Acute decrease in renal microvascular $\text{PO}_2$ during acute normovolemic hemodilution

Tanja Johannes,1,2 Egbert G. Mik,1,3 Boris Nohé,2 Klaus E. Unertl,2 and Can Ince1

1Department of Physiology, Academic Medical Center, University of Amsterdam, Amsterdam; 2Department of Anesthesiology and Critical Care, University Hospital Tuebingen, Tuebingen, Germany; and 3Department of Anesthesiology, Erasmus Medical Center, University of Rotterdam, Rotterdam, The Netherlands

Submitted 8 June 2006; accepted in final form 17 October 2006

Johannes T, Mik EG, Nohé B, Unertl KE, Ince C. Acute decrease in renal microvascular $\text{PO}_2$ during acute normovolemic hemodilution. Am J Physiol Renal Physiol 292:F796–F803, 2007. First published October 31, 2006; doi:10.1152/ajprenal.00206.2006.—Largedifferences in the tolerance of organ systems to conditions of decreased $\text{O}_2$ delivery such as hemodilution exist. The kidney receives $\sim25\%$ of the cardiac output and $O_2$ delivery is in excess of the oxygen demand under normal circumstances. In a rat model of acute normovolemic hemodilution (ANH), we studied the effect of reduced hematocrit on renal regional and microvascular oxygenation. Experiments were performed in 12 anesthetized male Wistar rats. Six animals underwent four steps of ANH (hematocrit 25, 15, 10, and <10%). Six animals served as time-matched controls. Systemic and renal hemodynamics and oxygenation parameters were monitored. Renal cortical (c) and outer medullary (m) microvascular $\text{PO}_2$ ($\mu\text{PO}_2$) and the renal venous $\text{PO}_2$ ($\text{PvO}_2$) were continuously measured by oxygen-dependent quenching of phosphorescence. Despite a significant increase in renal blood flow in the first two steps of ANH, $\mu\text{PO}_2$ and $\text{PvO}_2$ dropped immediately. From the first step onward oxygen consumption ($\text{VO}_{2\text{ren}}$) became dependent on oxygen delivery ($\text{DO}_{2\text{ren}}$). With a progressive decrease in hematocrit, a significant correlation between $\mu\text{PO}_2$ and $\text{VO}_{2\text{ren}}$ could be observed, as well as a $\text{PO}_2$ gap between $\mu\text{PO}_2$ and $\text{PvO}_2$. Furthermore, there was a high correlation between $\text{VO}_{2\text{ren}}$ and RBF over a wide range of flows. In conclusion, the oxygen supply to the renal tissue is becoming critical already in an early stage of ANH due to the combination of increased $\text{VO}_{2\text{ren}}$, decreased $\text{DO}_{2\text{ren}}$, and intrarenal $O_2$ shunt. This has clinical relevance as recent publications reporting that hemodilution during surgery forms a risk factor for postoperative renal dysfunction.

Hemodilution is a phenomenon occurring under various clinical circumstances. It can be seen during volume replacement in emergency or intensive care medicine (1, 15), is applied as standard practice during cardiopulmonary bypass (27), or is used in the form of normovolemic hemodilution as a clinical technique to reduce transfusion requirements in elective surgery (16, 19). Hemodilution reduces the oxygen-carrying capacity of the blood with a decrease in hematocrit followed by a drop in peripheral resistance and an increase in venous return and cardiac output. Large differences in the tolerance of organ systems to conditions of decreased $\text{O}_2$ delivery ($\text{DO}_{2\text{ren}}$) exist. However, as soon as the systemic oxygen delivery falls below a critical point, compensatory mechanisms are getting insufficient and oxygen consumption ($\text{VO}_{2}$) becomes dependent on supply (4, 28, 32, 36, 38). Although compensatory mechanisms preserve vital organ oxygenation over a wide range of decreasing hematocrit, they may impair tissue oxygenation of critical organs (34, 37).

The kidney receives approximately a quarter of the cardiac output (2) and its oxygen delivery is in excess of the oxygen demand under normal circumstances (4, 11). Therefore, the kidney might be regarded as being less prone to reduced oxygen delivery. For the kidney, it is known to be not only passively affected by hemodilution-induced changes in hematocrit (9) but that the organ itself is able to regulate the intraorganic distribution of blood flow (8, 22). This blood flow is highly heterogeneous with a nearly seven times higher flow per gram kidney weight in the cortex than in the inner medulla. Several studies have shown a renal flow distribution during hemodilution (11, 12, 22). However, the renal microcirculation is only partly understood and for a better understanding of the underlying mechanisms of the intrarenal flow distribution, the measurement of intrarenal oxygenation is mandatory. To our knowledge, there is as yet no study that comprehensively investigated the oxygenation of the renal microvasculature and oxygen extraction capabilities of the kidney during hemodilution.

