Preglomerular and Postglomerular Resistance Responses to Different Levels of Sympathetic Activation by Hypoxia

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Abstract. This study investigated the effects of graded reflex increases in renal sympathetic nerve activity (RSNA) on renal preglomerular and postglomerular vascular resistances. With the use of hypoxia to reflexly elicit increases in RSNA without affecting mean arterial pressure, renal function and stop-flow pressures were measured in three groups of rabbits before and after exposure to room air and moderate (14% O₂) or severe (10% O₂) hypoxia. Moderate and severe hypoxia increased RSNA, primarily by increasing the amplitude of the sympathetic bursts rather than their frequency. RSNA amplitude increased by 20 ± 6% (P < 0.05) and 60 ± 16% (P < 0.05), respectively. Moderate hypoxia decreased estimated renal blood flow (ERBF; 26 ± 7%; P = 0.07), whereas estimated glomerular capillary pressure (32 ± 1 versus 34 ± 1 mmHg; P < 0.05) and filtration fraction (FF; P < 0.01) increased. In response to moderate hypoxia, calculated preglomerular (~20%) and postglomerular (~70%) resistance both increased, but only the increase in postglomerular resistance was significant (P < 0.05). In contrast, severe hypoxia decreased ERBF (56 ± 8%; P < 0.01), GFR (55 ± 9%; P < 0.001), and glomerular capillary pressure (32 ± 1 versus 29 ± 1 mmHg; P < 0.001), with no change in FF, reflecting similar preglomerular (~240%; P < 0.05) and postglomerular (~250%; P < 0.05) contributions to the vasoconstriction and a decrease in calculated Kf (P < 0.05). These results provide evidence that reflexly induced increases in RSNA amplitude may differentially control preglomerular and postglomerular vascular resistances.

Previous renal micropuncture studies have suggested that electrical stimulation of the renal nerves predominantly constrict the preglomerular vessels (1–4); this is not surprising, because the afferent arteriole is much more densely innervated than the efferent arteriole (5). However, electrical stimulation may not fully mimic physiologic changes in nerve activity. For example, electrical stimulation at supramaximal voltages activates all nerves simultaneously, and the frequency at which the nerves are fired is altered, whereas it now clear that the number of nerves activated during a physiologic burst of nerve activity may vary widely (6–18). The bursting pattern of renal sympathetic nerve activity can be differentiated into changes in frequency (rate) of nerve firing and changes in amplitude (number of nerves recruited during the burst) (6,9,19). Physiologic stimuli such as hypoxia, air jet stress, white noise (sound that has a relatively wide continuous range of frequencies of uniform intensities), and thermal stimulation have been shown to reflexly increase the amplitude of the sympathetic bursts to the kidney (i.e., increased recruitment of nerves) without significantly changing the frequency of the discharges (11,12,20,21). Speculation has therefore arisen that different patterns of activation of the renal nerves may produce differential changes in renal function (22).

Physiologically induced graded increases in renal sympathetic nerve activity (RSNA) cause progressive renal vasoconstriction (11,13,23), but the effects of hypoxia on preglomerular and postglomerular vascular resistances have not been measured. This is of particular interest, given our recent identification of two distinct nerve types that are differentially distributed to afferent and efferent arterioles (24). Type I axons almost exclusively innervate the afferent arteriole and have larger axon diameters with atypical varicosities (5), whereas type II axons are distributed equally to both afferent and efferent arterioles and resemble those innervating other blood vessels with typical fusiform varicosities (5). The distribution of the two types of nerves to the renal arterioles raises the possibility that preglomerular and postglomerular vascular resistances may be regulated differentially, depending on the nerve type activated. We hypothesize that increased type I activation would constrict the afferent arteriole, whereas type II nerve activation would constrict both afferent and efferent arterioles.

To investigate this hypothesis, we have used two levels of hypoxia (14% and 10% O₂) as our stimulus, which is known to produce graded increases in RSNA and renal vascular resistance (11,23). Importantly, these levels of hypoxia do not alter mean arterial pressure (MAP), therefore avoiding the potentially confounding influence of renal autoregulation. We have determined the relative changes in preglomerular and postglomerular resistances to the hypoxic stimuli, using stop-flow pressure (SFP) measurements to estimate glomerular capillary pressure.

