ANG II-induced downregulation of RBF after a prolonged reduction of renal perfusion pressure is due to pre- and postglomerular constriction

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Sorensen, Charlotte Mehlin, Paul Peter Leyssac, Max Salomonsson, Ole Skott, and Niels-Henrik Holstein-Rathlou. ANG II-induced downregulation of RBF after a prolonged reduction of renal perfusion pressure is due to pre- and postglomerular constriction. Am J Physiol Regul Integr Comp Physiol 286: R865–R873, 2004. First published January 8, 2004; 10.1152/ajpregu.00424.2003.—Previous experiments from our laboratory showed that longer-lasting reductions in renal perfusion pressure (RPP) are associated with a gradual decrease in renal blood flow (RBF) that can be abolished by clamping plasma ANG II concentration ([ANG II]). The aim of the present study was to investigate the mechanisms behind the RBF downregulation in halothane-anesthetized Sprague-Dawley rats during a 30-min reduction in RPP to 88 mmHg. During the 30 min of reduced RPP we also measured glomerular filtration rate (GFR), proximal tubular pressure (Pprox,1), and proximal tubular flow rate (Qp,1). Early distal tubular fluid conductivity was measured as an estimate of early distal [NaCl] ([NaCl]ED), and changes in plasma renin concentration (PRC) over time were measured. During 30 min of reduced RPP, RBF decreased gradually from 6.5 ± 0.3 to 6.0 ± 0.3 ml/min after 5 min (NS) to 5.2 ± 0.2 ml/min after 30 min (P < 0.05). This decrease occurred in parallel with a gradual increase in PRC from 38.2 ± 11.0 × 10−5 to 87.1 ± 25.1 × 10−5 Goldblatt units (GU/ml after 5 min (P < 0.05) to 158.5 ± 42.9 × 10−5 GU/ml after 30 min (P < 0.01). GFR, Pprox, and [NaCl]ED all decreased significantly after 5 min and remained low. Estimates of pre- and postglomerular resistances showed that the autoregulatory mechanisms initially dilated preglomerular vessels to maintain RBF and GFR. However, after 30 min of reduced RPP, both pre- and postglomerular resistance had increased. We conclude that the decrease in RBF over time is caused by increases in both pre- and postglomerular resistance due to rising plasma renin and ANG II concentrations.

The present study aimed at investigating the mechanisms underlying the renal regulatory response to a maintained reduction of RPP to the lower limit of autoregulation (88 mmHg) for 30 min in Sprague-Dawley rats. During the 30 min we measured RBF, GFR, the proximal hydrostatic tubulus pressure (Pprox), the late proximal flow rate (Qp,1), and the early distal NaCl concentration ([NaCl]ED). Urine flow, Na+, and K+ excretion in the urine as well as changes in plasma renin concentration (PRC) over time were also measured. Using the measured parameters, we calculated the pre- and postglomerular resistances (Rpre and Rpost, respectively).

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The results show that during a long-lasting decrease in RPP within the autoregulatory limits RBF is gradually regulated to a lower level. The slow reduction in RBF occurs in conjunction with a gradual increase in PRC during the 30 min. GFR, P_{prox}, and early distal [NaCl] all decrease significantly after 5 min and remain at this new lower value throughout the 30 min, whereas the decrease in proximal tubular flow rate is more gradual. The gradual reduction in RBF seems to be caused by a continuous constriction of both the afferent and efferent arterioles due to increased plasma [ANG II]. The efferent constriction is most likely due to a direct effect of ANG II on the arteriole, whereas the afferent constriction could be mediated by several possible mechanisms. An increase in plasma [ANG II] enhances both the TGF and the myogenic response and at the same time can cause a downward resetting of the TGF response. These effects could, either alone or together, explain the constriction of the afferent arteriole when RPP is reduced for a longer period of time. Thus the downregulation of RBF appears to represent a resetting of nephron function so that GFR and, thereby renal sodium excretion, can be maintained at a new, lower level but still be autoregulated during acute perturbations occurring around the reduced RPP.

**METHODS**

**Animal Preparation**

The experiments were performed in male Sprague-Dawley rats weighing 250–330 g obtained from Møllergård, Lille Skensved, Denmark. The experimental protocol was approved by the Danish National Animal Experiments Inspectorate. The rats were fed standard rat chow and had free access to food and water until immediately before the experiments.