In the presented study, we measured noninvasively and continuously the microvascular $\text{PO}_2$ ($\mu\text{PO}_2$) simultaneously in two different depths in the rat kidney by recently described dual-wavelength phosphorimetry (14). With this technique in combination with the noninvasive detection of $\text{PO}_2$ values in the renal vein and renal blood flow readings, the oxygen supply ($\text{DO}_{2\text{ren}}$) and consumption ($\text{VO}_{2\text{ren}}$) of the total rat kidney could be monitored. This model was used to test the hypothesis that acute normovolemic hemodilution (ANH) is accompanied by distributional changes in $\mu\text{PO}_2$ in the rat kidney. Recent clinical reports have suggested that hemodilution may be associated with postoperative renal dysfunction (10, 17, 27). We hypothesized that this observation may reflect evolving mismatch between local oxygen supply and demand in the kidney.

MATERIALS AND METHODS

Phosphorescence lifetime measurements. For noninvasive detection of changes in $\mu\text{PO}_2$ and measurement of the $\text{PO}_2$ in the renal vein ($\text{PV}_2$), the technique of oxygen-dependent quenching of phosphorescence was applied. Therefore, the animal received an infusion of a water-soluble phosphorescent dye (Oxyporph-G2; Oxygen Enterprises, Philadelphia, PA). This palladium porphyrin dendrimer binds to...
albunin and therefore ensures the stay within the microcirculation (26, 42). When Oxyphor-G2 is excited by a flash of light, the phosphorescence (~800 nm) intensity decreases at a rate dependent on the surrounding oxygen concentration (5, 29, 31, 39).

The relationship between the measured decay-time and the PO2 is given by the Stern-Volmer relation: $1/\tau = 1/\tau_0 + k_q[O_2]$, where $\tau$ is the measured decay time, $\tau_0$ is the decay time at an oxygen concentration of zero, and $k_q$ is the quenching constant.

To measure the oxygenation within the rat renal cortex and outer medulla, a dual-wavelength phosphorimeter was used. A detailed description of the used phosphorimeter and the validation of the technique can be found in a recently published article by our group (14). In short, the phosphor-albumin complex (Oxyphor-G2) is excited with light of 440 and 632 nm allowing a continuous and near simultaneous measurement in two different depths. Ex vivo penetration depth experiments performed in the harvest rat kidney determined the catchments depth of the 440-nm excitation to be 700 μm, whereas the catchments depth of 632 nm is 4 mm. Therefore, the measurements differentiate between cortex and outer medulla (outer and inner stripe), respectively. In vitro calibrations were performed in a bicarbonate buffer containing 2% bovine serum albumin (Sigma, St. Louis, MO) and a concentration of 10 μM Oxyphor-G2. Using a system consisting of an oxygenator, gas-flow controllers, and a recirculation system PO2 values were regulated at 37°C and a pH of 7.4. On the basis of a high tissue penetration and the fact that the light absorbance of blood is low within the near-infrared spectrum, Oxyphor-G2 is also very well suited for oxygen measurements in full blood. By using a frequency-domain phosphorimeter and a very thin reflection probe, the noninvasive detection of the renal venous PO2 had been possible.

To prevent contribution of underlying tissue to the phosphorescence signal in the venous PO2 measurement, a 0.5 × 1.0-cm piece of aluminum foil was placed on the dorsal site of the renal vein. For detection of renal blood flow (RBF), a perivascular flow probe (type 0.7 RB; Transonic Systems, Ithaca, NY) was placed around the left renal artery and connected to a flow meter (T206; Transonic Systems) (41). Furthermore, the left ureter was isolated, ligated, and cannulated with a polyethylene catheter for urine collection. Throughout the entire experiment, the operation field was covered with saran wrap to prevent evaporation of body fluids. The temperature of the kidney surface was measured and kept at 37°C. At the end of the experiment, the animal was killed by infusion of 1 ml of 3 M potassium chloride and the correct placement of the catheters was checked postmortem.

