Evidence that renal arterial-venous oxygen shunting contributes to dynamic regulation of renal oxygenation

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Leong C-L, Anderson WP, O’Connor PM, Evans RG. Evidence that renal arterial-venous oxygen shunting contributes to dynamic regulation of renal oxygenation. Am J Physiol Renal Physiol 292: F1726–F1733, 2007.—Renal blood flow (RBF) can be reduced in rats and rabbits by up to 40% without significant changes in renal tissue PO2. We determined whether this occurs because renal oxygen consumption changes with RBF or due to some other mechanism. The relationships between RBF and renal cortical and medullary tissue PO2 and renal oxygen metabolism were determined in the denervated kidneys of anesthetized rabbits under hypoxic, normoxic, and hyperoxic conditions. During artificial ventilation with 21% oxygen (normoxia), RBF increased 32 ± 8% during renal arterial infusion of acetylcholine and reduced 31 ± 5% during ANG II infusion. Neither infusion significantly altered arterial pressure, tissue PO2 in the renal cortex or medulla, nor renal oxygen consumption. However, fractional oxygen extraction fell as RBF increased and the ratio of oxygen consumption to sodium reabsorption increased during ANG II infusion. Ventilation with 10% oxygen (hypoxia) significantly reduced both cortical and medullary PO2 (60–70%), whereas ventilation with 50% and 100% oxygen (hyperoxia) increased cortical and medullary PO2 (by 62–298 and 30–56%, respectively). However, responses to altered RBF under hypoxic and hyperoxic conditions were similar to those under normoxic conditions. Thus renal tissue PO2 was relatively independent of RBF within a physiological range (±30%). This was not due to RBF-dependent changes in renal oxygen consumption. The observation that fractional extraction of oxygen fell with increased RBF, yet renal parenchymal PO2 remained unchanged, supports the hypothesis that preglomerular arteriovenous diffusion shunting of oxygen from arteries to veins increases with increasing RBF, and so contributes to dynamic regulation of intrarenal oxygenation.

arteriovenous shunt; diffusional shunt; hypoxia; ischemia

Both hypoxia and hyperoxia can compromise tissue function and integrity, so tissue oxygenation must be tightly regulated (29). Functional imperatives dictate that the mechanisms regulating oxygenation of the kidney differ from those in other organs (3, 29, 30). For example, to drive glomerular filtration, renal blood flow (RBF) must greatly exceed that required to meet renal metabolic demand (3, 29, 30). Renal vasoconstriction reduces RBF and thereby O2 delivery to the kidney. It might therefore be expected that renal tissue PO2 would vary with RBF, provided renal O2 consumption remains relatively stable. This is the case in the brain (38, 47), retina (36, 37), and skeletal muscle (4, 19). Indeed, tissue and intravascular PO2 are considered useful surrogate markers of blood flow in the retina (49), brain (32), and kidney (18). However, we recently reported two situations in which renal tissue PO2 remained stable during moderate renal vasoconstriction (31). When RBF was reduced by ~15% by renal nerve stimulation in rabbits, or by ~40% by ANG II infusion in rats, we did not detect changes in either cortical or medullary tissue PO2 (31).

The two possible explanations for these observations are the hypotheses: 1) that the changes in O2 delivery induced by moderate changes in RBF are matched by changes in O2 consumption or 2) that some other mechanism acts to control and maintain O2 delivery to renal tissue in the face of changes in RBF, such as diffusional shunting of O2 from arterial blood to venous blood. The first hypothesis is supported by observations, made over 40 years ago, showing a direct relationship between RBF and O2 consumption (13, 20, 23–26, 45). However, the maneuvers that were used to change RBF directly altered O2 consumption (e.g., hypotension, cooling the kidney). The interpretation of these seminal and influential studies may therefore have been confounded by effects independent of RBF. Thus these hypotheses remain to be directly tested.

