Variable Responses of Regional Renal Oxygenation and Perfusion to Vasoactive Agents in Awake Sheep

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Running title: Hormonal control of renal oxygenation

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Abstract

Vasoactive agents are used in critical care to optimize circulatory function, but their effects on renal tissue oxygenation in the absence of anesthesia remain largely unknown. Therefore, we assessed the effects of multiple vasoactive agents on regional kidney oxygenation in awake sheep. Sheep were surgically instrumented with pulmonary and renal artery flow probes, and combination fiber-optic probes, in the renal cortex and medulla, comprising a fluorescence optode to measure tissue PO2 and a laser-Doppler probe to assess tissue perfusion. Carotid arterial and renal venous cannulae enabled measurement of arterial pressure and total renal oxygen delivery and consumption. Norepinephrine (0.1 or 0.8 µg/kg/min) dose-dependently reduced cortical and medullary laser Doppler flux (LDF) and PO2 without significantly altering renal blood flow (RBF), or renal oxygen delivery or consumption. Angiotensin II (9.8±2.1 µg/h) reduced RBF by 21%, renal oxygen delivery by 28%, oxygen consumption by 18% and medullary PO2 by 38%, but did not significantly alter cortical PO2 or cortical or medullary LDF. Arginine vasopressin (3.3±0.5 µg/h) caused similar decreases in RBF and renal oxygen delivery, but did not significantly alter renal oxygen consumption or cortical or medullary LDF or PO2. Captopril had no observable effects on cortical or medullary LDF or PO2, at a dose that increased renal oxygen delivery by 24%, but did not significantly alter renal oxygen consumption. We conclude that vasoactive agents have diverse effects on regional kidney oxygenation in awake sheep that are not predictable from their effects on LDF, RBF or total renal oxygen delivery and consumption.

Abstract word-count: 250

Key words: Intrarenal oxygenation, intrarenal perfusion, renal blood flow, hypoxia, angiotensin II, vasopressin, norepinephrine, captopril.
Introduction

Vasoactive agents are often used in a critical care setting with the aim of improving cardiovascular and renal function. One of the therapeutic goals of the use of vasoactive therapies is to maintain renal perfusion, particularly in patients with, or at risk of developing, acute kidney injury (AKI). For example, vasoconstrictor agents such as norepinephrine and arginine vasopressin (AVP) are used for hemodynamic resuscitation of patients with sepsis (4, 16). Similarly, patients undergoing cardiopulmonary bypass are managed with multiple vasoactive agents (14). Sepsis and cardiac surgery on cardiopulmonary bypass are both clinical settings in which patients are exposed to significant risk of development of AKI (11). Recently, considerable interest has been focused on the potential role of renal tissue hypoxia in the development of AKI (11). Consequently, there is a need for a better understanding of how vasoactive agents used in critical care settings affect regional kidney oxygenation.

Renal tissue oxygenation is determined by the balance between regional oxygen delivery and oxygen consumption (11). In an experimental setting, total renal oxygen delivery and consumption can be determined through measurement of total renal blood flow (RBF) together with arterial and renal venous blood oxygen content. The balance between total oxygen delivery and consumption has, for example, been used clinically to assess the impact on kidney oxygenation of resuscitation of patients, with vasodilatory shock after cardiac surgery, with norepinephrine (26) and AVP (1). However, because local perfusion within the kidney is heterogeneous, and blood flow to the renal medulla is regulated at least partly independently of blood flow to the renal cortex (5), this information has limited value in assessing local oxygen delivery or regional tissue oxygen tension (tPO2).

Renal tPO2 can be measured directly in experimental animals using electrochemical and optical methods (8). However, these techniques require the use of anesthesia and acute
surgical exposure of the kidney. We have recently developed a method that allows direct
measurement of regional tPO$_2$, together with tissue laser Doppler flux as an index of perfusion,
in awake sheep (3). This has the great advantage that it removes the confounding effects of
anesthesia, which alters systemic hemodynamics and is likely to also alter intra-renal
hemodynamics and tPO$_2$. In the current study, we applied this method to characterize the
effects of vasoactive agents used in the management of hemodynamics in clinical settings
(norepinephrine, AVP, angiotensin II (Ang II) and captopril), on tPO$_2$ in the renal cortex and
medulla. Simultaneously, we assessed the effects of these agents on cardiac output and total
RBF, whole kidney oxygen delivery and oxygen consumption, and cortical and medullary
laser Doppler flux.