**Hemodynamic and blood gas measurements.** Arterial pressure was continuously measured in the carotid artery. Mean arterial pressure (MAP) was calculated as MAP (mmHg) = diastolic pressure + (systolic pressure − diastolic pressure)/3. Furthermore, the blood flow of the renal artery (ml/min) was measured continuously. Five times an arterial blood sample (0.2 ml) was taken from the femoral artery at baseline and at the end of a 15-min period of stabilization following each hemodilution steps. The blood samples were replaced by the same volume of hydroxyethyl starch (Voluvene; 6% HES 130/0.4; Fresenius Kabi). The samples were used for determination of blood gas values (ABL505 blood gas analyzer; Radiometer), as well as for determination of hematocrit, hemoglobin concentration, hemoglobin oxygen saturation, sodium and potassium concentration (OSM 3; Radiometer). Additionally, for each measurement point a heparinized capillary was filled with blood and centrifuged for determining hematocrit.

**Calculation of renal Do2, VO2, and O2ER.** Renal oxygen delivery was calculated as $\text{Do}_2\text{ren} (\text{ml/min}) = \text{RBF} \times \text{arterial oxygen content} (1.31 \times Hb \times S_{A,O_2}) + (0.003 \times P_{A,CO_2})$. Renal oxygen consumption was calculated as $\text{VO}_2\text{ren} (\text{ml/min}^{-1} \cdot \text{g}^{-1}) = \text{RBF} \times (1 - \text{renal venous oxygen content difference})$. Renal venous oxygen content was calculated as $(1.31 \times Hb \times S_{v,O_2}) + (0.003 \times P_{v,CO_2})$. The $S_{v,O_2}$ was calculated using Hill’s equation with $p_{50} = 37 \text{ mmHg}$ and Hill coefficient $= 2.7$ (6).

![Fig. 1. Schema of renal oxygenation measurement. RBF, renal blood flow.](image-url)
The renal oxygen extraction ratio was calculated as $O_2ER_{\text{ren}} (%) = \frac{V_{O_2}\text{cort}}{D_{O_2}}$. The vascular resistance of the renal artery flow region was calculated as $MAP/RBF$ (U). $R_{\text{cort}}$, arterial resistance; $R_{\text{med}}$, resistance of the outer medulla; $R_{\text{med}}$, resistance of the inner medulla; $R_{\text{cort}}$, cortical $R_{\text{med}}$, medullary $R_{\text{cort}}$, cortical.$R_{\text{med}}$, medullary resistances.

**Colloid osmotic pressure and osmolality measurements.** The colloid osmotic pressure and the osmolality were determined in plasma samples taken at the end of a 15-min period of stabilization following each hemodilution step. Plasma colloid osmotic pressure (COP) was measured by using a membrane osmometer (Osmomat 050; Gonotec) with a molecular mass cut-off at 20 kDa. The osmolality of the plasma samples was determined using an osmotic pressure meter (OSMO STATION, OM-6050; Arkray).

**Experimental protocol.** After 60 min of surgery, two optical fibers for oxygen lifetime measurements were placed. One was positioned 1 mm above the decapsulated kidney surface, the other 1 mm above the renal vein. Then, a 15-min intravenous infusion of Oxyphor G2 (5 mg/kg; Oxygen Enterprises) was started. After 40 min the $\mu O_2$ and $P_{O_2}$ were continuously measured during the entire experiment. Ten mg/kg; Oxygen Enterprises) was started. After 40 min $\mu O_2$ and $P_{O_2}$ were continuously measured during the entire experiment. Ten minutes later, a baseline blood sample (0.2 ml) was taken via the femoral artery catheter for determination of blood-gas values, hematocrit, and hematocrit. At this time point, the rats were randomized between the hemodilution (n = 6) and control group (n = 6).

Normovolemic hemodilution was performed by withdrawal of blood from the femoral artery and simultaneous administration of a colloid (Voluven; 6% HES 130/0.4; Fresenius Kabi) at a rate of 20 ml/h via the femoral vein. Therefore, a double syringe pump (Harvard Apparatus, South Natick, MA) was used. During the entire hemodilution, the animal showed no hemodynamic instability. Normovolemic hemodilution was undertaken in four steps. Starting at baseline the first step of infusion withdrawal was stopped when a hematocrit of ~25% (H1) was reached. Each hemodilution step was followed by a 15-min period of stabilization. In the following steps, the animal was isovolemic hemodiluted to a hematocrit of 15% (H2), 10% (H3), and between 5 to 10% (H4). In most of the experiments, the animals showed hemodynamic instability following the final hemodilution step (H4). After H4 and 15 min of stabilization, the experiment was ended by 1 ml infusion of 3 M potassium chloride.

In four additional animals, plasma colloid osmotic pressure and osmolality were determined for baseline and all four hemodilution steps following the above described protocol.