Anesthesia was induced with 5% halothane delivered in 35% nitrogen and 65% oxygen. Polyethylene catheters were placed in the right jugular vein (PE-10) for infusion and in the left carotid artery (PE-50) for continuous systemic blood pressure measurement by a Statham P23-db pressure transducer (Gould, Oxnard, CA). A tracheostomy was performed, and the rat was placed on a servo-controlled heating table to maintain body temperature at 37°C. The rat was connected to and ventilated by a small animal respirator, tidal volume 1.7–2.5 ml to 250–330 g rats at a frequency of 60 breaths/min. The final halothane concentration needed to maintain sufficient anesthesia was ~1%. An intravenous bolus injection of 0.3 mg pancuronium bromide (Pavulon, Organon, Holland) in 0.4 ml 0.9% saline was given followed by continuous intravenous infusion of 0.6 mg/ml Pavulon at 20 μl/min. Additional saline was given continuously at a rate of 20 μl/min.

The rats were prepared for micropuncture as previously described in detail (39). In brief, the left kidney was exposed after laparotomy extended to the left flank and immobilized in a Lucite ring. The left ureter was catheterized (PE-10 connected to PE-50) to ensure free urine flow. The kidney was superfused with heated saline (37°C) during the experiment.

The left femoral artery was catheterized (PE-25) for measurements of RPP. The left renal artery was stripped of any fat or fascias, and a precalibrated electromagnetic perivascular flow sensor (Skalar Medical, model 1401, Holland) was placed around it (lumen diameter 0.6–0.8 mm depending on rat size). The aorta was dissected free. A servo-controlled aortic clamp (RPC-2 controller, Electronic Workshop, McGill University, Canada) was placed above the bifurcation of the renal arteries. The controller of the clamp was connected to a system in which the pressure signal from the femoral artery was compared with a reference signal corresponding to a preset pressure. The mean error signal was applied to a transducer operating the clamp. The presetting on the controller was found by trial-and-error on five different rats.

To ensure correct positioning of the aortic clamp and the flow sensor, the clamp was compressed to zero flow before initializing the experiment. The clamp was adjusted if the clamped femoral arterial pressure was >20 mmHg, and the renal blood flow meter was then adjusted to zero if necessary.

Arterial blood pressure, RBF, hydrostatic P_{prox}, and distal tubular fluid conductance were sampled online with a Powerlab/SSP system (ADInstruments) for later analysis on a personal computer. Proximal tubular fluid flow rate was recorded on VHS videotape for later analysis.

**GFR Measurements**

An intravenous bolus injection of 51Cr-EDTA (NEL Life Science Products, Belgium) containing 0.51 MBq in 1.75 ml saline was given, followed by continuous infusion of 51Cr-EDTA at 20 μl/min (0.00029 MBq/μl) for clearance measurement. An equilibration period of 45 min was allowed. Blood samples and urine samples were collected, and Cr51-EDTA concentration in urine and plasma samples was counted in a scintillation γ-counter.

**Hydrostatic Pressure Measurements**

Intratubular hydrostatic pressure in the proximal tubule was measured by a servomonitoring micropuncture system (Reditech) manufactured according to the descriptions of Intaglietta et al. (12) and connected to a Statham P23-db transducer. Sharpened micropipettes (OD 1–2 μm) were filled with 1 M NaCl solution colored with lissamine green (0.6 g%). The system was calibrated each day in a small pressure chamber and was linear over the range 0–100 mmHg within ±0.5 mmHg.

**Tubular Flow Measurements**

Late Q_{TP} was measured optically by a modification of the method of Marsh and colleagues (4, 9). The method has been described in detail in previous publications from this laboratory (16, 19). Briefly, small boluses (10–15 pl) of 1% rhodamine-labeled dextran in 0.9% saline were injected into the proximal tubule by a pneumatic picopump (model PV280, World Precision Instruments). The injections were made with a sharpened Pyrex micropipette (OD 1–3 μm) at a frequency of 30/min, each injection lasting 5 ms. A green He-Ne laser (1 mW, 534 nm, Melles Griot) excited the dye, and the fluorescent image was observed at ×50 magnification and passed through a red filter to an image intensifier (GENII SYS, Dage-MTI) connected to a charge-coupled device camera (model c72, Dage-MTI). The output from the camera was displayed on a video monitor and recorded on VHS videotape for later online analysis.

The video signal was routed to an image board (Matrox IP-8) mounted in a personal computer. This allowed two sample windows of variable size to be placed independently on the video image at two points along the nephron but distal to the injection pipette. Two digital signals were returned, each proportional to the light intensity defined by the sample window. The time delay for the passage of the dye bolus between the two windows was calculated for each pulse. On-screen measurement of tubular length and diameter allowed for calculation of tubular fluid flow rate.