Materials and Methods

Animal preparation

Experiments were conducted on 8 adult Merino ewes (33.0 ± 1.8 kg) aged 1.5 to 2 years.
Sheep were housed in individual metabolic cages, with free access to food and water. The
experimental procedures were approved by the Animal Experimental Ethics Committee of the
Howard Florey Institute under guidelines laid down by the National Health and Medical
Research Council of Australia.

The animals underwent two sterile surgical procedures under general anesthesia at an interval
of 2 weeks. Anesthesia was induced with intravenous sodium thiopental (10-15 mg/kg) for
intubation with an endotracheal tube (cuffed size 9). Maintenance of anesthesia was by means
of oxygen/air/isoflurane (end tidal isoflurane 1.5-2.0% v/v). In both operations, animals were
treated with procaine penicillin (900 mg administered intramuscularly, Ilium Propen; Troy
Laboratories, Smithfield, NSW, Australia or Mavlab, Qld, Australia) at the start of surgery and
then for 2 days postoperatively. Post-surgical analgesia was maintained with intramuscular injection of flunixin meglumine (1 mg/kg, Troy Laboratories or Mavlab, Qld, Australia) at the start of surgery and then 8 and 24 hours after surgery. At the first operation, a carotid arterial loop was created to facilitate subsequent arterial cannulation and a 20 mm transit-time flow probe (Transonic Systems, Ithaca, NY, USA) was placed around the pulmonary artery via a left thoracotomy through the 4th intercostal space. During the second procedure, a 4 mm transit-time flow probe was placed around the left renal artery. The renal vein was isolated and cannulated with a Tygon catheter (Cole-Parmer; Boronia, Australia; ID 1.0 mm, OD 1.5 mm).

At the same operation, two custom-built fiber-optic probes (CP-004-001, Oxford Optronix, Oxford, U.K.), with 20 mm of optical fiber extending from the outer sheath, were inserted into the kidney, as previously described (3). In brief, the outer sheath of the probes was glued to a plastic sheet, which was secured to the kidney capsule with cyanoacrylate adhesive. Probes were inserted at angles such that one probe tip was positioned within the cortex (~4 mm below the renal capsule) and one was in the medulla (~16 mm below the renal capsule). The positions of the probes were confirmed at post-mortem. Each probe contained a dual fiber laser Doppler probe (for estimation of tissue perfusion by measurement of laser Doppler flux (LDF)), a single fiber fluorescence optode (for measurement of tPO2) and a thermocouple (for measurement of tissue temperature) (3, 20, 24). The probes are pre-calibrated by the manufacturer. We have recently shown that the values of laser Doppler flux and tPO2 generated by these probes in the cortex and medulla of awake sheep are stable over many days and respond rapidly and reproducibly to changes in renal perfusion pressure (3). Provided the probes are not damaged during the experiment, values of PO2 obtained in saline solution saturated with CO2 (~0 mmHg) or room air (~160 mmHg) are similar before and after implantation.
Following placement of the fiber-optic probes, a Tygon catheter (ID 1.0 mm, OD 1.5 mm; Cole-Palmer; Boronia, Australia) was then inserted into the carotid arterial loop for measurement of arterial pressure and collection of arterial blood samples. Two polythene catheters (ID 1.19 mm, OD 1.7 mm; Portex®; Smiths Medical International Ltd. Hythe, Kent, UK) were inserted into a jugular vein; one for measurement of central venous pressure and one for drug infusion. The animals were then allowed five days to recover before the experimental observations commenced.

**Hemodynamic and renal variables**

The combination fiber-optic probes were connected to OxyLite™ 2000 and OxyFlo™ (Oxford Optronix) monitors to provide measures of tPO₂ (mmHg), tissue temperature (°C) and LDF (arbitrary units). These analog signals, as well as those for arterial pressure (MAP), central venous pressure (CVP), heart rate (HR), cardiac output (CO) and RBF were continuously recorded with Spike2 software (CED, Cambridge Electronic Design Limited, Cambridge, England) and averaged every 60 seconds.

Blood samples were simultaneously obtained from the carotid arterial and renal venous catheters for oximetry and measurement of blood lactate concentration. Standard formulae were used to calculate, for each sheep during each experimental period, cardiac index (CI = CO/body surface area (15)), renal oxygen delivery (RBF*Arterial O₂ concentration), renal oxygen consumption (RBF*[Arterial - venous O₂ concentration]), and oxygen extraction ([Arterial - venous O₂ concentration]/Arterial O₂ concentration).