To determine which hypothesis is correct, we altered renal O2 delivery in anesthetized and artificially ventilated rabbits by directly changing RBF through renal arterial infusion of vasoactive agents. We also varied inspired O2 concentration across the range from hypoxia to hyperoxia, at each level of RBF. We found that reducing or increasing RBF by ~30% had little effect on renal O2 consumption and renal tissue PO2, despite the total flux of O2 across the kidney changing in proportion with RBF. This phenomenon was observed during normoxemia, hypoxemia, and hyperoxemia. Calculations based on the assumption that capillary blood O2 content remained stable in the face of changes in RBF indicate that arterial-venous O2 shunting increases with RBF and so makes a major contribution to the dynamic regulation of renal parenchymal oxygenation.

MATERIALS AND METHODS

Eight male New Zealand White rabbits were used (3.23 ± 0.13 kg). Procedures accorded with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Animal Ethics Committee of the Department of Physiology, Monash University.

Surgical preparations. Catheters were placed in central ear arteries and veins (31). Rabbits were then anesthetized with pentobarbital sodium (90–150 mg plus 30–50 mg/h), intubated, and artificially ventilated. Throughout the surgery and experiment, a maintenance solution containing compound sodium lactate and a 10% polygeline/electrolyte solution in a 4:1 ratio was infused at 10.8 ml·kg⁻¹·h⁻¹ (22). The left kidney was exposed via a flank incision and a catheter

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was placed in the left ureter (31). The kidney was then denervated to avoid the potentially confounding effects of changes in renal sympathetic nerve activity during experimental maneuvers (22). Catheters were placed in the renal artery (35) and renal vein (10). RBF was measured by transit time ultrasound flowmetry (31). To measure medullary tissue oxygenation (MP02) and temperature, a PO2 optode/thermocouple (BF/OT, tip diameter 10-μm tip, Unisense, Aarhus, Denmark) was advanced into the kidney using a micromanipulator so that its tip lay ~10 mm below the midregion of the lateral surface of the kidney [i.e., within the inner medulla (15)]. For measurement of cortical tissue oxygenation (CP02) and temperature, both a second PO2 optode/thermocouple and a Clark electrode (10-μm tip, Unisense, Aarhus, Denmark) were inserted 2 mm into the kidney using micromanipulators. Ventilation rate and tidal volume were then adjusted so that arterial PO2, PCO2, and CPO2 (Clark) were 90–110 mmHg, 13–25 mmHg, and 3–7.5, respectively. Experimental manipulations commenced 90 min later.

Experimental protocol. The protocol comprised three major experimental periods where RBF was manipulated by renal arterial infusion of ANG II [3.6–53.4 ng·kg⁻¹·min⁻¹], titrated to decrease RBF by ~30% without altering MAP), acetylcholine (148–590 ng·kg⁻¹·min⁻¹), titrated to increase RBF by ~30% without altering MAP), or the saline vehicle (20 μl·kg⁻¹·min⁻¹; 154 mM NaCl). The order of these treatments was randomized. Once the doses of ANG II or acetylcholine were titrated to the target effect, they remained fixed at this level throughout the major experimental period.

At least 15 min was allowed after commencing the infusions, before responses to altered inspired O2 were tested. Within each of the major periods, there were four 15-min periods during which the rabbit was ventilated with 10, 21, 50, or 100% O2 in random order. During the final 10 min of each of the 12 ventilation periods, urine produced by the left kidney was collected. At the midpoint of the urine collection period, blood samples (0.3 ml) were obtained from the ear artery and renal vein for blood gas analysis and a 1-ml sample of arterial blood was collected for renal clearance measurements. Blood samples were replaced with washed red blood cells from previous samples and/or blood from a donor rabbit.

Measurement of hemodynamic variables and determination of renal function. Glomerular filtration rate (GFR) was measured as [14C]inulin clearance (22). Sodium concentrations in plasma and urine were determined by atomic absorption spectrophotometry (31). Blood gas analysis was performed using an ABL510 oximeter (Radiometer, Copenhagen, Denmark). Renal O2 consumption was calculated as the renal artery (35) and renal vein (10). RBF was 31 ± 5% less during infusion of ANG II compared with saline, and 32 ± 8% greater during infusion of acetylcholine compared with saline. Similar effects of these vasoactive infusions were observed under hypoxic (10% O2) and hyperoxic (50% and 100% O2) conditions. The vasoactive infusions did not significantly affect MAP (Fig. 1, Table 1).