**Tubular Fluid Electrical Conductivity**

Electrical conductivity in early distal tubular fluid was measured in situ by the microprobe method of Gutsche et al. (7). The microprobe was pulled from 0.9 mm (OD) Pyrex glass capillaries with filament (Clark Electromedical, Pangbourne, UK). The beveled tip was 6–8 μm (OD). The outer surface of the pipette was covered with platinum (Glantzplatin, Degussa, Germany) and dried at 180°C. The pipette was filled with isotonic saline, and a platinum wire was inserted into...
the pipette. The pipette holder was adapted to a microperfusion and suction pump (Fa. Hampel). Conductive silver paint (Leitsilber, Degussa) provided the electrical connection between the external platinum coat of the pipette and the lead. The leads from both electrodes were connected to an alternating-current (AC) voltage generator and a linear AC amplifier (Baumbach Electronics).

For measurements, fluid was continuously aspirated at a low rate (6 nl/min) into the tip of the pipette to provide electrical connection between the two electrodes. A constant voltage across the two electrodes generates a current proportional to the conductivity of the aspirated fluid that is expressed in millimoles of NaCl. The saline solution bathing the kidney surface served as a reference before and after each measurement. A calibration curve made from serial dilutions of a 154 mM NaCl solution gave a linear relation between the measured conductivity and corresponding NaCl concentration measured with flame emission photometry over a range from 15 to 154 mM (19).

Analysis

Plasma (100 μl) and the urine samples were counted in the scintillation γ-counter (Gamma analyzer, Moellsgaard Medical, Copenhagen, Denmark) to estimate 51Cr-EDTA content. The concentrations of sodium and potassium in urine and plasma were measured by flame photometry (IL243 LED flame photometer, Instrumentation Laboratory). The urine flow was measured gravimetrically. The PRC was measured by the method of Lykkegaard and Poulsen (23). Aliquots of plasma were diluted 20- to 80-fold with Tris buffer containing human albumin, and 5-μl portions of these samples were incubated for 24 h at 37°C with 20 ml of a reaction mixture that contained purified rat renin substrate (~1.200 ng ANG I equivalents/ml). This incubation was followed by radioimmunoassay of generated ANG I. PRC was measured in reference to renin standards obtained from the National Institute for Biological Standards and Control [Potters Bar, Herts, UK; 1 mGoldblatt unit (GU) = 160 pg ANG I ml⁻¹ h⁻¹].

Experimental Protocol

The experiments in series I were divided into eight 5-min periods. The first two periods were intrinsic controls before reduction of RPP and are presented as one mean control value. RPP was reduced in one step from basal to 88 mmHg and kept there for 30 min. In series II, III, and IV, a control period of a minimum 5 min preceded the 30-min period of reduced RPP.

Blood samples (~300 μl) were collected before and after the period of perfusion pressure reduction for measurement of plasma concentrations of renin, Na⁺, and K⁺. Ten microliters of 300 mM EGTA was added to the blood samples to avoid coagulation. The blood samples were centrifuged at 7,000 rpm for 5 min, and the plasma was kept frozen for later measurement. In series IV, smaller blood samples (50–75 μl) were collected every 5 min.

Series I

GFR. Seventeen rats were used. After the first blood sample was drawn, the RPP was reduced to 88 mmHg for 30 min. After the last blood sample was drawn, the left kidney was removed, drained, and weighed. Urine was collected in preweighed vials at 5-min intervals for measurement of urine flow, 51Cr-EDTA clearance, and Na⁺ and K⁺ concentrations.

Control experiments in 10 rats were made without reducing the RPP.

Series II

Late Q₁P and hydrostatic proximal tubulus pressure. Measurements in 13 nephrons were made in 13 rats. After surgery the rats were allowed to stabilize for 30 min. A proximal tubule was punctured with a pressure-recording pipette, and the downstream convolutions were identified by injection of a small amount of lissamine green-colored fluid. The fluorescent dye-containing pipette was inserted in a late convolution of the same proximal nephron. A nephron was selected for study only if the last convolution had a length permitting the video sampling windows to be positioned at some distance from each other. Late Q₁P was measured at the spontaneous RPP and after 5 min of stable recordings the first blood sample was drawn. RPP was reduced to 88 mmHg, and Q₁P and hydrostatic proximal tubulus pressure (P₁P) were measured for 30 min at the reduced RPP. If the shrinkage of the kidney had caused the pipettes to fall out, they were reinserted in the same nephron through the same puncture holes to avoid leakage. If possible, both P₁P and flow rate were measured continuously throughout the experiment. After 30 min, a second blood sample was drawn. Control experiments were made without reducing the RPP. Measurements in 12 nephrons were made in 9 rats.