**Experimental protocol**

The interventions were applied between 8 am and 6 pm over two consecutive days, 5 to 7 days after the implantation of the probes. During the experiments the animals were kept in a standing position in the cage and had no access to food and water. Animals were allowed at
least a 2 hour recovery period after completing each intervention, or until RBF and MAP had returned to baseline values. Following a 15 min baseline period, infusions of drugs were given for 30 min followed by a 30 min recovery period. Ang II and AVP (Auspep, Melbourne, Australia) were infused at rates titrated to reduce RBF by approximately 20%. Norepinephrine (Levophed®, Abbott, Kurnell, Australia) was infused at two doses (0.1 µg/kg/min and 0.8 µg/kg/min, 30 min infusion periods), to increase MAP by approximately 10 and 30 mmHg respectively. Captopril (25 mg; Squibb, Princetown, N.J.) was given at a dose that increased RBF by approximately 20%. The Ang II, AVP and norepinephrine treatments were given in random order; captopril treatment was given as the last intervention. Data were recorded continuously throughout the experimental periods. Hemodynamic and renal variables responded rapidly to the infusions, with a new baseline being reached, in most cases, within the first 10 min of the infusion. As an example, temporal responses to infusion of norepinephrine are shown in Figs 1 & 2. For simplicity, all data were averaged across the entire baseline (15 min), infusion (30 min each) and recovery (30 min) periods. Arterial and renal venous blood samples were collected, during the final 5 min of each baseline period, intervention period, and (except for captopril) recovery period.

**Histological analysis**

At the end of the studies, the animals were euthanized and the correct positioning of the tips of the fiber optic probes was verified. The tip of the medullary probe was 6-10 mm from the corticomedullary junction and the tip of the cortical probe was found to be 2-3 mm from the cortical surface. As we have found previously (3), Masson’s trichrome staining revealed little evidence of extensive scarring around the probe (data not shown).

**Statistical analysis**

Hemodynamic variables and tissue perfusion and PO2 are expressed as between-subject mean ± standard error of mean (SEM). Due to failure of equipment, not all variables were available
for all animals in all protocols, so \( n = 6-8 \) for all hemodynamic variables and regional kidney perfusion and oxygenation. Renal oxygen delivery, oxygen consumption and oxygen extraction were log transformed to achieve normality and are expressed as the geometric mean (95% confidence interval). Because the renal venous catheter was patent in only some experiments, relatively few observations were available for these data (\( n = 3-4 \)). Hypothesis testing was performed using the software package SYSTAT (Version 13, Systat Software, Chicago, IL, USA). Dichotomous comparisons between baseline and interventions were made using Student’s paired t-test (i.e. for Ang II, AVP and captopril). Responses to norepinephrine were analyzed using repeated measures analysis of variance. \( P \) values for within-subjects factors were conservatively adjusted by the Greenhouse-Geisser method (21). Two-tailed \( p \leq 0.05 \) was considered statistically significant.

**Results**

*Responses to Norepinephrine*

Norepinephrine increased MAP in a dose-dependent manner, such that it was increased by 6 ± 2 mmHg with the low dose (0.1 \( \mu g/kg/min \)) and by 30 ± 4 mmHg at the high dose (0.8 \( \mu g/kg/min \)) (Table 1, Figs. 1 & 3). Neither dose of norepinephrine significantly altered RBF (\( p = 1.0 \)), but it caused dose-dependent reductions in cortical LDF and tPO\(_2\) and in medullary LDF and tPO\(_2\) (Table 2, Figs 2 & 4). For example, at the high dose of norepinephrine cortical and medullary LDF were reduced by 17 ± 4 and 53 ± 10% respectively, while cortical and medullary tPO\(_2\) were reduced by 49 ± 9 and 72 ± 11% respectively (Table 2, Fig. 4).

A patent renal vein catheter was present in 3 of 8 animals at the time of the experiment. Renal oxygen delivery was not significantly altered by norepinephrine infusion. However, renal oxygen consumption was increased in a dose-dependent manner, by 14 ± 5% during infusion.
of 0.1 µg/kg/min and by 33 ± 9% during infusion of 0.8 µg/kg/min. However, the fractional extraction of oxygen was not significantly altered by norepinephrine infusion (Table 3, Fig. 5).