Because our major interest was to relate renal oxygenation to RBF, in subsequent analyses we assigned RBF as the independent variable (Figs. 2, 3, 4, 5). Alterations in inspired O2 concentration produced the expected changes in P02 of arterial blood, which were not influenced by the vasoactive infusions. Renal venous PO2 also increased with increasing inspired O2. Renal venous PO2 also increased as RBF was increased, at least under normoxic and hyperoxic conditions. Both (total) renal O2 delivery and renal venous O2 efflux increased with increasing RBF. The magnitudes of these changes were similar under normoxic, hyperoxic, and hypoxic conditions, but the absolute levels of renal O2 delivery and renal venous O2 efflux were markedly reduced under hypoxic conditions (Fig. 2, Table 1). Because arterial blood hemoglobin was nearly fully saturated under normoxic conditions, increasing inspired O2 from 21 to 100% during renal arterial infusion of saline increased arterial O2 content and renal O2 delivery by only 7.2 ± 2.5 and 5.0 ± 0.3%.

These analyses were performed using data obtained during ventilation with 10, 21, 50, and 100% O2. Because ventilation with 10% O2 reduced mean arterial pressure (Fig. 1), an additional analysis was performed that did not include data under hypoxic conditions. *Significant effects that are abolished by removal, from the analysis, of data obtained during hypoxia (10% O2). **MAP, mean arterial pressure; RBF, renal blood flow; AV, arterial-venous; GFR, glomerular filtration rate; P02 and P02*, levels of renal blood flow and inspired O2 content, respectively.

### RESULTS

#### Effects of altered inspired O2 concentration and renal arterial infusion of acetylcholine and ANG II.

Varying inspired O2 from 21 to 100% did not significantly alter RBF or MAP, but hypoxia (10% O2) reduced MAP by 5 ± 2 mmHg during salininfusion). During normoxia (21% O2), RBF was 31 ± 5% less during infusion of ANG II compared with saline, and 32 ± 8% greater during infusion of acetylcholine compared with saline. Similar effects of these vasoactive infusions were observed under hypoxic (10% O2) and hyperoxic (50 and 100% O2) conditions. The vasoactive infusions did not significantly affect MAP (Fig. 1, Table 1).

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### Table 1. Outcomes of analyses of variance for data shown in Figs. 1–5

<table>
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<tr>
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<th>Pnorm</th>
<th>Fgas</th>
<th>Pgas</th>
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<td>&lt;0.001*</td>
<td>0.95</td>
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<td>RBF</td>
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<td>Figure 2 Arterial PO2</td>
<td>0.82</td>
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<td>0.001</td>
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<td>&lt;0.001*</td>
<td>0.11</td>
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</tr>
<tr>
<td>Renal venous O2 efflux</td>
<td>&lt;0.001</td>
<td>&lt;0.001*</td>
<td>0.22</td>
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<tr>
<td>Figure 3 CPO2 (Clark)</td>
<td>0.09</td>
<td>&lt;0.001</td>
<td>0.50</td>
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<tr>
<td>CP02 (Fluorescence)</td>
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<td>&lt;0.001</td>
<td>0.95</td>
<td></td>
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<tr>
<td>MPO2 (Fluorescence)</td>
<td>0.88</td>
<td>&lt;0.001</td>
<td>0.48</td>
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<tr>
<td>Figure 4 Renal AV O2 difference</td>
<td>&lt;0.001</td>
<td>&lt;0.001*</td>
<td>0.92</td>
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<td>O2 extraction</td>
<td>&lt;0.001</td>
<td>0.16</td>
<td>0.91</td>
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<td>Renal O2 consumption</td>
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<td>&lt;0.001*</td>
<td>0.78</td>
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<tr>
<td>Figure 5 GFR</td>
<td>&lt;0.001</td>
<td>&lt;0.001*</td>
<td>0.94</td>
<td></td>
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<tr>
<td>Filtration fraction</td>
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<td>&lt;0.001*</td>
<td>0.76</td>
<td></td>
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<td>Sodium reabsorption</td>
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<td>&lt;0.001*</td>
<td>0.95</td>
<td></td>
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<tr>
<td>Renal energy efficiency</td>
<td>0.009</td>
<td>0.96</td>
<td>0.95</td>
<td></td>
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</table>
2.8%, respectively. In contrast, arterial blood hemoglobin was desaturated under hypoxic conditions, so decreasing inspired O₂ from 21 to 10% decreased arterial O₂ content and renal O₂ delivery by 42.6/9.1 and 37.9/8.7%, respectively, during renal arterial infusion of saline. The vasoactive infusions profoundly altered RBF but not arterial blood O₂ content. Consequently, renal O₂ delivery changed in proportion to the changes in RBF.

