Angiotensin II induced down regulation of RBF after a prolonged reduction of renal perfusion pressure is due to pre- and postglomerular constriction.

by

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Abstract

Previous experiments from our laboratory have shown 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 angiotensin II concentration ([Ang II]). The aim of the present study was to investigate the mechanisms behind the RBF downregulation in halothane-anaesthetized Sprague Dawley rats during a 30 min reduction in RPP to 88 mm Hg. During the 30 min of reduced RPP we also measured glomerular filtration rate (GFR), proximal tubular pressure (P_prox) and proximal tubular flow rate (Q_LP). 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 ml/min 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 plasma renin concentration from 38.2 ± 11.0 x 10^{-5} GU/ml to 87.1 ± 25.1 x 10^{-5} GU/ml after 5 min (P < 0.05) to 158.5 ± 42.9 x 10^{-5} GU/ml after 30 min (P < 0.01). GFR, P_prox, 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.

**Keywords:** Renal vascular resistance, autoregulation, GFR
Introduction

The myogenic response and the tubuloglomerular feedback (TGF) mechanism autoregulate renal blood flow (RBF) and glomerular filtration rate (GFR) when acute changes in renal perfusion pressure (RPP) occur (26; 36). Autoregulation ensures that the kidney accurately can control salt and water excretion and thereby maintain the extracellular fluid volume by separating kidney function from acute fluctuations in blood pressure.

A previous study from our laboratory showed that a longer lasting decrease in RPP is associated with a decrease in RBF that gradually develops a few minutes after the pressure reduction (38). This is also the case when the decrease in RPP is within the limits of autoregulation. This we refer to as regulation of RBF. Because this down regulation of RBF could be completely abolished by clamping the plasma angiotensin II concentration ([Ang II]) we hypothesized, that the decrease in RBF was dependent on an increase in local and plasma [Ang II].

The mechanism behind this down regulation of RBF is not clear. A direct effect of Ang II on the renal vessels is possible. As RPP is decreased and plasma renin increases, plasma [Ang II] also increases. Because the efferent arteriole constricts at lower Ang II concentrations (10^{-11} M) than the afferent arteriole (10^{-9} M) (14) this will primarily constrict the efferent arteriole, but possibly also the afferent arteriole if [Ang II] increases sufficiently. Another consequence of increased plasma [Ang II] is an increase in the sensitivity of the TGF response (24) leading to increased constriction of the afferent arteriole. TGF has also been shown to reset when perturbations in tubular flow last more than 10 min (32; 41) thus enabling autoregulation at the new tubular flow rate. This ensures that TGF can respond to changes in the new flow range with adequate effect. Therefore, through potentiation of TGF, the afferent arteriole could constrict resulting in the gradual decline in RBF.
The second autoregulatory mechanism, the myogenic response, is also affected by changes in [Ang II] (17). At concentrations low enough not to affect basal afferent resistance, Ang II potentiates the myogenic constriction of the afferent arteriole. Therefore, it is also possible that an effect of Ang II on the afferent arteriole, through a potentiation of the myogenic response, could play a role in the down regulation.

Lastly, a gradual change in the signal activating TGF i.e. in [Cl⁻] at macula densa due to changes in tubular reabsorption after RPP is decreased could also affect the renal vascular resistance.

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 mm Hg) for 30 min in Sprague Dawley rats. During the 30 minutes we measured RBF, GFR, the proximal hydrostatic tubulus pressure (P_{prox}), the late proximal flow rate (Q_{LP}) 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.

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 plasma renin concentration during the 30 min. GFR, proximal tubular pressure and early distal [NaCl] all decrease significantly after 5 min, and remain at this new lower value throughout the 30 min, while 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 down regulation 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 250-330g obtained from Møllegård, Lille Skensved, Denmark. The experimental protocol was approved by the Danish National Research Animal Committee. 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 renal perfusion pressure. 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 to 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 above 20 mmHg and the renal blood flow meter was then adjusted to zero if necessary.

Arterial blood pressure, renal blood flow, hydrostatic proximal tubular pressure and distal tubular fluid conductance were sampled online with a Powerlab/8SP system (ADinstruments) for later analysis on PC. Proximal tubular fluid flow rate was recorded on VHS videotape for later analysis.