Materials and Methods
Rabbits
Experiments were performed on male rabbits of a multicolored English strain (n = 20; mean, 3.0 ± 0.1 kg; range, 2.5 to 3.5 kg). The
experiments were performed in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved in advance by the Monash University Standing Committee on Ethics in Animal Experimentation.

Preparation

On the experimental day, catheters were placed in the ear central arteries and marginal veins under local anesthetic (Xylocaine, 0.5% wt/vol lidocaine; Astra Pharmaceuticals, North Ryde, NSW, Australia). Conscious arterial pressure was measured for 20 min. Anesthesia was then induced by intravenous administration of pentobarbitone sodium (90 to 150 mg plus 0.1 to 0.2 mg/kg per min intravenously; Nembutal, Boehringer Ingelheim, Artarmon, NSW, Australia) and the rabbits ventilated (small animal ventilator, Model 683; Harvard Apparatus, Holliston, MA). During surgery and throughout the experiment, fluid was infused intravenously at 0.17 ml/min per kg (Hartmann’s solution [compound sodium lactate]; Baxter Healthcare, Toongabbie, NSW, Australia) to replace fluid losses. Surgery was performed on a heated table, and esophageal temperature was maintained between 36°C and 38°C throughout the experiment via a servocontrolled infrared lamp (Digi-Sense Temperature Controller; Cole Palmer, Vernon Hills, IL). The rabbit was placed in an upright crouching position, and a left flank incision made to expose the left kidney. Silastic catheters were inserted into both ureters for the collection of urine, and the left kidney was placed in a micropuncture cup. Before the kidney was prepared for micropuncture, the renal nerves were identified and a 3- to 5-mm length freed from the surrounding tissue and carefully placed across a pair of hooked silver recording electrodes. The nerve and electrode were insulated from the surrounding tissue and efficiently from the micropuncture bath by use of Wacker Sil-Gel (Wacker-Chemie, Munich, Germany). The kidney was then prepared for micropuncture as described elsewhere (25).

After completion of surgery, [3H] inulin (4-μCi bolus plus 300 nCi/ml; NEN Research Products, Sydney, Australia) and [14C] para-aminomhippurate (1- and 110 mmHg. (300 mg).

Experimental Protocol

Measurements were made over three periods, a 60-min baseline period, a 60-min gas period, and a 10-min recovery period. There was a 20-min washout between each period (samples discarded). Each animal was randomly assigned to receive one of three gas mixtures (room air, 14% O2, or 10% O2). Each period consisted of a timed urine collection, with an arterial blood sample (2 ml total) taken at the midpoint for clearance, blood gas, hematocrit, and plasma renin activity measurements. At the conclusion of the experiment, the animal was killed with an intravenous overdose of pentobarbital (300 mg).

Data Acquisition

Arterial pressure was continuously measured by connecting the ear artery catheter to a pressure transducer (Cobe, Arvarda, CO) and the signal amplified (Model 7D, Grass Instruments, Quincy, MA). RSNA was amplified, filtered between 50 and 3000 Hz, full-wave rectified, and integrated by use of a low-pass filter with a time constant of 20 ms. The average voltage from the sympathetic neurotransmitter over 2-s periods was defined as total RSNA. Changes in voltage of the nerve signal above a defined threshold were classified as RSNA discharges (11).

Micropressure measurements were taken during the baseline and gas periods; micropressures were not measured during the recovery period. The micropressures were pooled for each period, with 3 to 6 SFP measurements and 3 to 5 proximal tubular pressures being taken in each period. The micropressures were measured by use of a servo-null pressure measuring device (Micropressure System, model 900A; WPI, Sarasota, Florida).

MAP, heart rate, total RSNA, RSNA frequency, and RSNA amplitude were all continuously recorded throughout the experiment and were sampled by use of an analog-to-digital data acquisition card (Lab-PC+, National Instruments, Melbourne, Australia). Calibrated signals were displayed on screen and saved to disk as 2-s averages of each variable by use of a program written in the LabVIEW graphical programming language (National Instruments) (11).