Under normoxic conditions during renal arterial infusion of saline, the PCO₂ and pH of arterial blood averaged 16.5 ± 1.9 mmHg and 7.40 ± 0.04 pH units, respectively. Corresponding values for renal venous blood were 17.9 ± 1.5 mmHg and 7.40 ± 0.04 pH units, respectively. These parameters were little affected by renal arterial infusions of acetylcholine and ANG II, and by hypoxia or hyperoxia.

CPO₂ measured both by Clark electrode and fluorescence optode, and MPO₂ measured by fluorescence optode, increased progressively as inspired O₂ concentration was varied from 10 to 100%. However, these variables did not vary in a systematic manner with RBF (Fig. 3, Table 1).

Renal O₂ consumption changed little as RBF changed. Both renal arterial-venous O₂ concentration difference and fractional O₂ extraction fell as RBF increased, similarly under normoxic and hyperoxic conditions. Hypoxia reduced the absolute level of renal O₂ consumption, presumably due chiefly to reduced GFR secondary to reduced MAP. Hypoxia also reduced renal arterial-venous O₂ concentration difference and increased fractional O₂ extraction. Nevertheless, these variables varied with RBF in a similar manner under hypoxic, normoxic, and hyperoxic conditions (Fig. 4, Table 1).

Changes in RBF induced by renal arterial infusion of vasoactive agents were positively associated with GFR and sodium reabsorption (Fig. 5, Table 1). Filtration fraction was less under hypoxic conditions than normoxic or hyperoxic conditions. It also tended to fall as RBF increased, at least under normoxic and hypoxic conditions. However, this apparent effect was not

Fig. 1. Responses of hemodynamic variables to alterations in inspired O₂ concentration and renal arterial infusion of acetylcholine and ANG II. MAP, mean arterial pressure; RBF, renal blood flow. See Table 1 for statistical analysis. Note that symbols showing MAP during infusion of saline, ANG II, and acetylcholine overlie each other.

Fig. 2. Responses of arterial and renal venous blood PO₂, renal O₂ delivery, and renal O₂ efflux to changes in RBF induced by renal arterial infusion of vasoactive agents, at 4 different levels of inspired O₂ concentration. Symbols show data during ventilation with 10% (●), 21% (○), 50% (▲), and 100% (□) O₂. The 3 sets of symbols joined by the dashed lines represent coordinates during infusion of ANG II, saline, and acetylcholine (left to right). See Table 1 for statistical analysis.
statistically significant across all levels of inspired O₂ (Fig. 5, Table 1). The ratio of renal O₂ consumption to sodium reabsorption was similar under hypoxic, normoxic, and hyperoxic conditions, but was inversely related to RBF (Fig. 5, Table 1). This effect was mostly attributable to the actions of the ANG II infusion. When averaged over all inspired gases, the ratio of renal O₂ consumption to sodium reabsorption averaged 0.51 ± 0.08 ml O₂/mmol during renal arterial infusion of saline. Infusion of ANG II increased this value to 0.78 ± 0.16 ml O₂/mmol (P = 0.02), but it was not significantly altered by infusion of acetylcholine (0.45 ± 0.10 ml O₂/mmol; P = 0.51).