**Glomerular Filtration Rate Measurements**

An intravenous bolus injection of $^{51}$Cr-EDTA (ethylenediamintetraacetat, NEN Life Science Products, Belgium) containing 0.51 MBq in 1.75 ml saline was given, followed by continuous infusion of $^{51}$Cr-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 $^{51}$-EDTA concentration in urine and plasma samples was counted in a scintillation $\gamma$-counter.
Hydrostatic Pressure Measurements

Intratubular hydrostatic pressure in the proximal tubule was measured by a servonulling micropuncture system (Reditech, Denmark) 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 mm Hg within ± 0.5 mm Hg.

Tubular Flow Measurements

Late proximal tubular flow rate was measured optically by a modification of the method of Chou and Marsh (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 (1mW, 534 nm, Melles Griot) excited the dye and the fluorescent image was observed at X50 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 PC. This allowed two sample windows of variable size to be placed independently on the video image at 2 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, England). The beveled tip was 6-8 µm (OD). The outer surface of the pipette was covered with platinum (Glantzplatin, Degussa, Germany) and dried at 180ºC. 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, Germany). 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, Denmark).

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 mM 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:** 100 µl plasma and the urine samples were counted in the scintillation γ-counter (Gamma analyzer, Moellsgaard Medical, Copenhagen, Denmark) to estimate $^{51}$Cr-EDTA content. The concentrations of sodium and potassium in urine and plasma were measured by flame photometry (IL243 LED flame photometer, Instrumentation Laboratory, MA). The urine flow was measured gravimetrically. The plasma renin concentration (PRC) was measured by the method of Lykkegaard & Poulsen (23). Aliquots of plasma were diluted 20-80 fold with tris(hydroxymethyl) aminomethane (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 (~1200 ng angiotensin I-equivalents/ml). This incubation was followed by radioimmunoassay of generated angiotensin I. PRC was measured in reference to renin standards obtained from the National Institute for Biological Standards and Control (Potters Bar, Herts, United Kingdom; 1 mGoldblatt unit = 160 pg angiotensin I/ml/h).

**Experimental Protocol**

The experiments in series I were divided into eight 5-min periods. The first two periods were intrinsic controls before reduction of renal perfusion pressure and are presented as one mean control value. Renal perfusion pressure 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 minimum 5 min preceded the 30 min period of reduced RPP.

Blood samples (app. 300 µl) were collected prior to and after the period of perfusion pressure reduction for measurement of plasma concentrations of renin, Na⁺ and K⁺. Ten µl 300 mM EGTA was added to the blood samples to avoid coagulation. The blood samples
were centrifuged at 7000 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

Glomerular Filtration Rate: 17 rats were used. After the first blood sample was drawn the RPP was reduced to 88 mm Hg for 30 min. After the last blood sample was drawn the left kidney was removed, drained and weighed. Urine was collected in pre weighed vials at 5 min intervals for measurement of urine flow, $^{51}$Cr-EDTA clearance and Na$^+$ and K$^+$ concentrations.

Control experiments in 10 rats were made without reducing the renal perfusion pressure.

Series II

Late Proximal Tubular Flow Rate ($Q_{LP}$) and Hydrostatic Proximal Tubulus Pressure ($P_{prox}$): 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. $Q_{LP}$ was measured at the spontaneous renal perfusion pressure and after 5 min of stable recordings the first blood sample was drawn. RPP was reduced to 88 mm Hg and $Q_{LP}$ and $P_{prox}$ were measured for 30 min at the reduced renal perfusion pressure. 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 proximal tubular pressure
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 renal perfusion pressure. Measurements in 12 nephrons were made in 9 rats.

Series III

Distal Conductivity ([\text{NaCl}]_{ED}) and Hydrostatic Proximal Tubular Pressure: 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. $P_{\text{prox}}$ 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 $P_{\text{prox}}$ the first blood sample was drawn. When the blood pressure had stabilized, the renal perfusion pressure was lowered with the aortic clamp from the spontaneous pressure to 88 mm Hg. 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 renal perfusion pressure 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 renal perfusion pressure. Measurements in 15 nephrons were made in 9 rats.

Series IV

Plasma Renin Concentrations over time: In 8 rats small blood samples (app. 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 mm Hg. 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, \( P_{\text{prox}} \), \( Q_{\text{LP}} \) and \([\text{NaCl}]_{\text{ED}}\) were tested for by analysis of variance (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. Plasma renin concentrations before and after the experiment were compared using a paired Students t-test.