**Statistical analysis.** Values are presented as means ± SD, unless otherwise indicated. Labview 6.1 software (National Instruments, Austin, TX) was used to develop a software environment to allow data acquisition and analysis of the phosphorescence decay curves. Statistics were performed using GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA). For data analysis within each group and intergroup differences (hemodilution vs. control), two-way ANOVA for repeated measurements with Bonferroni posttest was performed. $P$ values <0.01 were considered significant. Plots of $D_{O_2}$ vs. $V_{O_2}$ and cortical $\mu O_2$ or outer medullary $\mu O_2$ vs. $D_{O_2}$, respectively, were examined and quantified with single linear regression. The same applied to the relationship between RBF vs. $V_{O_2}$.

**RESULTS**

**Systemic hemodynamics.** As shown in Table 1, the hemodilution and control group showed no differences in baseline values. In the course of hemodilution, hematocrit decreased from 45 ± 2% at baseline to 7 ± 1% at H4. Hematocrit was significantly lower at H3 compared with baseline in the control group. In the experimental group, MAP decreased significantly from 124 ± 8 mmHg at baseline to 52 ± 10 mmHg at H4. Compared with the experimental group, the control group showed a slight but significant decrease in MAP over time. Heart rate and central venous pressure did not significantly change in the control group. Heart rate was significantly lower than baseline at H2 and H4 in the hemodilution group. Central venous pressure increased in the hemodilution group and was significantly higher than baseline at H3 and H4. During hemodilution, RBF significantly increased from baseline (6.0 ± 0.5 ml/min) to 7.9 ± 2.5 ml/min at H1 and to 7.5 ± 1.1 ml/min at H2. At H4 (1.4 ± 1.5 ml/min), RBF was significantly lower compared with control and baseline. RVR did not change in experimental and control group. The urine flow increased significantly at H1 and H2 in the hemodilution group. The averaged weight of the left kidney was 1.33 ± 0.09 g. There was no difference in kidney weight between the experimental

| Table 1. Systemic hemodynamics during normovolemic hemodilution |
|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
|                         | Baseline                | H1                      | H2                      | H3                      |
| Ht, %                   | 44.7±2.3                | 22.7±1.8*†              | 13.2±1.2*†              | 9.0±0.6*†               | 6.5±0.8*†               |
| MAP, mmHg               | 124±8                   | 104±8*                  | 96±12*                  | 75±7*†                  | 52±10*†                |
| HR, beats/min           | 279±36                  | 264±19                  | 240±25*                 | 248±16                  | 235±37*                |
| CVP, mmHg               | 4.3±1.6                 | 4.6±1.5                 | 4.9±1.7                 | 5.1±1.7*                | 5.3±2.0*               |
| RBF, ml/min             | 6.0±0.5                 | 7.9±2.5*†               | 7.5±1.1*†               | 5.0±1.4                 | 1.4±1.5*               |
| Urine flow, µl/min      | 12.9±4.7                | 137.8±70.4*†            | 55.8±33.7*              | 14.4±9.0                | 2.9±2.7                |

Values represent means ± SD. H, hemodilution group; C, control group; Ht, hematocrit; MAP, mean arterial blood pressure; HR, heart rate; CVP, central venous pressure; RBF, renal blood flow; RVR, renal vascular resistance. *P < 0.01 vs. baseline. †P < 0.01 vs. control.
and control group. At the end of an experiment on average an amount of 21.1 ± 0.6 ml blood had been exchanged for the same volume of Voluven. With a reduction in hematocrit below 10% at H4 most of the animals became hemodynamically instable. Therefore, next to the hemodilution, the findings in the H4 period are probably influenced to a large extent by the disintegrated whole animal physiology.

Renal oxygenation parameters. Data of the oxygenation parameters of the kidney are shown in Table 2. Cortical and outer medullary microvascular PO2 and P\textsubscript{r}O\textsubscript{2} decreased significantly in the course of hemodilution. Compared with control group, the readings in the hemodilution group were significantly lower from control from H1 to H4. In the experimental group, the renal oxygen delivery (DO\textsubscript{2ren}) decreased immediately during hemodilution from 1.39 ± 0.13 ml/min at baseline to 0.84 ± 0.27 ml/min at H1 and reached its lowest reading with 0.05 ± 0.5 ml/min at H4. In the control group DO\textsubscript{2ren} remained constant around 1.1 ml/min. VO\textsubscript{2ren} was significantly increased compared with baseline (0.13 ± 0.04 ml/min\textsuperscript{-1}•g\textsuperscript{-1}) at H1 (0.28 ± 0.14 ml/min\textsuperscript{-1}•g\textsuperscript{-1}) and decreased during hemodilution to reach its lowest value at H4 with 0.03 ± 0.03 ml/min\textsuperscript{-1}•g\textsuperscript{-1}. However, VO\textsubscript{2ren} increased slightly over the time in the control group, which was significant at H4 compared with baseline. In the course of hemodilution, the oxygen extraction (O\textsubscript{2}ER\textsubscript{ren}) of the kidney increased from 13% at baseline and to a maximum of 67% at H3.

