ delivery (Fig. 2). In contrast, percent excretion of glucose, phosphate, protein, and hemoglobin decreased until O$_2$ delivery exceeded 20–25 µmol·min$^{-1}$·g$^{-1}$ (Fig. 3).

We used 1% Hb to examine the interactions between P$_{50}$ and Po$_2$. Venous Po$_2$ was significantly lower with Hb-P$_{11}$ ($P < 0.001$, Table 1). Neither P$_{50}$ nor Po$_2$ influenced perfusate flow (Table 1) or GFR (Fig. 4) during the period from 60 to 120 min of perfusion (Fig. 5). The relationship between O$_2$ consumption and Na reabsorption was linear and virtually identical for the two types of Hb (Fig. 6). However, the low P$_{50}$ reduced both phosphate and glucose reexcretion (Figs. 4 and 5). Phosphate excretion was also sensitive to changes in Po$_2$ and O$_2$ delivery, but the effect of Po$_2$ on glucose reabsorption was negligible, when the same concentration of Hb-P$_{11}$ and Hb-P$_{35}$ was used. Reduction of percent Na excretion by Hb-P$_{11}$ cannot be ruled out ($P = 0.08$). In other respects, renal function with
high- and low-affinity Hb was indistinguishable: percent K excretion, percent protein excretion, urine osmolality, and free water clearance were not different (data not shown).

For comparison with the effects of different P_{50} values, we examined the interactions between arterial PO_{2} and concentration using 1 and 2% Hb-P_{35}. As expected, O_{2} delivery to the kidney was doubled by using 2% Hb (Table 2). Venous O_{2} may have been slightly higher when kidneys were perfused with 2% Hb-P_{35} (Table 2, \( P < 0.06 \)). Increasing Hb concentration from 1 to 2% was associated with increased GFR (Fig. 7), but GFR did not increase further when the concentration was raised to 3% (Fig. 2). The decrease in phosphate excretion was a function of both higher PO_{2} and higher Hb concentration. In contrast, the decrease of fractional glucose excretion was attributable to increased Hb concentration alone. If one compares Figs. 4 and 7, it appears that 1% Hb-P_{11} had the same capacity as 2% Hb-P_{35} to support phosphate and glucose reabsorption.

Distal nephron function as reflected by percent K excretion was not influenced by P_{50} (Tables 1 and 2) but was slightly reduced by increasing the concentration of Hb-P_{35} (Table 2). Percent free water clearance \( (C_{H_{2}O}/C_{\text{inulin}}) \) was not influenced by either P_{50} or Hb concentration.

Filtration and tubular uptake of Hb was examined by staining tissue sections for iron and by measuring the iron content of proximal tubules. Thick sections (20 µm) of paraffin-embedded tissue were examined for residual iron after the kidney had been flushed with 50 ml of saline and 10 ml of 10% buffered Formalin. There were very few scattered blue granules and no evidence of

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**Table 1. Oxygen content and delivery for Hb-P_{11} and Hb-P_{35}**