**Responses to Angiotensin II**

Infusion of Ang II (9.8 ± 2.1 µg/h) reduced RBF by 21 ± 1% and increased MAP by 12 ± 3 mmHg (Table 1, Fig. 3). These hemodynamic changes were associated with no significant change in medullary LDF, but a decrease in medullary tPO$_2$ of 38 ± 14% (Table 2, Fig. 4). There were no significant changes in cortical LDF or tPO$_2$.

A patent renal vein catheter was present in 4 of 8 animals at the time of the experiment. Ang II infusion decreased renal oxygen delivery by 28 ± 2%, but did not significantly change renal oxygen consumption or renal oxygen extraction ratio (Table 3, Fig. 5).

**Responses to Arginine Vasopressin**

Infusion of AVP (3.3 ± 0.5 µg/h, equivalent to 13.2 ± 2.0 Unit/h) reduced RBF by 18 ± 1% and increased MAP by 6 ± 1 mmHg (Table 1, Fig. 3). There were no significant changes in cortical or medullary LDF or tPO$_2$ ($p \geq 0.24$; Table 2, Fig. 4).

In 4 sheep, renal oxygen delivery significantly decreased by 36 ± 3% during infusion of AVP. Renal oxygen consumption did not change significantly, but fractional extraction of oxygen increased by 53 ± 16% from its baseline level (Table 3, Fig. 5).

**Responses to Captopril**

Infusion of captopril decreased MAP by 7 ± 1 mmHg and increased RBF by 16 ± 1% (Table 1, Fig. 3). No significant changes in cortical LDF ($p = 0.26$, paired t-test), medullary LDF ($p = 0.43$), cortical tPO$_2$ ($p = 0.82$) or medullary tPO$_2$ ($p = 0.19$) were observed (Table 2, Fig. 4).
A patent renal vein catheter was present in 4 of 8 animals at the time of the experiment. Captopril increased renal oxygen delivery (27 ± 7%) but did not significantly alter renal oxygen consumption, so the fractional extraction of oxygen was reduced by 19 ± 2% (Table 3, Fig. 5).

**Discussion**

Our major finding was that the vasoconstrictor agents norepinephrine, Ang II and AVP and the angiotensin converting enzyme inhibitor captopril had diverse and often divergent effects on regional renal tissue PO\(_2\) in awake sheep. Norepinephrine infusion, at a dose that increased MAP but had no significant effect on RBF or renal oxygen delivery, and increased renal oxygen consumption, decreased tissue LDF and PO\(_2\) in both the cortex and medulla. Infusion of Ang II, which reduced RBF and renal oxygen delivery, had no apparent effect on cortical or medullary LDF, but caused selective medullary hypoxia. In contrast, a dose of AVP that caused similar decreases in RBF and renal oxygen delivery did not reduce cortical or medullary LDF or PO\(_2\). Inhibition of angiotensin converting enzyme with captopril increased renal oxygen delivery, but not consumption, and did not significantly alter cortical or medullary tissue PO\(_2\). Taken together, our findings indicate that changes in regional renal oxygenation cannot be predicted from the effects of these drugs on total RBF, global renal oxygen delivery and renal oxygen consumption, or even regional perfusion as assessed by laser Doppler flowmetry.

Kidney oxygenation is determined by complex interactions between oxygen delivery in arterial blood, oxygen consumption (which is mainly driven by tubular sodium reabsorption (10)) and counter-current oxygen shunting in the renal cortex and medulla (7). Furthermore, because blood flow can be differentially regulated in the cortex and medulla (5), and because neurohumoral factors can alter sodium reabsorption within the various nephron segments (10),...
regional renal tissue oxygenation may be difficult to predict from global measures of renal
oxxygen delivery and oxygen consumption. Our current findings provide direct support for this
concept.