Estimates of single nephron proximal reabsorption rate (snAPR), glomerular capillary pressure (\( P_{\text{GC}} \)) and pre- and postglomerular vascular resistances 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 (BW) and initial RBF in rats from the 4 different series are shown in Tables 1, 2, 3 and 4. The rats used in the different experiments were of comparable physiological status.

*Renal Blood Flow*

When renal perfusion pressure was reduced from spontaneous pressure to 88 mm Hg, renal blood flow 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 Figure 1. RBF values for series I, II, III and IV respectively, are shown in Table 1, 2, 3 and 4.

RBF decreased significantly from $6.5 \pm 0.3 \text{ ml/min}$ to $5.2 \pm 0.2 \text{ ml/min} \ (N = 56)$ during 30 min of reduced RPP (Fig. 1B, $P < 0.05$). In the control rats ($N = 31$) RBF was $6.4 \pm 0.2 \text{ ml/min}$ and $6.0 \pm 0.2 \text{ ml/min}$ at the beginning and end of the 30 min control period, respectively (NS).

*Plasma Renin Concentration*

In all four experimental series a reduction of RPP to 88 mm Hg for 30 min lead to a significant increase in plasma renin concentration. 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 $38.4 \pm 4.9 \times 10^{-5} \text{ GU/ml}$ to $102.2 \pm 13.3 \times 10^{-5} \text{ GU/ml} \ (P < 0.01)$ in rats with reduced RPP ($N = 56$). In control rats ($N = 31$) PRC was $31.2 \pm 4.9 \times 10^{-5} \text{ GU/ml}$ and $33.3 \pm 4.5 \times 10^{-5} \text{ GU/ml} \ (\text{NS})$ before and after 30 min of spontaneous RPP.

In series IV ($N = 8$) PRC increased gradually over time (Fig. 1A) from $38.2 \pm 11.0 \times 10^{-5} \text{ GU/ml}$ in the control period to $87.1 \pm 25 \times 10^{-5} \text{ GU/ml}$ after 5 min ($P < 0.05$) to $158.5 \pm 42.3$
x $10^{-5}$ GU/ml (P < 0.05 vs. control and value after 5 min) after 30 min. PRC for the respective series are given in Tables 1, 2, 3 and 4.

**Glomerular Filtration Rate**

When renal perfusion pressure was reduced to 88 mm Hg for 30 min, GFR decreased from $934 \pm 85 \mu l/min/g$ kw in the control period to $764 \pm 67 \mu l/min/g$ kw after 5 min (Table 5, P < 0.01). After 30 min of reduced RPP, GFR remained at a value of $745 \pm 78 \mu l/min/g$ kw (P < 0.01, Table 5, Fig. 4).

In the control rats in series I, GFR was $895 \pm 110 \mu l/min/g$ kw in the control period and $921 \pm 92 \mu l/min/g$ kw after 30 min at the spontaneous perfusion pressure (NS, Table 1).

**Hydrostatic Proximal Tubular Pressure**

The response in $P_{prox}$ during 30 min with an RPP at 88 mm Hg is shown in Table 5. Because the rats in series II and III responded similarly to the RPP reduction, $P_{prox}$ values were pooled in Table 5. Respective $P_{prox}$ values for series II and III are shown in Table 2 and 3. When RPP was reduced to 88 mm Hg $P_{prox}$ significantly decreased from $15.1 \pm 0.5$ mm Hg to $13.8 \pm 0.6$ mm Hg after 5 min (N = 31, P < 0.01). $P_{prox}$ remained low and was $13.5 \pm 0.6$ mm Hg after 30 min of reduced RPP (P < 0.01, Table 5).

In the pooled control rats (N = 21) $P_{prox}$ was $15.1 \pm 0.5$ in the control period and after 30 min at the control renal perfusion pressure $P_{prox}$ was $14.6 \pm 0.6$ (NS).

**Late Proximal Tubular Flow**

The response in $Q_{LP}$ during 30 min of reduced RPP is shown in Figure 2 and Table 5. When RPP was reduced to 88 mm Hg for 30 min, $Q_{LP}$ decreased from $17.6 \pm 0.7$ nl/min in the
control period to $16.3 \pm 0.9$ nl/min after 5 min at 88 mm Hg (NS, Fig. 2, Table 5). After 30 min of reduced RPP, $Q_{LP}$ had decreased to $15.2 \pm 1.0$ nl/min ($P < 0.01$, Fig. 2, Table 5). $Q_{LP}$ was stable when measured for a period of 30 min at the spontaneous RPP ($16.8 \pm 0.6$ nl/min in the control period vs. $16.6 \pm 0.9$ nl/min after 30 min, NS, Fig. 2, Table 2).