<table>
<thead>
<tr>
<th></th>
<th>Hb-P_{11}</th>
<th>Hb-P_{35}</th>
<th>P_{50} Effect</th>
<th>P_{50} Effect</th>
<th>P_{50}/P_{28} Interaction</th>
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<tr>
<td>Venous O_{2}, mmHg</td>
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<td>0.001</td>
<td>0.072</td>
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<tr>
<td>5% O_{2}</td>
<td>10 ± 1</td>
<td>16 ± 1*</td>
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<tr>
<td>95% Air</td>
<td>28 ± 2</td>
<td>40 ± 2*</td>
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<tr>
<td>Perfusion flow, ml·min^{-1}·g^{-1}</td>
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<td></td>
<td>0.812</td>
<td>0.147</td>
<td>0.219</td>
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<tr>
<td>5% O_{2}</td>
<td>25 ± 1</td>
<td>25 ± 1</td>
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</tr>
<tr>
<td>95% Air</td>
<td>23 ± 1</td>
<td>27 ± 1</td>
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<tr>
<td>O_{2} delivery, µmol·min^{-1}·g^{-1}</td>
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<td>&lt;0.001</td>
<td>0.949</td>
<td>0.018</td>
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<td>5% O_{2}</td>
<td>10.9 ± 0.7</td>
<td>9.1 ± 0.6</td>
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<tr>
<td>95% Air</td>
<td>14.1 ± 0.8</td>
<td>15.9 ± 0.8</td>
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<tr>
<td>O_{2} consumption</td>
<td></td>
<td></td>
<td>0.397</td>
<td>0.632</td>
<td>0.476</td>
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<tr>
<td>5% O_{2}</td>
<td>5.7 ± 0.3</td>
<td>5.6 ± 0.4</td>
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<tr>
<td>95% Air</td>
<td>5.8 ± 0.4</td>
<td>6.3 ± 0.4</td>
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<tr>
<td>K excretion, %</td>
<td></td>
<td></td>
<td>0.943</td>
<td>0.376</td>
<td>0.787</td>
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<tr>
<td>5% O_{2}</td>
<td>126 ± 9</td>
<td>129 ± 8</td>
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<tr>
<td>95% Air</td>
<td>120 ± 11</td>
<td>118 ± 11</td>
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<td></td>
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<tr>
<td>C_{H_{2}O}/C_{\text{inulin}}</td>
<td></td>
<td></td>
<td>0.967</td>
<td>0.199</td>
<td>0.915</td>
</tr>
<tr>
<td>5% O_{2}</td>
<td>11.0 ± 1.2</td>
<td>9.1 ± 1.1</td>
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<tr>
<td>95% Air</td>
<td>10.9 ± 1.5</td>
<td>9.3 ± 1.5</td>
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</table>

Values are means ± SE for the 100th min of perfusion. No. of experiments: 9 for Hb-P_{11} with 5% O_{2}, 7 with 95% air; 11 for Hb-P_{35} with 5% O_{2}, 7 with 95% air. Values for O_{2} delivery and O_{2} consumption are in units of µmol·min^{-1}·g^{-1}. Data were analyzed by two-way ANOVA. *P < 0.05, Hb-P_{11} differs from Hb-P_{35} (Tukey's test).
iron in the tubular cells, tubular lumens, or interstitium. Iron content of proximal tubules from kidneys perfused with 1–2% Hb-P35 was similar to that in tubules from kidneys perfused with only BSA for 2 h (Table 3). There was no correlation between concentration of Hb-P35 in the perfusate and the proximal tubular iron content. Brush border and cellular structure was intact by light-microscopic examination in kidneys perfused with either Hb-P11 or Hb-P35 at low or high PO2.

DISCUSSION

Is O2 diffusivity within capillaries rate limiting for renal function? To examine this question, we exploited the fact that Hb in solution increases O2 diffusivity in proportion to its concentration and O2 affinity (1/P50) (13). Adding two different cross-linked Hbs with a threefold difference in O2 affinity to the kidney perfusate altered O2 diffusivity separately from arterial O2 content and delivery. The impact of P50 on diffusivity increases as PO2 approaches zero. Equation A16 (see APPENDIX) predicts that, at PO2 levels of 5 mmHg, 1% Hb-P11 would increase O2 diffusivity by 28%, and 1% Hb-P35 would increase O2 diffusivity by 11%. Theory also predicts that doubling the Hb concentration will not only double the quantity of O2 carried but also the effect on diffusivity.