For example, norepinephrine infusion substantially reduced both cortical and medullary tPO₂,
despite the fact that total RBF and renal oxygen delivery were well maintained during this
intervention, likely because of the significant increase in renal oxygen consumption and the
reduction in medullary perfusion (as assessed by LDF). During infusion of Ang II the
reduction in medullary tPO₂ might be explicable in terms of the decrease in RBF and
associated reduction in renal oxygen delivery as well as increased oxygen utilization within
the renal medulla. This could arise if Ang II were to increase sodium reabsorption, or to
reduce the efficiency of oxygen utilization for sodium reabsorption in the medullary thick
ascending limbs and/or the distal nephron. Such effects are likely, given that (i) glomerular
filtration rate is better maintained than RBF during infusion of Ang II in conscious sheep (27)
and (ii) Ang II stimulates superoxide production and thus sodium reabsorption in the thick
ascending limb (22). On the other hand captopril increased RBF and renal oxygen
consumption, but despite this hyperemia there were no significant changes in cortical or
medullary LDF or tPO₂. Similarly, we previously found that increases in RBF and cortical
LDF within the physiological range (up to 30%) in anesthetized rabbits, by renal arterial
infusion of acetylcholine, had little effect on renal cortical oxygenation (9, 19), even when
changes in global renal oxygen delivery far exceeded those in global renal oxygen
consumption. Interestingly, during infusion of AVP, at a dose that reduced RBF and renal
oxygen delivery to a similar extent to infusion of Ang II, cortical and medullary tPO₂ were
well maintained. These data indicate that total RBF and renal oxygen delivery are unreliable
predictors of cortical and medullary tissue oxygenation. This lack of predictive efficacy is
likely due to the fact that they do not take into account regional variations in blood flow and
oxygen delivery, changes in oxygen consumption associated with altered tubular sodium reabsorption, and potentially also changes in counter-current shunting of oxygen.

The effects of vasoactive infusions on cortical and medullary LDF also had variable efficacy in predicting changes in regional tPO_2_. On the one hand, reductions in cortical and medullary tPO_2_ induced by infusion of norepinephrine were associated with reductions in both cortical and medullary LDF. Furthermore, the maintenance of cortical and medullary tPO_2_ during infusion of AVP was associated with relatively well maintained cortical and medullary LDF. In contrast, infusion of Ang II reduced medullary tPO_2_ without reducing medullary LDF.

Maintenance of renal cortical oxygenation in the face of mis-matched changes in renal oxygen delivery and consumption has been observed previously. For example, in anesthetized rabbits renal cortical tissue PO_2_ changed little when RBF was reduced within the physiological range (i.e. by 30% or less) by renal arterial infusion of Ang II (9, 19) or electrical stimulation of the renal nerves (6). A candidate mechanism is altered diffusive counter-current exchange of carbon dioxide and/or oxygen between renal arteries and veins (6). According to this hypothesis, reduced arterial to venous oxygen shunting or increased venous to arterial shunting of carbon dioxide during ischemia could increase oxygen extraction and maintain oxygen transport to tissue even if total renal oxygen delivery is reduced. It has also been suggested that the coupling of proximal tubular sodium reabsorption with glomerular filtration rate (glomerulo-tubular balance) (23) might promote homeostasis of renal cortical oxygenation at the expense of changes in oxygen consumption in more distal segments of the nephron (9).

**Study Limitations**

In the context of the complexity of renal oxygenation, there are important limitations of the current study that decrease our ability to provide definitive explanations for our findings. First,
because of the relatively brief periods (30 min) over which we assessed the effects of each vasoactive agent, we were unable to measure glomerular filtration rate and sodium reabsorption. Thus, we have no direct measure of the major driver of renal oxygen consumption, tubular sodium reabsorption (10). Nevertheless, we were able to measure renal oxygen delivery and consumption and were able to assess the impact of the vasoactive agents we administered on the balance between oxygen supply and demand at the whole kidney level. However, due to failure of the renal venous catheter in some animals, our measurements of renal oxygen delivery and consumption were based in relatively small sample sizes (n = 3-4), thus increasing the risk of type 2 errors. Indeed, we encountered considerable between-sheep variability in renal oxygen delivery and consumption, although this was mainly attributable to variability of RBF. Consequently, fractional oxygen extraction, our major measure of the balance between renal oxygen delivery and consumption, was associated with relatively little between-sheep variation. The second limitation is that we have no measure of renal oxygen consumption specifically in the renal cortex or medulla. Unfortunately, the only available methods for assessment of local oxygen utilization have very limited spatial resolution (8, 18). Furthermore, because they rely on positron emission tomography, they can only be applied in experimental animals under anesthesia. The third limitation of our study arises from the use of laser Doppler flowmetry for measurement of regional perfusion. In highly perfused tissues such as the kidney, laser Doppler flux is chiefly a measure of mean erythrocyte velocity rather than total erythrocyte flux (and thus local oxygen delivery) (12). Thus, it cannot detect changes in the number of perfused capillaries which might have a major impact on oxygen delivery to tissue, particularly in the renal medulla (13). Collectively, these factors likely underlie the disparities in changes in total RBF and cortical LDF during infusion of vasoactive agents in the current study. Laser Doppler flowmetry is also insensitive to changes in the degree of heterogeneity of capillary perfusion, which would also be expected to have a
major impact on oxygen delivery to tissue (17). Effects of vasoactive infusions on heterogeneity of regional perfusion may at least partly explain why changes in cortical and medullary laser Doppler flux had variable efficacy in predicting changes in regional tissue PO$_2$. The between-subject variability in cortical and medullary laser Doppler flux is also noteworthy. This likely arises because the probe measures erythrocyte velocity within a relatively small volume of tissue (~1 mm$^3$). In the kidney, the spatial variability in local blood flow probably compounds this problem. However, the technique is valid when used for making comparisons on a within subject basis, as we have in the current study.