Measurements and Calculations

MAP, heart rate, total RSNA, frequency of RSNA, and RSNA amplitude were averaged over each clearance period. GFR was estimated via the clearance of [3H]-inulin, and estimated renal blood flow (ERBF) as the clearance of [14C]-para-aminomhippurate corrected for hematocrit. Renal vascular conductance was calculated as ERBF divided by MAP. Filtration fraction was calculated as GFR divided by estimated renal plasma flow. Plasma renin activity (PRA) was determined by RIA (26). Urinary sodium concentrations were determined by flame photometry (Model 943; Instrumentation Laboratory, Milan, Italy). Plasma oncotic pressure (πa) was calculated from arterial plasma protein concentration (Ca), determined by use of the Lowry assay (25). Estimated glomerular capillary pressure (Pgc) was calculated as SFP + πe. Preglomerular resistance was estimated as (MAP - Pgc)/ERBF and postglomerular resistance as Pgc/(ERBF - GFR). The preglomerular to postglomerular resistance ratio was determined as the preglomerular resistance divided by the postglomerular resistance. The mean net filtration pressure (Pnet) = Pgc - Pτ - πuc, and the glomerular ultrafiltration coefficient (Kf) = GFR/Pnet, where πuc equals the mean glomerular oncotic pressure calculated as (πa + πe)/2. The efferent oncocotic pressure (πe) was derived from the efferent protein concentration, which was calculated as Cτ/(1 - filtration fraction). However, Kf can only be calculated by the above formula if there is a net positive pressure at the efferent end of the glomerular capillaries (27). If there is not, the differential equation of Deen et al. (27) must be used, and then only a minimum value for Kf can be derived.

Statistical Analyses

All data are reported as mean ± SEM. P ≤ 0.05 was considered to be statistically significant. One-way ANOVA was used to test for differences between the groups during the baseline period. One-way repeated measures ANOVA was performed to compare the baseline and gas periods in each group.

Results

No significant differences were observed in any variable among the three treatment groups during the baseline (pre-gas mixture) period. Table 1 shows the baseline measurements in each group: the P values refer to an ANOVA for each variable. These results demonstrate that the groups were under similar resting conditions before administration of the gas mixture.
Table 1. Baseline variables in the three groups prior to gas administration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (Prior to Room Air)</th>
<th>Moderate (Prior to 14% O&lt;sub&gt;2&lt;/sub&gt;)</th>
<th>Severe (Prior to 10% O&lt;sub&gt;2&lt;/sub&gt;)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>83 ± 4</td>
<td>77 ± 6</td>
<td>77 ± 4</td>
<td>0.6</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>221 ± 8</td>
<td>224 ± 14</td>
<td>231 ± 10</td>
<td>0.8</td>
</tr>
<tr>
<td>PaO&lt;sub&gt;2&lt;/sub&gt; (mmHg)</td>
<td>90 ± 2</td>
<td>92 ± 3</td>
<td>93 ± 4</td>
<td>0.8</td>
</tr>
<tr>
<td>PaCO&lt;sub&gt;2&lt;/sub&gt; (mmHg)</td>
<td>22 ± 2</td>
<td>26 ± 3</td>
<td>22 ± 2</td>
<td>0.5</td>
</tr>
<tr>
<td>Total RSNA (μV)</td>
<td>3.7 ± 0.8</td>
<td>2.1 ± 0.6</td>
<td>2.5 ± 0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>RSNA amplitude (μV)</td>
<td>10.0 ± 2.0</td>
<td>9.1 ± 2.2</td>
<td>10.8 ± 2.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Frequency of RSNA (Hz)</td>
<td>9.5 ± 0.5</td>
<td>8.0 ± 1.1</td>
<td>8.7 ± 0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>30 ± 2</td>
<td>33 ± 2</td>
<td>30 ± 1</td>
<td>0.5</td>
</tr>
<tr>
<td>ERBF (ml/min)</td>
<td>17 ± 1</td>
<td>22 ± 4</td>
<td>20 ± 3</td>
<td>0.5</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>4.4 ± 0.5</td>
<td>4.1 ± 0.5</td>
<td>4.4 ± 0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Filtration fraction (%)</td>
<td>35 ± 3</td>
<td>28 ± 3</td>
<td>33 ± 4</td>
<td>0.4</td>
</tr>
<tr>
<td>UFR (ml/min)</td>
<td>0.11 ± 0.05</td>
<td>0.17 ± 0.07</td>
<td>0.16 ± 0.04</td>
<td>0.7</td>
</tr>
<tr>
<td>Na excretion (μmol/min)</td>
<td>11 ± 3</td>
<td>13 ± 3</td>
<td>20 ± 7</td>
<td>0.6</td>
</tr>
<tr>
<td>PRA (ng AngI/ml per h)</td>
<td>7.7 ± 1.9</td>
<td>5.8 ± 0.8</td>
<td>5.9 ± 1.1</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* Values are mean ± SEM. MAP, mean arterial pressure; HR, heart rate; PaO<sub>2</sub>, arterial partial pressure of oxygen; PaCO<sub>2</sub>, arterial partial pressure of carbon dioxide; RSNA, renal sympathetic nerve activity; EBRF, estimated renal blood flow; GFR, glomerular filtration rate; UFR, urine flow rate; Na excretion, sodium excretion rate; PRA, plasma renin activity. P values show the outcomes of one-way ANOVA, comparing levels in the three groups prior to exposure to the gas mixtures.