Na reabsorption drives the bulk of renal O2 consumption (12). Hb-O2 affinity did not alter the linear relationship between O2 consumption and Na reabsorption (Fig. 6) or between GFR and total Na reabsorption (Fig. 4); however, we cannot rule out a slightly higher fractional Na reabsorption (P = 0.084) in the Hb-P11 perfused kidneys. GFR and TNa were increased by changing from 1 to 2% Hb (Fig. 7), with no change in the ratio of Na reabsorption to O2 consumption (TNa/QO2; ANOVA, P = 0.975). Increased GFR might have been due to efferent arterial vasoconstriction related to scavenging of NO (1), although there was no significant difference in total vascular resistance, perfusate flow (Table 2), or filtration fraction (P = 0.185, ANOVA). GFR and TNa appeared to plateau above O2 delivery rates of 16 µmol·min⁻¹·g⁻¹ and were unaffected by increasing Hb-P35 concentration to 3%.

Na reabsorption coupled to glucose and phosphate transport is sensitive to small decreases in O2 supply (11) in proximal tubules. The late proximal tubule (S3 segment), in which Na transport is coupled 2:1 with glucose, is most sensitive to O2 deprivation. This segment is also most susceptible to hypoxic damage (4, 5). High O2 affinity (Hb-P11) significantly reduced glucose and phosphate excretion (Figs. 4 and 5). To obtain similar low rates of phosphate and glucose excretion
with Hb-P₃₅, it was necessary to increase O₂ delivery and diffusivity twofold by doubling Hb concentration in the perfusate (Fig. 7). Phosphate and glucose are largely reabsorbed by high-capacity, low-affinity transporters in the S₁ and S₂ segments. Low-capacity, high-affinity transporters in the S₃ segment are responsible.

### Table 2. Oxygen content and delivery for 1 and 2% Hb-P₃₅

<table>
<thead>
<tr>
<th></th>
<th>1% P₃₅</th>
<th>2% P₃₅</th>
<th>Po₂ Effect</th>
<th>Concentration Effect</th>
<th>Pₒₑₒ₂/P₅₀ Interaction</th>
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<tr>
<td>Venous Po₂, mmHg</td>
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<tr>
<td>5% O₂</td>
<td>16 ± 1</td>
<td>18 ± 2</td>
<td>&lt;0.001</td>
<td>0.060</td>
<td>0.438</td>
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<tr>
<td>95% Air</td>
<td>40 ± 2</td>
<td>45 ± 2</td>
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<tr>
<td>Perfusate flow, ml·min⁻¹·g⁻¹</td>
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<td>0.982</td>
<td>0.473</td>
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<tr>
<td>5% O₂</td>
<td>25 ± 1</td>
<td>28 ± 1</td>
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<tr>
<td>95% Air</td>
<td>27 ± 1</td>
<td>26 ± 1</td>
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<tr>
<td>O₂ delivery, µmol·min⁻¹·g⁻¹</td>
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<tr>
<td>5% O₂</td>
<td>8.8 ± 0.7</td>
<td>16.0 ± 1.1*</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>95% Air</td>
<td>15.9 ± 0.9</td>
<td>28.9 ± 1.1*</td>
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<tr>
<td>O₂ consumption, µmol·min⁻¹·g⁻¹</td>
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<tr>
<td>5% O₂</td>
<td>5.7 ± 0.3</td>
<td>5.9 ± 0.7</td>
<td>0.011</td>
<td>0.047</td>
<td>0.107</td>
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<tr>
<td>95% Air</td>
<td>6.3 ± 0.5</td>
<td>8.6 ± 0.7*</td>
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<tr>
<td>K excretion, %</td>
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<tr>
<td>5% O₂</td>
<td>129 ± 9</td>
<td>93 ± 15</td>
<td>0.043</td>
<td>0.862</td>
<td>0.473</td>
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<tr>
<td>95% Air</td>
<td>118 ± 11</td>
<td>100 ± 15</td>
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<tr>
<td>C₅₀/₆₆/Cinulin, %</td>
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<tr>
<td>5% O₂</td>
<td>9.1 ± 1.0</td>
<td>8.3 ± 1.7</td>
<td>0.261</td>
<td>0.0633</td>
<td>0.320</td>
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<tr>
<td>95% Air</td>
<td>9.3 ± 1.3</td>
<td>11.4 ± 1.7</td>
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</table>