Our current studies are the first, to our knowledge, to assess responses of regional kidney tissue oxygenation to vasoactive agents in unanesthetized animals. Consequently, it is difficult to compare our current findings with those of previous studies performed in anesthetized animals (e.g. (6, 25)). Such comparisons would require matched experiments, performed while animals are conscious and under a variety of anesthetic regimens.

It is also important to note that these studies were carried out in healthy animals with normal blood pressure and the responses may be different in hypotensive settings, such as sepsis, where these vasopressor agents are used. Indeed, using the methods employed in the current study, we recently demonstrated renal ischemia and hypoxia restricted to the medulla during septic acute kidney injury in sheep (2). In future studies it will be imperative to determine whether medullary ischemia and hypoxia in septic AKI are exacerbated by vasoactive therapies.

**Perspectives**

Our findings emphasize the complexity of the regulation of intrarenal oxygenation and the dangers of drawing conclusions regarding kidney oxygen status from anything but direct
measurement of tPO$_2$. Specifically, they show that the responses of regional tissue oxygenation to vasoactive agents cannot be reliably predicted from measurement of total RBF and global renal oxygen delivery and consumption, nor from estimation of regional tissue perfusion by laser Doppler flowmetry. Thus, methods for direct measurement of regional tPO$_2$, as described herein, are indispensable for assessment of regional kidney oxygenation. Our findings also demonstrate that, in awake sheep, tPO$_2$ in both the renal cortex and medulla is better preserved during infusion of AVP than during infusion of Ang II, to achieve a similar (~20%) reduction in RBF, or during infusion of norepinephrine, to achieve a similar (~6%) increase in MAP. These findings may have important implications for the choice of vasoactive agents for hemodynamic support in the setting of critical care, particularly for patients with kidney failure in whom reductions in intra-renal PO$_2$ may worsen the condition. Further studies are required to determine the effects of these vasoconstrictor agents on renal cortical and medullary PO$_2$ in models of acute and chronic kidney injury.

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References


Table 1. Hemodynamic responses to infusion of vasoactive agents

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Arterial Pressure (mmHg)</th>
<th>Heart Rate (beats/minute)</th>
<th>Cardiac Index (L/min/m²)</th>
<th>Renal Blood Flow (mL/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>92 ± 4</td>
<td>80 ± 5</td>
<td>4.00 ± 0.19</td>
<td>221 ± 13</td>
</tr>
<tr>
<td>Norepinephrine (low)</td>
<td>98 ± 4*</td>
<td>73 ± 3*</td>
<td>3.95 ± 0.14</td>
<td>221 ± 14</td>
</tr>
<tr>
<td>Norepinephrine (high)</td>
<td>122 ± 4***</td>
<td>81 ± 4</td>
<td>4.26 ± 0.18</td>
<td>221 ± 16</td>
</tr>
<tr>
<td>Recovery</td>
<td>106 ± 4**</td>
<td>98 ± 7*</td>
<td>5.12 ± 0.26**</td>
<td>245 ± 17*</td>
</tr>
<tr>
<td>Baseline</td>
<td>88 ± 3</td>
<td>79 ± 4</td>
<td>3.98 ± 0.19</td>
<td>221 ± 17</td>
</tr>
<tr>
<td>Ang II</td>
<td>100 ± 4**</td>
<td>71 ± 3**</td>
<td>3.81 ± 0.14</td>
<td>174 ± 13***</td>
</tr>
<tr>
<td>Recovery</td>
<td>93 ± 5</td>
<td>75 ± 3</td>
<td>3.99 ± 0.13</td>
<td>206 ± 18</td>
</tr>
<tr>
<td>Baseline</td>
<td>90 ± 4</td>
<td>85 ± 5</td>
<td>4.16 ± 0.18</td>
<td>241 ± 17</td>
</tr>
<tr>
<td>AVP</td>
<td>96 ± 5**</td>
<td>63 ± 3***</td>
<td>2.92 ± 0.23**</td>
<td>198 ± 14***</td>
</tr>
<tr>
<td>Recovery</td>
<td>92 ± 4</td>
<td>69 ± 6*</td>
<td>3.51 ± 0.19*</td>
<td>214 ± 15***</td>
</tr>
<tr>
<td>Baseline</td>
<td>90 ± 5</td>
<td>86 ± 8</td>
<td>4.28 ± 0.22</td>
<td>233 ± 16</td>
</tr>
<tr>
<td>Captopril</td>
<td>83 ± 4**</td>
<td>100 ± 11*</td>
<td>4.46 ± 0.24*</td>
<td>270 ± 19***</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of n = 6-8. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 compared with baseline (from paired t-test). Low dose and high dose for norepinephrine: 0.1 µg/kg/min and 0.8 µg/kg/min respectively. AVP = arginine vasopressin; Ang II = angiotensin II.
Table 2. Regional kidney perfusion and oxygenation during infusion of vasoactive agents