Renal O₂ consumption was positively correlated with RBF, GFR, and sodium reabsorption (Fig. 6). On their own, RBF, GFR, and sodium reabsorption only accounted for 7–17% of the variance observed in renal O₂ consumption (Table 2).

Adding the categorical variable “Rabbit” to the analyses increased the proportion of the variance explained by the models to 50–53%. In contrast, adding the categorical variables “Gas” and “Flow” only increased the proportion of the variance explained by the models to 16–32 and 7–21%, respectively. This analysis suggests that renal O₂ consumption varies between different rabbits because RBF, GFR, and sodium reabsorption vary between rabbits. The small increases in r² achieved by the addition of Gas to the models are likely attributable to the fact that hypoxia reduced renal O₂ consumption (Fig. 4). The fact that addition of Flow to the model had little impact on the values of r² likely reflects the fact that changes in RBF induced by the vasoactive agents had little impact on renal O₂ consumption (Fig. 4).

Tissue temperature in both the cortex and medulla varied slightly with RBF. Under normoxic conditions, infusion of acetylcholine increased cortical temperature by 0.3 ± 0.2°C (from 35.9 ± 0.6°C during saline infusion) and medullary temperature by 0.2 ± 0.2°C (from 37.8 ± 0.5°C during saline infusion). Infusion of ANG II reduced cortical temperature by

Fig. 3. Responses of renal tissue PO₂ to changes in RBF induced by renal arterial infusion of vasoactive agents, at 4 different levels of inspired O₂ concentration. Symbols as for Fig. 2. The 3 sets of symbols joined by the dashed lines represent coordinates during infusion of ANG II, saline, and acetylcholine (left to right). See Table 1 for statistical analysis. CPO₂ and MPO₂, cortical and medullary tissue PO₂.

Fig. 4. Responses of renal tissue PO₂ to changes in RBF induced by renal arterial infusion of vasoactive agents, at 4 different levels of inspired O₂ concentration. AV, arterial-to-venous. Symbols as for Fig. 2. The 3 sets of symbols joined by dashed lines represent coordinates during infusion of ANG II, saline, and acetylcholine (left to right). See Table 1 for statistical analysis.
DISCUSSION

We found that RBF could be reduced or increased by ~30%, without detectable changes in tissue \( \text{Po}_2 \) in the cortex or medulla. This was observed under normoxic, hypoxic, and hyperoxic conditions. Changes in RBF induced by renal arterial infusion of vasoactive agents, at 4 different levels of inspired \( \text{O}_2 \) concentration. The 3 sets of symbols joined by dashed lines represent coordinates during infusion of ANG II, saline, and acetylcholine (left to right). See Table 1 for statistical analysis.

0.5 ± 0.2°C but had little effect on medullary temperature (−0.1 ± 0.3°C change).

The conventional understanding of regulation of intrarenal oxygenation might predict relatively stable renal tissue \( \text{Po}_2 \) in the face of changes in RBF, because of concomitant changes in renal \( \text{O}_2 \) consumption. This view is based on studies performed over 40 years ago, showing little change in fractional \( \text{O}_2 \) extraction with altered RBF (23–26). Because renal \( \text{O}_2 \) consumption is the product of RBF and \( \text{O}_2 \) extraction, renal \( \text{O}_2 \) consumption. Thus renal tissue oxygenation is maintained in the face of relatively large changes in RBF, and so renal \( \text{O}_2 \) delivery. Under the conditions of our current experiment, this phenomenon appears not to be mediated by changes in renal \( \text{O}_2 \) consumption.

Fig. 5. Responses of glomerular filtration rate (GFR), filtration fraction, total sodium reabsorption, and the ratio of \( \text{O}_2 \) consumption to total sodium reabsorption (the inverse of energy efficiency) to changes in RBF induced by renal arterial infusion of vasoactive agents, at 4 different levels of inspired \( \text{O}_2 \) concentration. The 3 sets of symbols joined by dashed lines represent coordinates during infusion of ANG II, saline, and acetylcholine (left to right). See Table 1 for statistical analysis.