Responses to Graded Hypoxia

Room Air (Time Control). No significant time-related changes in MAP, PaO<sub>2</sub>, RSNA (total, frequency, or amplitude), renal vascular resistance (RVC), EBRF, GFR, filtration fraction, urine flow rate, or sodium excretion rate were seen throughout the duration of the experiment (Figures 1 and 2). Heart rate (221 ± 8 versus 218 ± 8 beats/min), arterial partial pressure of carbon dioxide (PaCO<sub>2</sub>) (20 ± 1.3 versus 20 ± 1.7 mmHg) and arterial pH (7.41 ± 0.02 versus 7.41 ± 0.015) were unaffected by time. PRA decreased by 16 ± 7% (P = 0.04; Figure 3) with time. Estimated glomerular capillary pressure was 32.2 ± 0.5 mmHg during baseline and 32.5 ± 0.4 mmHg during room air (P = 0.4). Plasma protein concentrations were 4.0 ± 0.1 g% during baseline and 3.9 ± 0.1 g% during room air (P = 0.4). Estimated glomerular capillary pressure, preglomerular and postglomerular vascular resistances, and K<sub>e</sub> were unaffected by time (Figure 4).

14% Oxygen (Moderate Hypoxia). Moderate hypoxia caused PaO<sub>2</sub> to decrease to 48 ± 7 mmHg (P = 0.003; Figure 1) and total RSNA to increase by 89 ± 22% (P = 0.03; Figure 1). This increase in total RSNA was associated with a significant increase in the RSNA amplitude (20 ± 6%; P = 0.03; Figure 1) but no significant change in the frequency of RSNA (7 ± 4%; P = 0.2; Figure 1). MAP and heart rate did not change significantly after moderate hypoxia (Figure 1). PaCO<sub>2</sub> levels were 26 ± 3 mmHg during baseline and 26 ± 2 mmHg during 14% O<sub>2</sub> (P = 0.6), pH levels were unchanged between baseline (7.36 ± 0.033) and 14% O<sub>2</sub> (7.36 ± 0.034). PRA levels were not significantly different between baseline and moderate hypoxia, 5.8 ± 0.8 ng angiotensin I (AngI)/ml per h and 7.6 ± 2.0 ng AngI/ml per h, respectively (P = 0.3; Figure 3).