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cortical Perfusion (Units)</th>
<th>Cortical Tissue PO$_2$ (mmHg)</th>
<th>Medullary Perfusion (Units)</th>
<th>Medullary Tissue PO$_2$ (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1167 ± 214</td>
<td>32.7 ± 4.6</td>
<td>809 ± 107</td>
<td>37.4 ± 3.7</td>
</tr>
<tr>
<td>Norepinephrine (low)</td>
<td>1106 ± 214</td>
<td>27.4 ± 4.6</td>
<td>654 ± 105</td>
<td>28.6 ± 4.4**</td>
</tr>
<tr>
<td>Norepinephrine (high)</td>
<td>966 ± 193**</td>
<td>16.9 ± 3.9**</td>
<td>360 ± 74**</td>
<td>10.5 ± 3.7***</td>
</tr>
<tr>
<td>Recovery</td>
<td>1164 ± 228</td>
<td>28.4 ± 5.0*</td>
<td>439 ± 64*</td>
<td>13.6 ± 3.8**</td>
</tr>
<tr>
<td>Baseline</td>
<td>1126 ± 181</td>
<td>33.7 ± 4.6</td>
<td>642 ± 128</td>
<td>32.4 ± 4.4</td>
</tr>
<tr>
<td>Ang II</td>
<td>1280 ± 240</td>
<td>31.2 ± 5.5</td>
<td>636 ± 144</td>
<td>22.3 ± 6.1*</td>
</tr>
<tr>
<td>Recovery</td>
<td>1231 ± 172</td>
<td>35.5 ± 5.0</td>
<td>672 ± 144</td>
<td>30.6 ± 4.9</td>
</tr>
<tr>
<td>Baseline</td>
<td>1068 ± 208</td>
<td>31.2 ± 2.6</td>
<td>750 ± 85</td>
<td>38.7 ± 4.6</td>
</tr>
<tr>
<td>AVP</td>
<td>1203 ± 249</td>
<td>31.6 ± 4.5</td>
<td>926 ± 173</td>
<td>40.0 ± 4.4</td>
</tr>
<tr>
<td>Recovery</td>
<td>1167 ± 237</td>
<td>36.8 ± 4.7</td>
<td>881 ± 169</td>
<td>42.0 ± 3.7*</td>
</tr>
<tr>
<td>Baseline</td>
<td>1395 ± 312</td>
<td>35.6 ± 3.2</td>
<td>878 ± 121</td>
<td>34.7 ± 4.8</td>
</tr>
<tr>
<td>Captopril</td>
<td>1504 ± 317</td>
<td>34.6 ± 3.9</td>
<td>828 ± 125</td>
<td>38.4 ± 4.1</td>
</tr>
</tbody>
</table>

Values are mean ± SEM of n = 6-8. *$p$ ≤ 0.05, **$p$ ≤ 0.01, ***$p$ ≤ 0.001 compared with baseline (from paired t-test). Low-dose and high-dose norepinephrine (0.1 µg/kg/min and 0.8 µg/kg/min respectively). AVP = arginine vasopressin; Ang II = angiotensin II.
Table 3. Responses of renal oxygen delivery, consumption and extraction ratio to infusion of vasoactive agents