Values are means ± SE for the 100th min of perfusion. No. of experiments: 11 for 1% Hb-P₃₅ with 5% O₂, 7 with 95% air; 4 for 2% Hb-P₃₅ with 5% O₂, 4 with 95% air. Data were analyzed by two-way ANOVA. Values for O₂ delivery and O₂ consumption are in units of µmol·min⁻¹·g⁻¹. *P < 0.05, 1% Hb-P₃₅ differs from 2% Hb-P₃₅ (Tukey’s test).
sible for producing low urinary concentration of glucose and phosphate (19). High-affinity 2:1 sodium-glucose cotransport in the S3 segment consumes twice as much ATP as 1:1-coupled reabsorption in the early proximal tubule (19). 

\[ K_m \text{ for glucose reabsorption in } S1–2 \text{ segments is } 1.6 \text{ mM, and, in the S3 segment, it is } 0.35 \text{ mM (19). Kidneys perfused with 1% Hb-P11 produced urine glucose concentrations well below the } K_m \text{ for the S3 segment (0.17 mM; 0.16–0.37 mmol/l; median, 25th–75th percentile). Kidneys perfused with 1% Hb-P35 produced urine glucose concentrations that were twice the } K_m \text{ value for the S3 segment (0.77 mM; 0.61–1.11 mmol/l) (P < 0.001). The S3 segment also contains a high-affinity, low-capacity phosphate transporter (17). These results strongly suggest that Na-coupled glucose and phosphate reabsorption in the S3 segment were significantly greater with Hb-P11 than with Hb-P35. Kidneys perfused with Hb-P11 and Hb-P35 had similar O}_2 \text{ delivery and consumption rates, but O}_2 \text{ delivery to S3 segments in the corticomedullary region was presumably enhanced with Hb-P11, as indicated by the difference in glucose and phosphate reabsorption.}

Delivery of O}_2 \text{ for ATP production and Na transport depends on the steep O}_2 \text{ gradient from capillaries to mitochondria and is influenced by resistance to O}_2 \text{ diffusion (20). Inner cortical capillary } P_{o2} \text{ is unlikely to have been higher with Hb-P11 than with Hb-P35, because venous } P_{o2} \text{ was significantly lower (Table 1). Nonetheless, improved Na-coupled reabsorption strongly suggests that O}_2 \text{ delivery to tubules in the corticomedullary region was increased, due to improved O}_2 \text{ diffusivity. The effect of cross-linked Hb on O}_2 \text{ diffusion probably occurred almost exclusively within vascular lumens, which is where up to 90% of the resistance to O}_2 \text{ diffusion has been found in 3- to 10-μm layers adjacent to the walls of erythrocyte perfused capillaries (7). The uniform distribution of cross-linked Hb throughout the capillary lumen could facilitate diffusion of O}_2 \text{ to capillary walls. It is unlikely that significant amounts of Hb passed from capillaries into the interstitial space, since no iron staining was seen in the renal interstitium, very little Hb passed through glomerular capillaries into the urine (Fig. 3), and there was no evidence of tubular iron uptake (Table 3). Reduced resistance to O}_2 \text{ diffusion is probably insufficient to completely account for improved O}_2 \text{ delivery in}

Table 3. Iron content of proximal tubules from isolated perfused kidneys

<table>
<thead>
<tr>
<th>Iron, µg/mg protein</th>
<th>BSA 1% Hb-P35</th>
<th>1% Hb-P35</th>
<th>2% Hb-P35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median</td>
<td>0.96</td>
<td>0.91</td>
<td>1.18</td>
</tr>
<tr>
<td>25th–75th Percentile</td>
<td>0.33–2.35</td>
<td>0.56–1.95</td>
<td>0.63–2.5</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