ERBF decreased by 26 ± 7% (P = 0.05; Figure 2), and RVC decreased by 30 ± 5% (P = 0.04; Figure 2) in response to moderate hypoxia. GFR did not change significantly (4.1 ± 0.5 versus 3.9 ± 0.9 ml/min; P = 0.5; Figure 2), but filtration fraction rose significantly from 28 ± 3 to 39 ± 5% (P = 0.006; Figure 2) in response to moderate hypoxia. Urine flow rate (P = 0.7), sodium excretion (P = 0.2), and fractional sodium excretion (P = 0.9) did not change significantly (Figure 2).

SFP was 21.4 ± 0.2 mmHg during the baseline period and rose significantly to 23.4 ± 0.3 mmHg during moderate hypoxia (P = 0.007; Figure 4). Plasma protein concentration was not different during baseline and 14% O<sub>2</sub> (3.8 ± 0.1 versus 3.8 ± 0.1 g%, P = 0.1). Estimated glomerular capillary pressure increased by 2.1 ± 0.5 mmHg during 14% O<sub>2</sub> (P = 0.007; Figure 4). Proximal tubule pressure was 10.7 ± 0.3 mmHg during baseline and 11.5 ± 0.6 mmHg during 14% O<sub>2</sub> (P = 0.4). Estimated preglomerular resistance increased in response to moderate hypoxia, being 2.7 ± 0.6 mmHg/ml per min and 3.7 ± 1.3 mmHg/ml per min, respectively, but this did not reach statistical significance (P = 0.4; Figure 4). Postglomerular resistance increased significantly, being 2.1 ± 0.4 mmHg/ml per min during baseline and 3.4 ± 0.5 mmHg/ml per min during moderate hypoxia (P = 0.016; Figure 4). The preglomerular to postglomerular resistance ratio decreased by 25 ± 13% during 14% O<sub>2</sub>, but this was not significant (P = 0.14). The rabbits were all in a state of filtration disequilibrium (i.e. ΔP > π<sub>e</sub>) during both periods of the experiments. The calculated glomerular ultrafiltration coefficient was 0.97 ± 0.24 ml/min per mmHg during baseline and 0.84 ± 0.13 ml/min per mmHg during 14% O<sub>2</sub> (P = 0.6; Figure 4).

10% Oxygen (Severe Hypoxia). Severe hypoxia caused PaO<sub>2</sub> to decrease to 36 ± 4 mmHg (P = 0.001; Figure 1),
whereas total RSNA increased by $162 \pm 34\% (P = 0.04$; Figure 1). This increase in total RSNA was associated with a significant increase in the RSNA amplitude ($60 \pm 16\%; P = 0.05$; Figure 1) and with a small but significant increase in the frequency of RSNA ($10 \pm 2\%; P = 0.002$; Figure 1). MAP did not change significantly after severe hypoxia, being $77 \pm 4$ and $74 \pm 5$ mmHg during baseline and severe hypoxia, respectively (Figure 1). Heart rate increased significantly during
severe hypoxia, from 230 ± 10 beats/min to 242 ± 12 beats/min (P = 0.04). PaCO$_2$ levels were 22 ± 2 mmHg during baseline and 24 ± 2 mmHg during 10% O$_2$ (P = 0.2). pH levels were 7.37 ± 0.021 during baseline and 7.35 ± 0.024 during 10% O$_2$ (P = 0.3). PRA rose in response to severe hypoxia, levels being 5.9 ± 1.1 ng AngI/ml per h and 9.42 ± 1.4 ng AngI/ml per h during baseline and severe hypoxia, respectively (P = 0.017; Figure 3).

ERBF decreased by 56 ± 8% (P = 0.005; Figure 2), and renal vascular conductance decreased by 60 ± 10% (P = 0.04; Figure 2) in response to severe hypoxia. GFR decreased significantly in response to severe hypoxia, falling from 4.7 ± 0.9 ml/min during the baseline period to 2.0 ± 0.5 ml/min during severe hypoxia (P = 0.001; Figure 2), but filtration fraction was unchanged (33 ± 4 versus 32 ± 4%, respectively; P = 0.6; Figure 2). Urine flow rate decreased significantly in response to severe hypoxia, falling from 0.16 ± 0.04 to 0.11 ± 0.02 ml/min (P = 0.008; Figure 2), as did sodium excretion rate, falling from 20 ± 7 to 7 ± 2 μmol/min (P = 0.022). The decrease in fractional sodium excretion in response to severe hypoxia was not statistically significant (45 ± 12% versus 25 ± 5%; P = 0.09).