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oxygen Delivery (ml/min)</th>
<th>Oxygen Consumption (ml/min)</th>
<th>Extraction Ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>17.5 (8.4 – 36.7)</td>
<td>2.21 (1.18 – 4.12)</td>
<td>12.6 (9.9 - 16.0)</td>
</tr>
<tr>
<td>Norepinephrine (low)</td>
<td>18.2 (8.7 – 38.0)</td>
<td>2.52 (1.35 – 4.70)</td>
<td>13.9 (10.9 - 17.6)</td>
</tr>
<tr>
<td>Norepinephrine (high)</td>
<td>21.6 (10.3 – 45.3)</td>
<td>2.92 (1.56 – 5.44)</td>
<td>13.5 (10.6 - 17.1)</td>
</tr>
<tr>
<td>Ang II</td>
<td>16.5 (9.0 – 30.1)</td>
<td>2.33 (1.25 – 4.31)</td>
<td>14.1 (11.3 - 17.7)</td>
</tr>
<tr>
<td>A VP</td>
<td>11.9 (6.5 – 21.7)</td>
<td>1.88 (1.01 – 3.46)</td>
<td>15.4 (12.6 - 19.7)</td>
</tr>
<tr>
<td>Captopril</td>
<td>18.6 (9.9 – 34.8)</td>
<td>2.67 (1.49 – 4.78)</td>
<td>14.4 (11.7 - 17.7)</td>
</tr>
<tr>
<td>Captopril</td>
<td>11.9 (6.4 – 22.4)</td>
<td>2.58 (1.44 – 4.63)</td>
<td>21.7 (17.6 - 26.7)</td>
</tr>
<tr>
<td>Baseline</td>
<td>19.8 (12.4 – 29.0)</td>
<td>2.86 (1.94 – 4.22)</td>
<td>15.1 (12.4 - 18.4)</td>
</tr>
<tr>
<td>Captopril</td>
<td>23.3 (15.3 – 35.7)</td>
<td>2.56 (1.94 – 4.21)</td>
<td>12.2 (10.0 - 14.9)</td>
</tr>
</tbody>
</table>

Data are geometric means and 95% confidence limits of n = 3-4. P Values are the outcomes of Student’s unpaired t-test (Protocols 1, 2 & 4) or repeated measures analysis of variance (Protocol 3) performed on logarithmically transformed data. P values derived from repeated measures analysis of variance were conservatively adjusted using the Greenhouse-Geisser method (21). Low-dose and high-dose norepinephrine (0.1 µg/kg/min and 0.8 µg/kg/min respectively). AVP = arginine vasopressin; Ang II = angiotensin II.
**Figure Legends**

**Fig. 1** Hemodynamic response to intravenous infusion of norepinephrine. Thick lines represent the mean, while thin lines represent the SEM, of one minute averages of each variable (n = 6-8). Low dose = 0.1 µg/kg/min and high dose = 0.8 µg/kg/min.

**Fig. 2** Responses of regional renal tissue perfusion (laser Doppler flux) and oxygenation to intravenous infusion of norepinephrine. Thick lines represent the mean, while thin lines represent the SEM, of one minute averages of each variable (n = 6-8). Low dose = 0.1 µg/kg/min and high dose = 0.8 µg/kg/min.

**Fig. 3** Percentage changes (%Δ) in hemodynamic variables during infusion of vasoactive agents. Data are mean ± SEM of n = 6-8. Ang II = angiotensin II, infused at a rate titrated to reduce renal blood flow by ~20% (9.8 ± 2.1 µg/h). AVP = arginine vasopressin, infused at a rate titrated to reduce renal blood flow by ~20% (3.3 ± 0.5 µg/h). NE = norepinephrine, infused at 0.1 and 0.8 µg/kg/min. CAP = captopril, given as a bolus of 25 mg. *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001 compared with baseline by paired t-test.

**Fig. 4** Percentage changes (%Δ) in regional kidney perfusion as assessed by laser Doppler flux and oxygenation during infusion of vasoactive agents. Data are mean ± SEM of n = 6-8. Abbreviations and statistical symbols are as for Fig. 1.

**Fig. 5** Percentage changes (%Δ) in global renal oxygen delivery, consumption and fraction extraction during infusion of vasoactive agents. Data are mean ± SEM of n = 3-4. Abbreviations and statistical symbols are as for Fig. 1.
Fig. 5

Renal Oxygen Delivery (%)

Renal Oxygen Consumption (%)

Fractional Oxygen Extraction (%)

NE  Ang II  AVP  CAP

***  **  *  **