Series III

Distal conductivity ([NaCl]ED) and hydrostatic P₁P. Measurements in 20 nephrons were made in 18 rats. After surgery the rats were allowed to stabilize for 30 min. A proximal tubule was punctured with a pressure-recording pipette, and the corresponding distal tubule was identified by injection of a small amount of lissamine green-colored fluid from the pressure pipette. P₁P was measured throughout the experiment if possible. The conductivity pipette was inserted in the earliest possible distal convolution. After 5 min of stable recordings of distal conductivity and P₁P, the first blood sample was drawn. When the blood pressure had stabilized, the RPP was lowered with the aortic clamp from the spontaneous to 88 mmHg. If the pipettes had fallen out due to shrinkage of the kidney, they were reinserted in the same nephron through the same puncture holes to avoid leakage. Recordings at the reduced RPP continued for 30 min after which the aortic clamp was released and a second blood sample was drawn.

Control experiments were made without reducing the RPP. Measurements in 15 nephrons were made in 9 rats.

Series IV

PRC over time. In eight rats small blood samples (~50 μl) were sampled at 5-min intervals. After surgery, the rats were allowed to stabilize for 30 min. One control blood sample was drawn, and RPP was then reduced to 88 mmHg. After 5 min the second blood sample was drawn and so forth until the 30-min period had ended.

Calculations

Results are means ± SE of original data. Differences in the mean values of RBF, GFR, P₁P, Q₁P, and [NaCl]ED were tested for by ANOVA for repeated measurements. If a difference between means was found in the ANOVA, points at which there were significant differences from the respective control values were found with a Newman-Keuls test. PRCs before and after the experiment were compared using a paired Student’s t-test.

Estimates of single nephron proximal reabsorption rate (snAPR), glomerular capillary pressure (Pgc), and R₁P and R₁P were calculated as outlined in Appendix A. The calculated values were tested with a one-way ANOVA for repeated measurements, and Newman-Keuls test was performed as post hoc test. P < 0.05 was considered significant.

RESULTS

Mean arterial pressure (MAP), body weight, and initial RBF in rats from the four different series are shown in Tables 1–4. The rats used in the different experiments were of comparable physiological status.
Table 1. Physiological status of rats used in series I

<table>
<thead>
<tr>
<th></th>
<th>Reduced RPP (n = 17, body wt = 266 ± 7 g, kidney wt = 1.2 ± 0.0 g)</th>
<th>Control (n = 10, body wt = 267 ± 7 g, kidney wt = 1.2 ± 0.0 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After 30 min</td>
</tr>
<tr>
<td>RPP, mmHg</td>
<td>102±2</td>
<td>88</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>6.1±0.6</td>
<td>5.6±0.5*</td>
</tr>
<tr>
<td>GFR, µl·min⁻¹·g kidney wt⁻¹</td>
<td>934±85</td>
<td>745±78†</td>
</tr>
<tr>
<td>Vᵢ, µl·min⁻¹·g kidney wt⁻¹</td>
<td>12.5±1.2</td>
<td>8.8±1.2†</td>
</tr>
<tr>
<td>Uₙa, µmol·min⁻¹·g kidney wt⁻¹</td>
<td>2.4±0.2</td>
<td>1.9±0.4</td>
</tr>
<tr>
<td>Uₜ, µmol·min⁻¹·g kidney wt⁻¹</td>
<td>1.9±0.2</td>
<td>1.6±0.2*</td>
</tr>
<tr>
<td>P₃Na, mM</td>
<td>145±1</td>
<td>148±2*</td>
</tr>
<tr>
<td>PₚK, mM</td>
<td>4.2±0.1</td>
<td>4.5±0.2*</td>
</tr>
<tr>
<td>PRC, 10⁻⁵ GU/ml</td>
<td>34.5±9.0</td>
<td>65.2±16.4*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Vᵢ, urine flow rate; Uₙa, urinary sodium excretion rate; Uₜ, urinary potassium excretion rate; P₃Na, plasma sodium concentration; PₚK, plasma potassium concentration. GU, Goldblatt units; RPP, renal perfusion pressure; GFR, glomerular filtration rate; RBF, renal blood flow. *P < 0.05 vs. baseline. †P < 0.01 vs. baseline.

RBW

When RPP was reduced from spontaneous pressure to 88 mmHg, RBF showed a gradual decline during the 30 min the reduction lasted (Fig. 1B). This was true for all four experimental series; therefore, RBF results were pooled in Fig. 1. RBF values for series I, II, III, and IV, respectively, are shown in Tables 1–4.