SFP was 21.1 ± 0.2 mmHg during the baseline period and fell to 18.5 ± 0.3 mmHg during severe hypoxia (P = 0.001; Figure 4). Plasma protein concentration was not different during baseline and 10% O$_2$ (3.9 ± 0.1 versus 3.8 ± 0.1 g%, P = 0.3). Estimated glomerular capillary pressure decreased by 3.0 ± 0.5 mmHg during 10% O$_2$ (P = 0.001; Figure 4). Proximal tubule pressure was 11.0 ± 0.7 mmHg during baseline and 10.9 ± 0.3 mmHg during 10% O$_2$ (P = 0.9). Estimated preglomerular resistance was increased in response to severe hypoxia, being 2.7 ± 0.4 mmHg/ml per min and 7.0 ± 1.8 mmHg/ ml per min, respectively (P = 0.05; Figure 4). Postglomerular resistance also increased significantly, being 2.5 ± 0.3 mmHg/ml per min during baseline and 6.9 ± 1.5 mmHg/ml per min during moderate hypoxia (P = 0.03; Figure 4). The preglomerular to postglomerular resistance ratio was not different from baseline during 10% O$_2$ (1.15 ± 0.07 versus 1.19 ± 0.17; P = 0.3). The rabbits were all in a state of filtration disequilibrium (i.e. ΔP > πc) during both periods of the experiments; therefore, $K_f$ could be calculated. $K_f$ was significantly decreased during 10% O$_2$, being 0.85 ± 0.20 ml/min per mmHg during baseline and 0.40 ± 0.10 ml/min per mmHg during 10% O$_2$ (P = 0.034; Figure 4).

**Recovery from Hypoxia**

After a return to breathing room air, the rabbits were followed for 30 min. During the final 10 min of this period, measurements were taken. Measurements of MAP, heart rate, PaO$_2$, PaCO$_2$, and RSNA (total, frequency, or amplitude) were made in all rabbits, but clearance measurements were only made in five rabbits exposed to room air or 14% O$_2$ and four rabbits exposed to 10% O$_2$. All variables, MAP, heart rate, PaO$_2$, PaCO$_2$, RSNA (total, frequency, or amplitude), RVR,
ERBF, GFR, filtration fraction, urine flow rate, and sodium excretion rate had returned toward baseline values in all three groups (Figures 1 and 2).

**Discussion**

The two levels of hypoxia caused graded increases in total RSNA and decreases in renal blood flow in these experiments, with no change in MAP (11,13,23). The increases in total RSNA evoked by both moderate and severe hypoxia were due almost entirely to increases in the amplitude of RSNA rather than to increases in the frequency of the bursts (11,13,23). The amplitude of the sympathetic nerve activity signal reflects the number of activated fibers (6–17); therefore, an increase in the amplitude of the signal reflects recruitment of additional nerve fibers in response to hypoxia (11). The conditions were therefore established to test the aim of this study of how changes in renal nerve recruitment affect preglomerular and postglomerular vascular resistances.

This study demonstrated that graded physiologic activation of the renal nerves evokes different preglomerular and postglomerular responses. In response to moderate hypoxia, the RSNA amplitude increased by 20% and renal blood flow fell by 25%. Despite this marked renal vasoconstriction, GFR was well maintained; therefore, filtration fraction was increased. The renal vasoconstriction was due to increases in both preglomerular (~20%) and postglomerular (~70%) resistance, and a small increase in glomerular capillary pressure, as estimated via stop-flow pressure measurement, was seen. During severe hypoxia (10% O2), when RSNA amplitude increased by 60%, there was even greater renal vasoconstriction (56%) and now GFR also fell, but filtration fraction was unchanged. Both estimated preglomerular (~240%) and postglomerular (~250%) resistance increased, but the glomerular ultrafiltration coefficient decreased by almost 50%. These results demonstrate that lower levels of increased renal nerve activation, evoked reflexly, produced a greater increase in postglomerular vascular resistance than in preglomerular resistance. This response would tend to conserve GFR, despite renal vasoconstriction. Higher levels of RSNA, evoked reflexly by severe hypoxia, resulted in a greater preglomerular component to the renal vasoconstriction and a reduction in the glomerular ultrafiltration coefficient, with consequent reductions in GFR. Thus, in severe hypoxia, there is both marked renal vasoconstriction and loss of GFR.