Medians are not significantly different by one-way ANOVA (P = 0.589). BSA is perfusate without hemoglobin.
Hb-P₁₁ perfusions. High Hb O₂ affinity might favor even distribution of P O₂ throughout the cortex and outer medulla by reducing release of O₂ in the usually well-oxygenated outer cortex and increasing availability of O₂ in the more hypoxic corticomedullary region. The combination of redistribution and facilitated diffusion enabled Hb-P₁₁ to deliver O₂ to the corticomedullary region without significantly depriving the outer cortex of O₂. This is shown by the similarity of GFR and T Na obtained with 1% Hb-P₁₁ and 1% Hb-P₃₅ (Figs. 4 and 5).

Our observations indicate that Hb O₂ affinity will significantly influence kidney function under these conditions. The results demonstrate that the relationship between P₅₀ and O₂ delivery is different when Hb is in solution rather than encapsulated within erythrocytes. Hb in solution facilitates O₂ diffusion through plasma to the capillary wall, and Hb with a high O₂ affinity (low P₅₀) facilitates O₂ diffusion more than Hb with lower O₂ affinity. In contrast, lowering Hb P₅₀ within erythrocytes lowers the P O₂ at which O₂ is released into the plasma and decreases the gradient that drives O₂ diffusion to the mitochondria (14).

The corticomedullary region, which includes the S3 proximal tubules, is most at risk for hypoxic damage (5). Under hypoxic conditions, Hb-P₁₁, with high O₂ affinity, improved O₂ delivery to tubular cells in the corticomedullary region, without evidence of an adverse effect on cortical or distal tubular function. This beneficial effect was probably related to enhanced O₂ diffusivity at low P O₂ and may also have been related to redistribution of O₂ from the cortex to the corticomedullary junction (13). We conclude that O₂ diffusivity can be rate limiting for O₂ delivery when P O₂ is low. Furthermore, cross-linked Hb with a low P₅₀ should be more effective in preserving kidney function under conditions of hypoxia than cross-linked Hb with P₅₀ similar to that found in erythrocytes.

APPENDIX

Facilitated Diffusion of Oxygen by Hemoglobin

Assume O₂ and hemoglobin bind and dissociate rapidly in aqueous solution by the following mechanism

\[ O_2 + Hb \rightleftharpoons HbO_2 \]  \hspace{1cm} (A1)

The “Hb” refers to a single O₂ binding site, of which there are four per hemoglobin molecule.

The equilibrium constant, K, is defined by the relationship in Eq. A2

\[ K = \frac{C_{HbO_2}}{C_{Hb} \cdot C_{O_2}} \]  \hspace{1cm} (A2)

where

\[ C_{HbO_2} = \text{conc. of bound O}_2 \]
\[ C_{Hb} = \text{conc. of unliganded sites on Hb} \]

\[ \text{conc. of dissolved O}_2 \text{ in aqueous solutions} \]

Let

\[ \bar{C} = C_{HbO_2} + C_{Hb} = \text{total molar concn. of Hb bindings sites} \]

K is not a constant in this case but varies as a result of multiple sites and cooperative binding. However, an “overall equilibrium constant” (Kₐᵥ) is specified here, to be used as an approximation. At the conditions where half of the population of Hb sites are bound to O₂

\[ K_{av} = \frac{\frac{1}{2} \bar{C}}{C_{O_2,50\%}} = \frac{1}{C_{O_2,50\%}} \]  \hspace{1cm} (A3)

C₂₅₅% is the equilibrium concentration of dissolved O₂ at which 50% of the Hb binding sites are occupied. This is related to the P₅₀, the partial pressure of O₂ (mmHg) at which the 50% bound condition occurs, by Eq. A4

\[ C_{O_2,50\%} = H \cdot P_{50} \]  \hspace{1cm} (A4)

For dilute aqueous solutions of O₂ at 37°C, the Henry's law constant, H, is equal to 1.4 × 10⁻⁶ M/mmHg. Combining Eqs. A3 and A4

\[ K_{av} = \frac{1}{H \cdot P_{50}} \]  \hspace{1cm} (A5)

The concentration of C₂₅₅% of O₂ in aqueous solution is assumed to be in equilibrium with a gaseous source at a known partial pressure of O₂, P O₂

\[ C_{O_2} = H \cdot P_{O_2} \]  \hspace{1cm} (A6)

Equation A6 is the more general form of Eq. A4.