A typical example of an experiment is shown in Fig. 2. During 150 min, the hematocrit was diminished by hemodilution from 45% at baseline to 6% at H4. Pa\textsubscript{o}2 increased from 13% at baseline and to a maximum of 67% at H3. During 150 min, the hematocrit was diminished by hemodilution from 1.39 ± 0.13 ml/min at baseline to 0.84 ± 0.27 ml/min at H1 and reached its lowest reading with 0.05 ± 0.5 ml/min at H4. In the control group DO\textsubscript{2ren} remained constant around 1.1 ml/min. VO\textsubscript{2ren} was significantly increased compared with baseline (0.13 ± 0.04 ml/min\textsuperscript{-1}•g\textsuperscript{-1}) at H1 (0.28 ± 0.14 ml/min\textsuperscript{-1}•g\textsuperscript{-1}) and decreased during hemodilution to reach its lowest value at H4 with 0.03 ± 0.03 ml/min\textsuperscript{-1}•g\textsuperscript{-1}. However, VO\textsubscript{2ren} increased slightly over the time in the control group, which was significant at H4 compared with baseline. In the course of hemodilution, the oxygen extraction (O\textsubscript{2}ER\textsubscript{ren}) of the kidney increased from 13% at baseline and to a maximum of 67% at H3.

A typical example of an experiment is shown in Fig. 2. During 150 min, the hematocrit was diminished by hemodilution from 45% at baseline to 6% at H4. Pa\textsubscript{o}2 increased from 140 mmHg at baseline to 192 mmHg at H4. In this experiment, Pa\textsubscript{r}O\textsubscript{2} starts at a higher value than both cortical and outer medulla PO2 at baseline. Both cortical and outer medulla PO2 and P\textsubscript{r}O\textsubscript{2} dropped immediately with start of hemodilution. With hemodilution step two, a PO2 gap between Pa\textsubscript{r}O\textsubscript{2} and Pa\textsubscript{o}2 can be observed which now consistently persists for both the cortex and outer medulla. The Pa\textsubscript{r}O\textsubscript{2} which is defined as the difference in cortical and outer medullary PO2, decreased significantly (17.5 ± 11.5 mmHg at baseline and 2.4 ± 1.8 mmHg at H4).

Figure 3A shows the response of VO\textsubscript{2ren} on subsequent hemodilution. VO\textsubscript{2ren} significantly increased during the first hemodilution step from 0.13 ± 0.04 at baseline to 0.28 ± 0.14 at H1. The correlation between DO\textsubscript{2ren} and VO\textsubscript{2ren} is shown in Fig. 3B. VO\textsubscript{2ren} became dependent DO\textsubscript{2ren} already during the first hemodilution step (r\textsuperscript{2} = 0.8; P < 0.01). The correlation between \mu\textsubscript{PO2} and VO\textsubscript{2ren} is illustrated in Fig. 3C, D. With diminished microvascular oxygenation during progressive hemodilution, a significant correlation between cortical and outer medullary \mu\textsubscript{PO2} and VO\textsubscript{2ren} could be observed (cortical: r\textsuperscript{2} = 0.6; P < 0.01; medullary: r\textsuperscript{2} = 0.6; P < 0.01).

Correlation between renal oxygen consumption (VO\textsubscript{2ren}) and the RBF is demonstrated in Fig. 4. There was a significant correlation between RBF and VO\textsubscript{2ren} over a wide range of different flows (r\textsuperscript{2} = 0.6; P < 0.01).

Colloid osmotic pressure and osmolality. In Fig. 5, the plasma COP and osmolality are shown for baseline and all four hemodilution steps. The COP did not change during the first two steps of hemodilution. At H3 and H4 COP was significantly lower than baseline (P < 0.01). The plasma osmolality increased with start of hemodilution and was for all hemodilution steps significantly higher than baseline.