RBF decreased significantly from 6.5 ± 0.3 to 5.2 ± 0.2 ml/min (n = 56) during 30 min of reduced RPP (Fig. 1B, P < 0.05). In the control rats (n = 31) RBF was 6.4 ± 0.2 and 6.0 ± 0.2 ml/min at the beginning and end of the 30-min control period, respectively [not significant (NS)].

PRC

In all four experimental series, a reduction of RPP to 88 mmHg for 30 min led to a significant increase in PRC. Control rats showed no increase in PRC. This was true for all experimental series; therefore, PRC values before and after RPP reduction have been pooled. PRC increased from 38.4 ± 4.9 × 10⁻⁵ to 102.2 ± 13.3 × 10⁻⁵ GU/ml (P < 0.01) in rats with reduced RPP (n = 56). In control rats (n = 31) PRC was 31.2 ± 4.9 × 10⁻⁵ and 33.3 ± 4.5 × 10⁻⁵ GU/ml (NS) before and after 30 min of spontaneous RPP.

In series IV (n = 8) PRC increased gradually over time (Fig. 1A) from 38.2 ± 11.0 × 10⁻⁵ GU/ml in the control period to 87.1 ± 25 × 10⁻⁵ GU/ml at 5 min (P < 0.05) to 185.8 ± 42.3 × 10⁻⁵ GU/ml (P < 0.05 vs. control and value after 5 min) after 30 min. PRC for the respective series is given in Tables 1–4.

Table 2. Physiological status of rats used in series II

<table>
<thead>
<tr>
<th></th>
<th>Reduced RPP (n = 13, body wt = 277 ± 4 g)</th>
<th>Control (n = 12, body wt = 289 ± 4 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>After 30 min</td>
</tr>
<tr>
<td>RPP, mmHg</td>
<td>103±2</td>
<td>88</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>6.6±0.5</td>
<td>5.6±0.5*</td>
</tr>
<tr>
<td>P₃Na, mmHg</td>
<td>14.5±0.9</td>
<td>13.0±1.0</td>
</tr>
<tr>
<td>QₚK, nl/min</td>
<td>17.6±0.7</td>
<td>15.2±1.0</td>
</tr>
<tr>
<td>PRC, 10⁻⁵ GU/ml</td>
<td>50.8±16.1</td>
<td>107±31.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE. P₃Na, proximal tubular pressure; QₚK, proximal tubular flow rate; *P < 0.05 vs. baseline; †P < 0.01 vs. baseline.

GFR

When RPP was reduced to 88 mmHg for 30 min, GFR decreased from 934 ± 85 µl·min⁻¹·g kidney wt⁻¹ in the control period to 764 ± 67 µl·min⁻¹·g kidney wt⁻¹ after 5 min (Table 5, P < 0.01). After 30 min of reduced RPP, GFR remained at a value of 745 ± 78 µl·min⁻¹·g kidney wt⁻¹ (P < 0.01, Table 5, see Fig. 4).

In the control rats in series I, GFR was 895 ± 110 µl·min⁻¹·g kidney wt⁻¹ in the control period and 921 ± 92 µl·min⁻¹·g kidney wt⁻¹ after 30 min at the spontaneous perfusion pressure (NS, Table 1).

Hydrostatic Pₚprox

The response in Pₚprox during 30 min with an RPP at 88 mmHg is shown in Table 5. Because the rats in series II and III responded similarly to the RPP reduction, Pₚprox values were pooled in Table 5. Respective Pₚprox values for series II and III are shown in Tables 2 and 3. When RPP was reduced to 88 mmHg, Pₚprox significantly decreased from 15.1 ± 0.5 mmHg to 13.8 ± 0.6 mmHg after 5 min (n = 31, P < 0.01). Pₚprox remained low and was 13.5 ± 0.6 mmHg after 30 min of reduced RPP (P < 0.01, Table 5).

In the pooled control rats (n = 21) Pₚprox was 15.1 ± 0.5 in the control period and after 30 min at the control RPP Pₚprox was 14.6 ± 0.6 (NS).

Late Proximal Tubular Flow

The response in QₚL during 30 min of reduced RPP is shown in Fig. 2 and Table 5. When RPP was reduced to 88 mmHg for
Table 4. Physiological status of rats used in series IV

<table>
<thead>
<tr>
<th>Reduced RPP (n = 8 body wt = 283 ± 8 g)</th>
<th>Baseline</th>
<th>After 30 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPP, mmHg</td>
<td>102±2</td>
<td>95±3*</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>5.6±0.5</td>
<td>4.7±0.6*</td>
</tr>
<tr>
<td>PRC, 10⁻⁵ GU/ml</td>
<td>38.2±11.0</td>
<td>158.5±42.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 vs. baseline. †P < 0.01 vs. baseline.