At steady state, the concentration gradient of O₂ across the film of thickness, L, is a constant given by

\[ \frac{dC_{O_2}}{dz} = \frac{C_{O_2} - C_{O_2,L}}{L} = \frac{\Delta C_{O_2}}{L} \]  \hspace{1cm} (A7)

Assume that O₂ consumption at the opposite side of the stagnant layer is very rapid and produces locally very low P O₂, then we may assume the dissolved O₂ concentration C₂₅₅% = 0.

\[ \frac{dC_{O_2}}{dz} = \frac{C_{O_2}}{L} \]  \hspace{1cm} (A8)

The flux of O₂, both bound (J hbo₂) and unbound (J o₂) across the unstirred layer is given by the following expressions (see Ref. 8, p. 398). Equation A9 describes simple diffusion of dissolved O₂ through water, whereas Eq. A10 describes facilitated diffusion of O₂ by Hb. The O₂ diffusivity used here is assumed to be unaffected by changes in protein concentration

\[ J_{O_2} = \frac{D}{L} \Delta C_{O_2} = \frac{DC_{O_2}}{L} \]  \hspace{1cm} (A9)

\[ J_{Hbo_2} = \frac{DC_{O_2}}{L} \left( K \frac{C}{1 + KC_{O_2}} \right) \]  \hspace{1cm} (A10)

The total O₂ flux, Jₜ, is

\[ J_T = J_{O_2} + J_{Hbo_2} \]  \hspace{1cm} (A11)
The total molar concentration of O₂ binding sites, C (in mol/l), is given by

\[ \frac{DC_{o2}}{L} \left( 1 + \frac{K C}{1 + K C_{o2}} \right) \]  

(A12)

D is the diffusivity of O₂ in aqueous solution and is a function of temperature.

The term in parentheses in Eq. A12 may be referred to as the "diffusivity enhancement factor" or \( f_D \). The value of \( f_D \) is always \( \geq 1 \), and a value of 2 would represent a situation where O₂ transport is twice as fast, due to facilitated diffusion

\[ f_D = 1 + \frac{K C}{1 + K C_{o2}} \]  

(A13)

By substituting \( K_{av} \) for \( K \) (Eq. A5), using the Henry's Law constant at 37°C, and combining with Eq. A6 for \( C_{o2} \), we obtain the following expression

\[ f_D = 1 + \frac{C}{H \cdot P_{so} + H \cdot P_{o2}} \]  

(A14)

The total molar concentration of O₂ binding sites, C (in mol/l), is given by

\[ \frac{\text{total Hb wt\%} \times 10}{\text{mol wt of Hb}} \times \frac{4}{\text{mol binding sites}} \times \frac{1}{\text{mol Hb}} \]  

(A15)

Finally, the factor \( f_D \) may be expressed most simply as

\[ f_D = 1 + \frac{443 \cdot \text{Hb} \%}{(P_{so} + P_{o2})} \]  

(A16)

where the partial pressures are expressed in mmHg. Equation A16 can only be considered to be semiquantitative as a result of the numerous assumptions made. It does, however, qualitatively reflect the effect of \( P_{so} \) on O₂ delivery in hypoxic tissue.

We are very grateful to Diane Wiffen and Chris Talpas for the preparation of Hb-P₁₁.

This research was supported by a National Science and Engineering Research Council Strategic Grant and by a grant from Hemosol.

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Received 25 June 1997; accepted in final form 5 January 1998.

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