DISCUSSION

In a model of ANH, we studied the effect of reduced hematocrit on regional and microvascular oxygenation of the rat kidney. The hypothesis we tested was that ANH is accompanied by distributional changes in microvascular PO2 in the rat kidney. The main findings of the present study are that a significant increase in RBF in the first two steps of ANH, cortical \mu\textsubscript{PO2} and outer medullary \mu\textsubscript{PO2} dropped immediately and VO\textsubscript{2ren} became supply dependent early during hemodilution. ANH was associated with occurrence of PO2 gap (Pa\textsubscript{r}O\textsubscript{2}-\mu\textsubscript{PO2}) and redistribution of microvascular PO2 from cortex to outer medulla. Furthermore, early hemodilution was accompanied by an increase in renal oxygen consumption. With a

Table 2. Renal oxygenation parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>H1</th>
<th>H2</th>
<th>H3</th>
<th>H4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical \mu\textsubscript{PO2}, mmHg</td>
<td>70.9±12.0</td>
<td>37.0±6.1*†</td>
<td>19.8±4.3*†</td>
<td>17.4±8.6*†</td>
<td>11.8±3.7*†</td>
</tr>
<tr>
<td>C</td>
<td>67.0±15.0</td>
<td>63.6±15.2</td>
<td>64.4±12.4</td>
<td>60.8±13.1</td>
<td>58.1±12.1*</td>
</tr>
<tr>
<td>Medullary \mu\textsubscript{PO2}, mmHg</td>
<td>53.4±2.5</td>
<td>28.5±2.3*†</td>
<td>14.8±1.6*†</td>
<td>11.7±2.2*†</td>
<td>9.5±2.5*†</td>
</tr>
<tr>
<td>C</td>
<td>55.3±5.9</td>
<td>53.0±5.3</td>
<td>52.2±5.7</td>
<td>49.5±5.4*</td>
<td>48.0±4.6*</td>
</tr>
<tr>
<td>P\textsubscript{r}O2, mmHg</td>
<td>71.4±9.5</td>
<td>39.9±9.9*†</td>
<td>32.9±7.9*†</td>
<td>26.6±3.2*†</td>
<td>20.4±4.4*†</td>
</tr>
<tr>
<td>C</td>
<td>68.6±8.1</td>
<td>61.4±7.3</td>
<td>59.3±5.5</td>
<td>53.3±7.5*</td>
<td>48.9±8.2*</td>
</tr>
<tr>
<td>DO\textsubscript{2ren}, ml/min</td>
<td>H</td>
<td>1.39±0.13</td>
<td>0.84±0.27*</td>
<td>0.46±0.08*†</td>
<td>0.22±0.07*†</td>
</tr>
<tr>
<td>C</td>
<td>1.12±0.18</td>
<td>1.13±0.24</td>
<td>1.08±0.20</td>
<td>1.03±0.20</td>
<td>0.98±0.20</td>
</tr>
<tr>
<td>VO\textsubscript{2ren}, ml/min\textsuperscript{-1}•g\textsuperscript{-1}</td>
<td>H</td>
<td>0.13±0.04</td>
<td>0.28±0.14*†</td>
<td>0.20±0.08</td>
<td>0.11±0.03*</td>
</tr>
<tr>
<td>C</td>
<td>0.14±0.05</td>
<td>0.19±0.06</td>
<td>0.20±0.05</td>
<td>0.23±0.08</td>
<td>0.26±0.10*†</td>
</tr>
<tr>
<td>O\textsubscript{2}ER\textsubscript{ren}, %</td>
<td>H</td>
<td>13.0±4.8</td>
<td>44.9±14.7*†</td>
<td>56.0±13.3*†</td>
<td>66.9±6.0*†</td>
</tr>
<tr>
<td>C</td>
<td>15.7±4.5</td>
<td>22.1±5.8</td>
<td>23.6±4.3</td>
<td>28.7±7.5</td>
<td>34.7±10.8</td>
</tr>
</tbody>
</table>

Values represent means ± SD. \mu\textsubscript{PO2}, microvascular partial pressure of oxygen; P\textsubscript{r}O2, renal venous partial pressure of oxygen; DO\textsubscript{2ren}, renal oxygen delivery; VO\textsubscript{2ren}, renal oxygen consumption; O\textsubscript{2}ER\textsubscript{ren}, renal oxygen extraction ratio. *P < 0.01 vs. baseline. †P < 0.01 vs. control.

AJP-Renal Physiol • VOL 292 • FEBRUARY 2007 • www.ajprenal.org
progressive decrease in renal \( \mu \text{Po}_2 \) during ANH, a significant correlation between \( \mu \text{Po}_2 \) and \( \text{mPo}_2 \) and \( \text{VO}_2 \) could be observed. Furthermore, there was a high correlation between \( \text{VO}_2 \) and RBF over a wide range of different flows.