*Early Distal [NaCl]*

The response in [NaCl]$_{ED}$ to 30 min of reduced RPP is shown in Table 5. In the control period [NaCl]$_{ED}$ was $43.9 \pm 1.9$ mM and after 5 min of reduced RPP, [NaCl]$_{ED}$ was significantly reduced to $37.0 \pm 2.7$ mM ($P < 0.01$, Table 5). After 30 min of reduced RPP [NaCl]$_{ED}$ remained at $37.9 \pm 2.4$ mM ($P < 0.01$, Table 5). In the control rats [NaCl]$_{ED}$ was $43.9 \pm 2.8$ mM in the control period and $43.8 \pm 2.5$ 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 mm Hg urine excretion decreased significantly from $12.5 \pm 1.2$ µl/min/g kw in the control period to $8.8 \pm 1.2$ µl/min/g kw after 30 min (Table 1, $P < 0.01$). Sodium excretion was $2.4 \pm 0.2$ µmol/min/g kw in the control period and $1.9 \pm 0.4$ µmol/min/g kw after 30 min of reduce RPP (Table 1, NS). Potassium excretion decreased from $1.9 \pm 0.2$ µmol/min/g kw to $1.6 \pm 0.2$ µmol/min/g kw (Table 1, $P < 0.05$).

In the control rats urine excretion was $11.4 \pm 1.1$ µl/min/g kw in the control period and $13.2 \pm 2.0$ µl/min/g kw after 30 min (Table 1, NS). Sodium excretion was $2.5 \pm 0.3$ µmol/min/g kw in the control period and $3.1 \pm 0.5$ µmol/min/g kw after 30 min (NS) while potassium
excretion was 2.0 ± 0.2 µmol/min/g kw in the control period and 1.9 ± 0.2 µmol/min/g kw 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 mM to 148 ± 2 mM (P < 0.05) and 4.2 ± 0.1 mM to 4.5 ± 0.2 mM, respectively (P < 0.05, Table 1). In the control rats plasma sodium and potassium remained unchanged (142 ± 2 mM vs. 141 ± 2 mM and 4.0 ± 0.3 mM vs. 3.9 ± 0.2 mM, respectively, NS, Table 1).

Glomerular Capillary Pressure (P\(_{GC}\)), Pre- and Postglomerular Vascular Resistance

Estimates of glomerular capillary pressure (P\(_{GC}\)) and pre- and postglomerular vascular resistance are shown in Table 6 and Figure 4. The estimated value of P\(_{GC}\) corresponds well to values measured directly in the glomerular capillary (20; 21).

Five min after reducing RPP P\(_{GC}\) had decreased from 47.2 ± 2.0 mm Hg to 45.6 ± 1.8 mm Hg (P < 0.05) and remained low during the 30 min of reduced RPP (Table 6). Preglomerular resistances decreased after 5 min of reduced RPP from 0.234 ± 0.02 mm Hg/ml/min to 0.184 ± 0.02 mm Hg/ml/min (Fig. 4, P < 0.05) whereas postglomerular resistance was unchanged (Table 6). After 30 min of reduced RPP pregglomerular resistance had increased towards the control value (0.205 ± 0.02 mm Hg/ml/min, P < 0.05 vs. baseline and value after 5 min) while postglomerular resistance had increased to a value above the control value (0.196 ± 0.02 mm Hg/ml/min, P < 0.05 vs. baseline and value after 5 min, Table 6, Fig. 4).
Total renal vascular resistance (RVR) also decreased after 5 min of reduced RPP from 17.6 ± 1.2 mm Hg/ml/min to 15.6 ± 1.0 mm Hg/ml/min (P < 0.05, Table 6). After 30 min of reduced RPP RVR had returned to 17.3 ± 1.1 mm Hg/ml/min (P < 0.05 vs. value after 5 min).
Discussion

We have previously shown that a reduction of renal perfusion pressure to just above the lower limit of autoregulation (88 mm Hg) for 30 min induces a gradual decrease in renal blood flow that can be abolished when plasma [Ang II] is clamped by simultaneous infusion of the angiotensin converting enzyme (ACE) inhibitor captopril and 4 ng/min Ang II (38). We therefore hypothesized that the decrease seen in RBF was caused by a gradual increase in renal vascular resistance due to the increasing plasma [Ang II]. However, the exact mechanism is not known. The present study aimed to test the mechanisms underlying the renal response to a 30 min reduction in RPP to 88 mm Hg. After 30 min of reduced RPP, RBF had gradually decreased while plasma renin concentration gradually increased (Fig. 1). Late proximal tubular flow rate also decreased gradually when RPP was reduced (Fig. 2). Glomerular filtration rate, proximal hydrostatic pressure and early distal [NaCl] significantly decreased after 5 min of reduced RPP and remained low during the 30 min (Table 5, Fig. 3).