30 min, Q_Lp decreased from 17.6 ± 0.7 nl/min in the control period to 16.3 ± 0.9 nl/min after 5 min at 88 mmHg (NS, Fig. 2, Table 5). After 30 min of reduced RPP, Q_Lp had decreased to 15.2 ± 1.0 nl/min (P < 0.01, Table 5).

Q_Lp was stable when measured for a period of 30 min at the spontaneous RPP (16.8 ± 0.6 nl/min in the control period vs. 16.6 ± 0.9 nl/min after 30 min, NS, Fig. 2, Table 2).

[NaCl]_ED

The response in [NaCl]_ED to 30 min of reduced RPP is shown in Table 5. In the control period, [NaCl]_ED was 43.9 ± 1.9 mM and after 5 min of reduced RPP, [NaCl]_ED was significantly reduced to 37.0 ± 2.7 mM (P < 0.01, Table 5). After 30 min of reduced RPP, [NaCl]_ED remained at 37.9 ± 2.4 mM (P < 0.01, Table 5). In the control rats [NaCl]_ED was 43.9 ± 2.8 mM in the control period and 43.8 ± 2.5 mM after 30 min at the control RPP (NS, Table 3).

Excretion of Na⁺, K⁺, and Water

During 30 min with an RPP reduced to 88 mmHg, urine excretion decreased significantly from 12.5 ± 1.2 μl·min⁻¹·g kidney wt⁻¹ in the control period to 8.8 ± 1.2 μl·min⁻¹·g kidney wt⁻¹ after 30 min (Table 1, P < 0.01). Sodium excretion was 2.4 ± 0.2 μmol·min⁻¹·g kidney wt⁻¹ in the control period and 1.9 ± 0.4 μmol·min⁻¹·g kidney wt⁻¹ after 30 min of reduce RPP (Table 1, NS). Potassium excretion decreased from 1.9 ± 0.2 μmol·min⁻¹·g kidney wt⁻¹ to 1.6 ± 0.2 μmol·min⁻¹·g kidney wt⁻¹ (Table 1, P < 0.05).

In the control rats, urine excretion was 11.4 ± 1.1 μl·min⁻¹·g kidney wt⁻¹ in the control period and 13.2 ± 2.0 μl·min⁻¹·g kidney wt⁻¹ after 30 min (Table 1, NS). Sodium excretion was 2.5 ± 0.3 μmol·min⁻¹·g kidney wt⁻¹ in the control period and 3.1 ± 0.5 μmol·min⁻¹·g kidney wt⁻¹ after 30 min (NS), whereas potassium excretion was 2.0 ± 0.2 μmol·min⁻¹·g kidney wt⁻¹ in the control period and 1.9 ± 0.2 μmol·min⁻¹·g kidney wt⁻¹ after 30 min (Table 1, NS).

Plasma Na⁺ and K⁺

Plasma sodium and potassium increased slightly but significantly in the rats with reduced RPP from 145 ± 1 to 148 ± 2 mM (P < 0.05) and 42 ± 0.1 to 4.5 ± 0.2 mM, respectively (P < 0.05, Table 1). In the control rats, plasma sodium and potassium remained unchanged (142 ± 2 vs. 141 ± 2 mM and 4.0 ± 0.3 vs. 3.9 ± 0.2 mM, respectively, NS, Table 1).

Glomerular Capillary Pressure, R_pre, and R_post

Estimates of glomerular capillary pressure (P_GC) and R_pre and R_post are shown in Table 6 and Fig. 3. The estimated value of P_GC corresponds well to values measured directly in the glomerular capillary (20, 21).

Five minutes after reducing RPP, P_GC had decreased from 47.2 ± 2.0 to 45.6 ± 1.8 mmHg (P < 0.05) and remained low during the 30 min of reduced RPP (Table 6). R_pre decreased...
Table 6. Changes in estimated snAPR, $P_{G\text{G}}$, $R_{P\text{re}}$, $R_{P\text{post}}$, and RVR after 5 min and 30 min of reduced RPP