Fig. 6. Scattergram of the relationships between renal \( \text{O}_2 \) consumption and RBF (top), GFR (middle), and total sodium reabsorption (bottom). Lines of best fit were determined by the least-products method. Data points show coordinates of individual observations during renal arterial infusion of saline (○), acetylcholine (■), and ANG II (●). See Table 2 for statistical analyses.
consumption was found to vary directly with RBF. In contrast, we found that fractional O₂ extraction fell progressively as RBF increased, so that renal O₂ consumption remained relatively constant. This likely reflects an important methodological difference between our current approach and those of the classic studies in this field (23–26, 45), in which RBF was altered by maneuvers that alter O₂ consumption independently of changes in RBF. For example, RBF was altered by chronic uninephrectomy (25, 45), by changes in renal perfusion pressure that would greatly alter GFR and tubular load (13, 20, 23, 24, 26), or by cooling the kidney, which would reduce tissue metabolic rate (23). In contrast, we acutely altered RBF by infusion of vasoactive agents, which did not significantly alter MAP (and so renal perfusion pressure) and only slightly altered renal tissue temperature. Thus our findings call for revision of the dogma that blood flow and O₂ consumption are necessarily tightly linked in the kidney.

Nevertheless, tubular sodium reabsorption and renal O₂ consumption are tightly linked (21). Our data are consistent with this concept, since renal O₂ consumption was positively correlated with RBF, GFR, and sodium reabsorption. Nevertheless, under normoxic conditions, ANG II infusion reduced sodium reabsorption by 27 ± 15% and acetylcholine infusion increased sodium reabsorption by 18 ± 18%, yet neither treatment significantly altered O₂ consumption. In contrast, we detected decreased renal O₂ consumption during hypoxia (24 ± 11% during saline infusion), reflecting reduced GFR secondary to a small (5 ± 2 mmHg) fall in MAP. Because we could detect this physiologically relevant change in renal O₂ consumption, we can be confident of our measurements of renal O₂ consumption.

Infusion of ANG II reduced the efficiency of renal O₂ consumption, as shown by a significant increase in the ratio of renal O₂ consumption to sodium reabsorption. Presently, we can only speculate about the mechanisms mediating this effect. Potential mechanisms include 1) effects on mitochondrial O₂ utilization, mediated by potential actions of ANG II on nitric oxide bioavailability (3); 2) differential effects of ANG II and/or nitric oxide on tubular transport mechanisms along the nephron that differ in their efficiency of O₂ utilization (3); and even potentially 3) effects of ANG II on O₂ consumption by renal vascular smooth muscle (16, 41, 42, 44, 48).

O₂ transport to tissue occurs not just in capillaries, but also from arteries, arterioles, and sometimes venules (34, 44). Blood Po₂ falls progressively along the arterial tree (7, 44). Indeed, approximately two-thirds of total O₂ extraction occurs in the precapillary network in resting skeletal muscle (34). O₂ losses from arterial blood occur from transport to parenchymal tissue, vessel wall O₂ consumption (41), and where arteries and veins are in a countercurrent arrangement (e.g., kidney, skeletal muscle, and gut), arterial-venous O₂ shunting (29, 30, 39, 46).

The relative contributions of these three O₂ sinks likely differ in different tissues (30, 34). Mathematical models predict that the progressive fall in blood Po₂ along the arterial tree is inversely related to blood flow (40). Thus increased blood flow should lead to increased blood Po₂ in downstream vascular elements and so an increased driving force for diffusion of O₂ to tissue. In the absence of changes in O₂ consumption, this should increase tissue Po₂. Neither tissue Po₂ nor O₂ consumption varied with RBF in the current study, but fractional O₂ extraction fell as RBF increased, suggesting that a mechanism operates to maintain longitudinal Po₂ gradients in the face of changes in RBF.