The renal responses to reflexly induced increases in nerve activity via hypoxia were different from those seen previously in response to electrical stimulation of the renal nerves (1–4). Postglomerular increases in resistance were as great or greater for both levels of hypoxia than preglomerular responses, unlike the situation with electrical stimulation, in which greater preglomerular effects have been documented to all but the lowest stimulation frequencies (1–4). Particularly, the responses to moderate hypoxia were at odds with previous whole kidney and micropuncture studies in which the renal nerves were electrically stimulated (1–4,28,29). Electrical stimulation studies have demonstrated that a stimulus sufficient (~3 Hz) to reduce renal blood flow by 20% to 30% causes a predominant increase in preglomerular vascular resistance and decreased filtration (1–3,28,29). This is in contrast to the present study, in which a 25% decrease in renal blood flow evoked reflexly in response to moderate hypoxia caused greater postglomerular vasoconstriction and maintenance of GFR. We suggest that an explanation lies in the way in which the increases in renal nerve activity in the two circumstances are evoked. Electrical stimulation at supramaximal voltages results in the synchronous firing of all renal sympathetic nerves, with the frequency of firing being varied. This does not appear to be the case physiologically, where it is an increase in the amplitude of RSNA (recruitment) that occurs in response to a wide range of physiologic stimuli, including hypoxia (22). In this regard, it is interesting to note that some recent studies that have attempted to mimic the bursting pattern of renal nerve activity have elicited different renal responses to that of standard square wave stimuli (15,17,30).

The glomerular ultrafiltration coefficient (Kf) calculated in this study by use of whole-kidney GFR values should be regarded with caution. Although significant changes in Kf when calculated in this manner are likely to be real, small changes in Kf are unlikely to be detected. The fall in Kf in response to severe hypoxia is in accord with reports elsewhere of decreased Kf in response to electrical stimulation (1–4). Whether this effect is due to a direct neural action on the mesangium, as suggested by Kon and Ichikawa (2), or the result of an indirect action of a vasoactive agent remains unanswered. However, in the three-dimensional ultrastructural analysis of the innervation of the juxtaglomerular region by Luff et al. (5), no axons were seen close to the extraglomerular mesangium, making the decrease in Kf unlikely to be due to a direct neural action.

The results of this study support our hypothesis that reflex changes in renal nerve activity may differentially evoke changes in preglomerular and postglomerular vascular resistances by activating different populations of nerves (22). As indicated in the introduction, we have previously shown that two types of axons, types I and II, innervate the afferent and efferent arterioles, with type I axons almost exclusively innervating the afferent arteriole, whereas type II axons are distributed at similar innervation densities to both afferent and efferent arterioles (24). Thus, activation of type I nerves would cause almost exclusive preglomerular resistance increases. In contrast, we suggest that type II axon stimulation would be expected to constrict both the afferent and efferent arterioles. In turn, this would be predicted to result in a greater increase in postglomerular resistance than preglomerular resistance, on the basis of a consideration of Poiseuille’s relationship, as we have argued elsewhere (31). Briefly, the resting diameter of the efferent arteriole is less than that of the afferent arteriole; therefore, similar changes in afferent and efferent arteriole radius in response to increased RSNA would increase resistance to a greater extent in the efferent arteriole (resistance being proportional to the fourth power of the radius [31]).