Tissue \( \text{Po}_2 \) values, traditionally measured by microelectrodes (3, 20, 40), range from 50 to 70 mmHg (33) in the rat kidney cortex and from 10 to 20 mmHg (2) in the renal medulla. We used the technique of oxygen-dependent quenching of phosphorescence to noninvasively measure the microvascular \( \text{Po}_2 \) in the kidney cortex and outer medulla and detected \( \mu \text{Po}_2 \) to be \( \sim 70 \) and \( \text{mPo}_2 \) \( \sim 50 \) mmHg. Using phosphorescence quenching Norman et al. (25) found the \( \mu \text{Po}_2 \) to be 49 mmHg (\( \text{FI}_2 = 20\% \)), 20 mmHg lower than in our study (\( \text{FI}_2 = 40\% \)). \( \text{FI}_2 \) has marked effect on \( \mu \text{Po}_2 \) values in the kidney (14) and at a \( \text{FI}_2 \) of 20% using monoexponential signal analysis we found a \( \mu \text{Po}_2 \) values similar to Norman and colleagues. Another study by our group (23) using two-photon excitation to detect renal oxygen tensions in different depths showed comparable values.

Surprisingly, in our model cortical and outer medullary \( \mu \text{Po}_2 \) dropped immediately with start of hemodilution, despite an initial increase in RBF. This is in contradiction to the theory that the renal oxygen supply is well in excess to oxygen demand (4, 11). An explanation for the sudden drop in \( \mu \text{Po}_2 \)

---

Fig. 2. Example experiment. During 150 min of acute normovolemic hemodilution (ANH), the hematocrit decreased from 45% at baseline (BL) to 6% at H4 (hemodilution step 4). \( \text{PaO}_2 \) increased from 140 mmHg at BL to 192 mmHg at H4. Cortical, outer medullary \( \mu \text{Po}_2 \) and \( \text{PrO}_2 \) dropped immediately with start of ANH. With hemodilution step 2, an oxygen gap between \( \mu \text{Po}_2 \) and \( \text{PrO}_2 \) could be observed which increased to the end of the experiment.

Fig. 3. Response of renal oxygen consumption on subsequent hemodilution. Rats are individually presented and connected by lines. The mean is denoted by horizontal line. *\( P < 0.001 \) vs. BL. A: relationship between renal oxygen delivery (\( \text{DO}_2 \)) and renal oxygen consumption (\( \text{VO}_2 \)). \( \text{VO}_2 \) became dependent on supply already during the first step of ANH and a significant correlation between \( \text{DO}_2 \) and \( \text{VO}_2 \) could be demonstrated (\( r^2 = 0.89 ; P < 0.01 \)). The correlation between cortical and outer medullary \( \mu \text{Po}_2 \) and renal oxygen consumption (\( \text{VO}_2 \)) is illustrated in C and D (cortical: \( r^2 = 0.53 ; P < 0.01 \); outer medullary: \( r^2 = 0.64 ; P < 0.01 \)).
might be a relative increase in diffusive oxygen shunt in relation to oxygen transport capacity. This concept is supported by the finding of a paradoxical difference in $P_{\text{r}}O_2$ and $\mu P_{\text{O}_2}$. While at baseline conditions this $P_O_2$ gap ($P_{\text{r}}O_2-\mu P_{\text{O}_2}$) was limited to the outer medulla, at low hematocrit it also occurred between $P_{\text{r}}O_2$ and $c\mu P_{\text{O}_2}$. Previously, $O_2$ diffusion shunt was demonstrated in the renal cortex under physiological conditions (18, 33, 40).

One actually could argue that the $P_O_2$ gap ($P_{\text{r}}O_2-\mu P_{\text{O}_2}$) should decrease or become inversed as hematocrit declines, reflecting reduced $D_{\text{O}_2}$ and maintained or enhanced $V_{\text{O}_2}$. Our finding of a paradoxical increase in $P_O_2$ gap from H1 is probably explained by $O_2$ shunt. The concept of $O_2$ shunt explains our results when regarding the $\mu P_{\text{O}_2}$ as being determined by the balance between $O_2$ supply and consumption at the microcirculatory level (43). A decline in $V_{\text{O}_2}$ as found in our model from H2 onwards would be expected to counterbalance a decrease in $\mu P_{\text{O}_2}$ due to decreased oxygen supply. By such a mechanism, a decrease in $V_{\text{O}_2}$ should actually reduce the $P_O_2$ gap. Therefore, the decrease in $\mu P_{\text{O}_2}$ to values well below $P_{\text{r}}O_2$ (and therefore the increase in $P_O_2$ gap) is more likely to reflect a profound decrease in $O_2$ supply at the microcirculatory level. This could be explained by a diffusive shunt (driven by plasma $P_O_2$ and nearly independent on $Hb$) before the capillary bed in combination with a decline in oxygen content in the microcirculation behind the shunt (depending more on $Hb$ than plasma $P_{\text{O}_2}$). The negative effect of the diffusive shunt on renal microcirculatory oxygenation will therefore increase with decreasing $Hb$, resulting in a lowering of $\mu P_{\text{O}_2}$ while keeping $P_{\text{r}}O_2$ relatively high.