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After 5 min reduction</th>
<th>After 30 min reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>snAPR, nl/min</td>
<td>24.8±3.0*</td>
<td>17.7±2.9*</td>
<td>19.4±3.6*</td>
</tr>
<tr>
<td>$P_{G\text{G}}$, mmHg</td>
<td>47.2±2.0*</td>
<td>45.6±1.8*</td>
<td>44.8±1.9*</td>
</tr>
<tr>
<td>$R_{P\text{pre}}$, mmHg·nl⁻¹·min⁻¹</td>
<td>0.234±0.02‡</td>
<td>0.184±0.02*‡</td>
<td>0.205±0.02†‡</td>
</tr>
<tr>
<td>$R_{P\text{post}}$, mmHg·nl⁻¹·min⁻¹</td>
<td>0.185±0.02</td>
<td>0.177±0.01</td>
<td>0.196±0.02*‡</td>
</tr>
<tr>
<td>RVR, mmHg·ml⁻¹·min⁻¹</td>
<td>17.6±1.2†</td>
<td>15.6±1.0*</td>
<td>17.3±1.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE. snAPR, single nephron proximal reabsorption rate. RVR, renal vascular resistance; $R_{P\text{re}}$ and $R_{P\text{post}}$, preglomerular and postglomerular resistances, respectively; $P_{G\text{C}}$, glomerular capillary pressure. *$P < 0.05$ vs. baseline. ‡$P < 0.05$ vs. value after 5 min.

After 5 min of reduced RPP from 0.234±0.02 to 0.184±0.02 mmHg·nl⁻¹·min⁻¹ (Fig. 3, $P < 0.05$), whereas $R_{P\text{post}}$ was unchanged (Table 6). After 30 min of reduced RPP, $R_{P\text{pre}}$ had increased toward the control value (0.205±0.02 mmHg·nl⁻¹·min⁻¹, $P < 0.05$ vs. baseline and value after 5 min), whereas $R_{P\text{post}}$ had increased to a value above the control value (0.196±0.02 mmHg·nl⁻¹·min⁻¹, $P < 0.05$ vs. baseline and value after 5 min, Table 6, Fig. 3).

Total RVR also decreased after 5 min of reduced RPP from 17.6±1.2 to 15.6±1.0 mmHg·ml⁻¹·min⁻¹ ($P < 0.05$, Table 6). After 30 min of reduced RPP, RVR had returned to 17.3±1.1 mmHg·ml⁻¹·min⁻¹ ($P < 0.05$ vs. value after 5 min).

**DISCUSSION**

We previously showed that a reduction of RPP to just above the lower limit of autoregulation (88 mmHg) for 30 min induces a gradual decrease in RBF that can be abolished when plasma [ANG II] is clamped by simultaneous infusion of the angiotensin converting enzyme (ACE) inhibitor captopril and 400% increase in arterial plasma renin activity (PRA) was found (30).

A direct effect of [ANG II] on the renal vessels can contribute to the constriction of the afferent and efferent arterioles. Cortical afferent arterioles constrict at [ANG II] II of $10^{-9}$ M (31, 43), and at $10^{-10}$ M intracellular [Ca²⁺] starts to increase (15). Cortical efferent arterioles constrict at $10^{-14}$ (14). Juxtamedullary afferent arterioles constrict at [ANG II] II of $10^{-10}$ M (11, 13) as does juxtamedullary efferent arterioles (10). Thus the cortical efferent arterioles constrict at [ANG II] II 10–100 times lower than the afferent arteriole. Recent studies...
found the tissue [ANG II] in both medulla and cortex to be $\sim 10^{-10}$ M (42). Measurements of interstitial [ANG II] showed the concentration to be in the nanomolar range (2, 29) and plasma [ANG II] is $10^{-10}$ M (27). Thus the plasma [ANG II] under control conditions is at a level where the efferent arterioles are most sensitive to changes in plasma [ANG II], whereas the afferent arterioles will require an increase in plasma [ANG II] before they are affected.

The constriction of the afferent and efferent arterioles happens simultaneously (Fig. 3). Due to the above-mentioned differences in sensitivity to ANG II, it is most likely that the efferent constriction is a direct vascular effect of increased [ANG II]. However, the afferent constriction is most likely caused by another mechanism that might be indirectly influenced by the increase in plasma [ANG II]. An increase in plasma [ANG II] will increase TGF sensitivity (24). Accordingly, at low flow rates, the afferent arteriole is more constricted than it would be at the same flow rate when [ANG II] is low. This could be one mechanism by which the afferent arteriole constricts when RPP is reduced. Also, a longer-lasting change in proximal tubular flow has been shown to reset the TGF mechanism after $\sim 20$ min (40). The resetting shifts the turning point of the TGF curve, i.e., the point where TGF is most sensitive, toward a lower $Q_{LP}$. A reduced RPP by itself decreases TGF sensitivity (33). However, as shown by Selen and Persson (35), after 20 min of RPP reduction the sensitivity returns to the control value. Thus a reduction in RPP resets the TGF mechanism to operate effectively at the new lower tubular flow rate. Together with an increased sensitivity of TGF, the resetting could cause constriction of the afferent arteriole despite an unchanged [NaCl] at the macula densa.