What is the nature of this mechanism? One possibility is that changes in precapillary arterial-venous O₂ shunting contribute to dynamic regulation of renal parenchymal oxygenation. O₂ in renal venous effluent comes from only two sources: 1) O₂ from blood within the renal capillaries and 2) O₂ shunted directly from precapillary renal arteries to adjacent veins. If renal arterial O₂ delivery and renal venous O₂ concentration increase while renal O₂ consumption remains stable, kidney tissue Po₂ should increase unless some of the increased O₂ delivered in the renal artery never actually gets to kidney tissue. This most plausible explanation for the “missing oxygen” is increased arterial-to-venous O₂ shunting. Our observation of stable renal parenchymal Po₂ therefore indicates that changes in the amount of arterial-venous O₂ shunting may offset changes in renal O₂ delivery induced by changes in RBF and so maintain homeostasis of renal parenchymal O₂ delivery. Three caveats must be applied to this conclusion. First, at present we must limit our conclusions to the context of the current experimental conditions; since changes in RBF induced by different vasoactive factors from those used in the current study, or through changes in renal perfusion pressure, may have very different effects on arterial-to-venous O₂ shunting than changes in RBF induced by renal arterial infusion of ANG II and acetylcholine.

Second, we cannot exclude the possibility that changes in the pH of renal tissue and capillary blood, induced by changes in RBF, may have altered the Po₂/hemoglobin saturation relationship. However, this seems unlikely since although we did not measure renal tissue pH in the current study, the Pco₂ and pH of arterial and renal venous blood were not altered by changes in RBF or inspired O₂ content. Third, our experiment was performed under conditions of hypocapnia. This arose because we needed to set arterial blood Po₂ at ~100 mmHg during ventilation with room air. Although the pH of arterial and renal venous blood was normal (i.e., 7.4) in the current study, our experiment must be replicated under conditions of normocapnia before we can confidently generalize the concept that arterial-to-venous O₂ shunting contributes to dynamic regulation of intrarenal oxygenation.

These caveats aside, our data indicate that the O₂ content of blood within renal capillaries remained relatively stable across the range of RBF examined. This allows us to estimate the

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### Table 2. Values of Pearson’s product-moment correlation coefficient ($r^2$) for analyses of covariance performed on the data shown in Fig. 6

<table>
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<tr>
<th>Covariant Variable</th>
<th>Covariant Only</th>
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<th>+ Gas</th>
<th>+ Flow</th>
<th>Full Model</th>
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<td>RBF</td>
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<td>0.75</td>
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<td>GFR</td>
<td>0.17</td>
<td>0.53</td>
<td>0.25</td>
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<td>0.51</td>
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<td>0.07</td>
<td>0.68</td>
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Values of $r^2$ represent the proportion of the variance that can be accounted for by the relationships between renal oxygen consumption (ordinate) and RBF, GFR, or sodium reabsorption (abscissa/covariant). Values of $r^2$ are shown for the simple correlation of the abscissal and ordinal variables and when the categorical variables “Rabbit,” “Gas,” and “Flow” are included in the analysis. Values of $r^2$ are also shown for the full model, which includes all 3 categorical variables plus all interaction terms that do not include the factor Rabbit.
contribution of arterial-venous O₂ shunting to dynamic regulation of renal oxygenation using the equation:

\[
\text{Change in renal arterial-venous O}_2 \text{ shunting} = [\text{Renal arterial-venous O}_2 \text{ difference (high flow)} - \text{renal arterial-venous O}_2 \text{ difference (low flow)}] * \text{RBF (high flow)}
\]

That is, if we assume the contribution of O₂ within renal capillaries, to renal venous O₂ efflux, changes in direct proportion with RBF, then changes in arterial-venous O₂ concentration difference must be due to changes in arterial-venous O₂ shunting. Based on this formula, we calculate that 0.17 ± 0.06 ml/min more O₂ was shunted during saline infusion than during ANG II infusion, and 0.37 ± 0.09 ml/min more O₂ was shunted during acetylcholine infusion than during ANG II infusion (across all gas mixtures). This equates to ~15% of the difference in renal O₂ delivery in each case. Thus arterial-venous O₂ shunting may make an important contribution to maintenance of homeostasis of intrarenal oxygenation.