The explanation of our results as outlined above finds agreement in the findings by Rosenberger et al. (30). They demonstrated in hypoxic and ischemic rat kidney that anemia or $CO$ poisoning causes a heterogeneous pattern of hypoxia-inducible factor (HIF) induction that is different from the pattern seen after total ischemia. Hypoxia was leading to a marked acute increase in expression of HIF-1$\alpha$ in the cell nuclei in both the renal cortex and outer medulla particularly in tubular segments, whereas ischemia induced a marked upregulation of HIF-1$\alpha$ in cells in the direct vicinity of necrotic tissue first 1 day after induction of renal infarction. Overall, it seems that the reduction of functional $Hb$ content results in hypoxic areas in the kidney even when arterial and venous $P_O_2$ values are well maintained.

The change in distribution of microvascular $P_O_2$ from cortex toward the outer medulla during hemodilution was quantified in our studies as a decrease in $\Delta \mu P_{\text{O}_2}$. A possible explanation for the diminishing $\Delta \mu P_{\text{O}_2}$ might be that with the increase in RBF known to occur as blood viscosity decreases (21) the renal oxygen consumption in the cortical renal tubules increases due to an increase in tubular sodium reabsorption (7). This explanation fits very well with our finding of an initial rise in $V_{\text{O}_2}$ at H1, assumingly reflecting activation of energy consuming adaptive cellular responses. It is questionable if the increase in RBF can be held solely responsible for the observation that the urine flow increased tremendously in the first two steps of ANH in our model. That acute change in COP during hemodilution may have contributed to the marked diuresis in H1 can be excluded. Experiments determining COP showed no significant reduction for the first two steps of ANH. A speculative explanation might be a hypoxia-related altered countercurrent system, for example diminished active chloride reabsorption in the medulla or increased urea permeability in the cortical part of the collecting tubule.

In the course of hemodilution, we found a rise in arterial $P_O_2$ while the animal was ventilated with fixed $P_{\text{F}_2}$ of 0.4. The...
theoretical arterial $P_O_2$ with a $F_0_2$ of 40% is somewhere \(~250\) mmHg. The fact that during baseline conditions the $P_O_2$ was \(~140\) mmHg indicates that either a ventilation-perfusion mismatch was present in our model or that the pulmonary transit time was too short to fully saturate the blood/plasma with oxygen. Lowering the hematocrit has had therefore beneficial effects on the first by hemorheological changes and on the latter by decreasing the oxygen buffer capacity of the blood (allowing relatively more oxygen to be dissolved in the plasma). This is reflected by a steady increase in arterial $P_O_2$ values following the subsequent hemodilution steps.

Hemorheological changes during hemodilution cause renal hematocrit to be \(~90\)% of systemic hematocrit under physiological conditions (35). Therefore, it is likely that filtration and reabsorption processes may lead to heterogeneity in hematocrit in the kidney. And, in light of our study, it is interesting to consider if hemodilution changes the overall pattern of heterogeneity. Hellberg et al. (12) actually compared fractional red cell volume in the renal cortex, outer stripe, inner stripe, and the inner medulla in control vs. mild hemodiluted animals. They found that the red blood cell volume in all areas of the kidney decreased in proportion to the systemic hematocrit.

Although caution must be exercised in extrapolating data from animal experiments to the clinic, our results strongly suggest that the oxygen supply to the renal tissue becomes critical already in an early stage of ANH. These findings may be relevant in explaining the findings in a number of recent publications that report about hemodilution during surgery being a risk factor for postoperative renal dysfunction. A critical $DO_2$ could be defined for development of acute renal failure after cardiopulmonary bypass (27). Another study reports about an increased likelihood of renal injury when hematocrit was below <24\% (10). Furthermore, it has been shown that a low hematocrit is an independent risk factor for contrast-induced nephropathy, a hypoxia-mediated type of acute kidney failure (24). On the basis of our results and those of other clinical studies, there is a need of determining an “optimal” degree of hemodilution (17) to minimize the risk of acute renal failure after standardized clinical procedures.

GRANTS
This work was supported in part by a grant of the fortune-program to T. Johannes (No. 1168–0-0, Medical Faculty, University of Tuebingen).

REFERENCES