It is important to emphasize that although the present results are compatible with our hypothesis, there are a number of important considerations that require further work. First, it is
possible that some of the actions within the kidney are due to the hypoxia itself. However, we have demonstrated elsewhere that in conscious and anesthetized rabbits, the renal response to similar levels of hypoxia are due to the reflex activation of the sympathetic nerves, because renal denervation abolishes the renal responses (11,23,32). We are therefore confident that changes in preglomerular and postglomerular vascular resistances are due to reflex increases in sympathetic nerve activity and that there are no significant direct effects of hypoxia on the renal circulation. The rapid return of all variables to control levels during the recovery phase of the experiment strengthens this conclusion.

Second, apart from the direct actions of renal nerves on the renal vasculature, indirect vasoconstriction via activation of vasoactive agents may occur. In particular, the renin-angiotensin system plays an important role in modulating the effects of the renal sympathetic nerves (33) and may be directly responsible for the changes seen in preglomerular and postglomerular resistances in response to hypoxia. Studies that have examined the effect of AngII inhibition on the response to electrical stimulation of the renal nerve have shown conflicting findings. Pelayo et al. (1) found that both afferent and efferent vasoconstrictor responses to electrical stimulation were attenuated during AngII inhibition, whereas Handa and Johns (34) inferred from whole kidney studies that AngII was predominantly responsible for the constriction of the efferent arteriole in response to electrical stimulation. Neylon et al. (35,36) examined the renal responses to hypoxia in rats; however, RSNA was not measured, and marked changes in arterial pressure, PaCO₂, and pH occurred in response to hypoxia, confounding the results. In this study, moderate hypoxia did not result in a significant change in plasma renin activity. On this basis, the observed predominant postglomerular effects of increased RSNA in response to moderate hypoxia do not appear to be due to the actions of AngII. However, changes in plasma renin levels may not reflect intrarenal AngII levels; thus, this conclusion requires caution. Further experiments in AngII-inhibited animals to elucidate the contribution of the renin-angiotensin system to the effects of reflex activation of the renal sympathetic nerves should be conducted. Similarly, it is possible that hypoxia is altering catecholamine or adenosine release, which may indirectly influence the renal circulation (33).

Finally, the experimental setting is an important consideration and potentially confounding. In this anesthetized rabbit preparation, arterial BP and kidney function are closely matched to that in the conscious rabbit (25). Estimated glomerular capillary pressures in this study were comparable to those measured previously in rabbits, by use of both stop-flow estimates and direct capillary puncture in superficial glomeruli (25,37,38). It is recognized that the use of SFP to estimate glomerular capillary pressure theoretically has inherent problems, in that flow is interrupted to the macula densa, which may cause afferent arteriole vasodilatation and reduce myogenic responsiveness. This may have led to underestimation of preglomerular resistance. However, a detailed study has shown that this effect is small (39). Also, in this study in a single rabbit with superficial glomeruli, directly measured glomerular capillary pressures were 30 mmHg in response to room air, 35 to 38 mmHg in response to 14% hypoxia, and 25 mmHg to 10% hypoxia, in complete agreement with the stop-flow estimates of glomerular capillary pressure. Importantly, arterial pressure was unaffected by the hypoxic stimuli; therefore, autoregulatory changes in renal blood flow and GFR have not confounded the interpretation of the results. As reported elsewhere (12), a further methodological consideration is that setting the threshold for the determination of nerve activity has the potential to bias the results. Therefore, as indeed we saw in this study, an increase in the amplitude of small bursts of renal nerve activity, previously just below the threshold to above threshold, can result in an apparent increase in the frequency of nerve activity.

In conclusion, we have shown that reflex activation of the sympathetic nerves by hypoxia produces different preglomerular and postglomerular effects, depending on the severity of the hypoxia and extent of increase in RSNA amplitude. Moderate hypoxia increased postglomerular resistance predominantly, whereas more severe hypoxia markedly increased preglomerular and postglomerular resistance. These results provide evidence that the renal sympathetic nerves can differentially regulate preglomerular and postglomerular resistances. Thus, glomerular pressure and filtration may be differentially regulated, depending on the nature of change in renal nerve activity.

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