Our current and previous (31) observations suggest that renal tissue Pₒ₂ remains remarkably stable when RBF is altered within the physiological range, although tissue hypoxia does occur when RBF is reduced by more than ~30% (31). How can we reconcile these findings with those of previous studies showing changes in renal tissue Pₒ₂ in response to vasoactive factors? An important difference between our current study, and most previous studies of the relationship between RBF and renal tissue oxygenation (5, 6, 8, 12, 28), is our provision of detailed information on both RBF and renal O₂ consumption. Thus it is equally possible that the changes in CPO₂ observed in these previous experiments resulted from altered renal metabolism rather than the direct effects of altered RBF. Our data might also appear at odds with those of Juillard et al. (18) who observed progressive desaturation of hemoglobin (assessed by functional magnetic resonance imaging) in both the cortex and medulla when RBF was reduced by renal artery constriction. However, blood O₂ levels do not necessarily reflect tissue Pₒ₂, as evidenced by the fact that renal venous Pₒ₂ varied with RBF in our study, yet renal tissue Pₒ₂ did not.

As we have found previously (31), cortical Pₒ₂ measured by fluorescence optode was always less than that measured by Clark electrode. Nevertheless, responses of cortical tissue Pₒ₂ to changes in RBF and arterial blood Pₒ₂, assessed using Clark electrodes and fluorescence optodes, were qualitatively similar. Furthermore, the relationship between simultaneous measurements of cortical Pₒ₂ made with the two methods in the current study could be fitted to a straight line with proportional bias (i.e., slope ≠ 1) but no fixed bias (i.e., no zero offset). Thus it seems very unlikely that the conclusions we draw from our current experiment are confounded by the techniques we used to measure tissue Pₒ₂.

Although changes in RBF had little effect on renal tissue Pₒ₂, changes in arterial Pₒ₂ profoundly altered renal tissue Pₒ₂. These observations reflect the importance of gradients in O₂ partial pressure in tissue O₂ delivery. Thus changes in atmospheric O₂ content would likely profoundly alter renal tissue Pₒ₂, and thus the signaling cascades that mediate erythropoietin release (43). Our observations suggest that changes in RBF within the physiological range will likely have little impact on these signaling cascades. Dissociation of erythropoietin synthesis and RBF makes adaptive sense, since it allows independent regulation of extracellular fluid volume and blood O₂-carrying capacity.

In conclusion, tissue Pₒ₂ must be regulated within tight limits to maintain cellular respiration, prevent hypoxic damage, prevent excessive production of reactive oxygen species, and regulate O₂-dependent gene expression. This is no less true in the kidney than in other organs (1, 3, 9, 29, 30, 43). However, the mechanisms regulating tissue oxygenation in the kidney differ somewhat from those operating in other organs. In particular, unlike organs such as skeletal muscle and the heart (11), brain (2), and retina (36), changes in tissue Pₒ₂ in the kidney do not profoundly alter renal vascular tone (11, 22). This allows control of glomerular filtration, the primary function of the kidney, to dominate control of renal vascular tone. However, this could result in large fluctuations in renal tissue Pₒ₂, unless additional mechanisms operate to maintain homeostasis of renal tissue oxygenation. Our current results indicate that renal tissue Pₒ₂ changes little when RBF is altered within the physiological range (± ~30%). This occurs despite relatively stable renal O₂ consumption and may be mediated by changes in the efficiency of pregglomerular arterial-venous O₂ shunting. We speculate that this mechanism could be adaptive, by allowing RBF and GFR to change in response to physiological requirements, without concomitant changes in tissue Pₒ₂. Diabetes (33) and hypertension (46) are both associated with renal tissue hypoxia, at least in part due to reduced efficiency of O₂ utilization by the kidney. Future studies should examine whether malfunction of renal arterial-venous O₂ shunting also contributes to renal hypoxia under these pathological conditions. Renal arterial-venous O₂ shunting may also contribute to development of renal hypoxia during acute hemodilution (17).