ANG II also has a potentiating effect on the second autoregulatory mechanism, the myogenic response (17). At increasing RPP, the afferent diameter is decreased to a further degree when ANG II is present. This effect could also contribute to the gradual constriction of the afferent arteriole when plasma [ANG II] increases. It should, however, be emphasized that the present data do not allow an assessment of the quantitative contributions of these various mechanisms to the afferent arteriolar constriction seen between 5 and 30 min after the reduction in RPP.

From the estimated $R_{pre}$ and $R_{post}$ it can be seen that postglomerular arteriolar resistance increases to a level above the control level, whereas afferent arteriolar resistance increases back toward the control level. This is consistent with a larger direct effect of ANG II on the efferent arteriolar resistance (3, 5). However, it might also suggest that there is a continuous dilating effect of the autoregulatory mechanisms on the preglomerular arterioles if the constrictor effect of ANG II is equal on the afferent and efferent arteriole.

The $Q_{LP}$ decreases steadily during the 30 min of reduced RPP (Fig. 2). $Q_{LP}$ decreases to a lesser extent than GFR, indicating a reduced absolute proximal reabsorption rate (APR) in the beginning of the period of reduced RPP. The estimated single nephron APR (snAPR, see APPENDIX A) also indicates that proximal tubular reabsorption rate decreases significantly after 5 min when RPP is reduced for a prolonged period (Table 6). The decrease in APR did not cause [NaCl]$_{ED}$ to increase. Therefore, we can exclude the possibility that a changed tubular reabsorption caused an activation of TGF through an increased NaCl delivery to the macula densa.

We conclude that the gradual decrease in RBF is induced by a gradual constriction of both the afferent and efferent arterioles most likely caused by an increase in plasma [ANG II]. Both autoregulatory mechanisms are potentiated by the increase in plasma [ANG II], and, furthermore, TGF may reset during a longer-lasting change in RPP. Together, these changes may explain the constriction of the afferent arteriole, which, if unopposed, will cause a severe decrease in GFR. The decrease in GFR is, however, offset by constriction of the efferent arteriole, which helps to maintain GFR at a reasonable level. Thus the gradual decrease in RBF after a prolonged reduction of RPP can be seen as a consequence of the need to maintain autoregulatory efficiency and GFR at the same time.

APPENDIX A

Calculations to estimate $R_{pre}$ and $R_{post}$. We calculated the $R_{pre}$ and $R_{post}$ using the equations from Hall et al. (8)
\( R_{\text{pre}} = \frac{(RPP - P_{gc})/RBF} \)

\( R_{\text{post}} = \frac{(P_{gc} - P_i)/(RBF - GFR)} \)

Assuming that the kidney in Sprague-Dawley rats is composed of 25,000 nephrons (1, 44), the \( P_{gc} \) used in equations 1 and 2 was estimated from the equation

\[ P_{gc} = (\text{snGFR}/k_i) + P_{\text{prox}} + \Pi_u \]

where single nephron GFR was calculated from GFR by dividing by 25,000. The ultrafiltration coefficient \( (k_i) \) was assumed to be 0.08 \( \text{nl}/(\text{mmHg}\cdot\text{min}) \) (6), and \( P_{\text{prox}} \) was measured directly in the single nephron.

Average colloid osmotic pressure in the glomerular capillary \( (\Pi_c) \) was approximated from

\[ \Pi_c = (\Pi_a + \Pi_b)/2 \]

\( \Pi_a \) and \( \Pi_b \) were calculated from an assumed arterial protein concentration \( (CP_A) \) of 5.5 g/dl in the rat (28), and an efferent arteriolar protein concentration \( (CP_E) \) calculated as: \( CP_E = CP_A/(1-FF) \). \( \Pi_a \) and \( \Pi_b \) used in equation 4 to was found from the following relation (28)

\[ \Pi = 1.825 \cdot CP + 0.164 \cdot CP^2 + 0.012 \cdot CP^3 \]

where CP is the protein concentration in either the afferent or the efferent end of the glomerular capillaries.

Filtration fraction \( (FF) \) was calculated from

\[ FF = \frac{GFR}{RPF} \]

where RPF is renal plasma flow estimated from an assumed hematocrit of 45%.

The average peritubular capillary pressure, \( P_C \), used in equation 2 was calculated as

\[ P_C = P_{\text{prox}} - 4 \text{mmHg} \]

snAPR was calculated as

\[ \text{snGFR} - Q_{LP} \]

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