An acute decrease in RPP is compensated for by two autoregulatory mechanisms. The myogenic response dilates the afferent arteriole in response to a decrease in RPP. The TGF mechanism reacts to a decrease in [Cl⁻] at macula densa (34), and further dilates the afferent arteriole. These mechanisms maintain RBF and GFR as long as the RPP reduction is of a shorter duration. In accordance with this, the estimated preglomerular resistance is significantly decreased after 5 min (Table 6, Fig. 5) corresponding to the autoregulatory response. However, this only maintains RBF at the control level, while GFR has decreased significantly after 5 min (Table 5).

Both RBF and GFR are influenced by the afferent and efferent arteriolar resistances. GFR is directly influenced by changes in glomerular capillary pressure (P_{GC}) and proximal tubular pressure. Initially, when RPP is reduced the autoregulatory mechanisms, as described
above, will also attempt to maintain $P_{GC}$, but after 5 min GFR, $P_{prox}$ (Table 5) and the estimated $P_{GC}$ (Table 6) have decreased significantly. After 30 min GFR has not changed from the value seen after 5 min, but RBF has decreased significantly when compared to the value after 5 min (Fig. 3, Table 5). $P_{GC}$ and $P_{prox}$ remain unchanged at the lower values seen after 5 min (Table 5 and 6). Thus, both the afferent and the efferent arteriole gradually constrict during the last 25 min of the reduced RPP leading to increased total renal vascular resistance (Table 6). This is also seen from the calculated values for $R_{pre}$ and $R_{post}$, which both increased from 5 to 30 min (Fig. 5).

Clamping plasma [Ang II] completely abolished the gradual decline in RBF seen during 30 min of reduced RPP (38). It is therefore likely that the increase in plasma [Ang II] induces the constriction of both the afferent and efferent arteriole. This is further supported by the time course of the changes in RBF and PRC, where the gradual increase in PRC parallels the gradual decrease in RBF (Fig. 1). The increased plasma renin concentrations are consistent with both the decrease in RPP, and the decrease in $[NaCl]_{ED}$ (Table 3). $[NaCl]_{ED}$ is an estimate of the chloride concentration at macula densa, which has been shown to be a stimulus for renin release (22; 37), and an inverse relationship between PRC and $[NaCl]_{ED}$ has been demonstrated in vivo (18). Despite the fact that both RPP and $[NaCl]_{ED}$ remain unchanged from 5 to 30 min, there is a gradual increase in PRC, and most likely also of plasma [Ang II], in the entire period. Possibly, the gradual increase in PRC reflects both the distribution and accumulation of renin in the extracellular fluid, and a gradual activation of the renin release mechanisms. It has previously been shown, that when RPP is reduced to 70 mm Hg, it takes 5 min before renal venous plasma renin activity is maximal (25). After 20 min of reduced RPP to approximately 80 mmHg, a 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] of $10^{-9}$ M (31; 43) and at $10^{-10}$ M intracellular [Ca$^{++}$] starts to increase (15). Cortical efferent arterioles constrict at $10^{-11}$ (14). Juxtamedullary afferent arterioles constrict at [Ang II] of $10^{-10}$ M (11; 13) as does juxtamedullary efferent arterioles (10). Thus, the cortical efferent arterioles constrict at Ang II concentrations 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. 4). 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 which 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 approximately 20 min (40). The resetting shifts the turning point of the TGF curve, i.e. the point where TGF is most sensitive, toward lower proximal tubular flow rates. A reduced RPP by itself decreases TGF sensitivity (33). However, as shown by Selen & 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 does not allow an assessment of the quantitative contributions of these various mechanisms to the afferent arteriolar constriction seen between 5 and 30 minutes following the reduction in RPP.

From the estimated $R_{\text{pre}}$ and $R_{\text{post}}$ it can be seen that postglomerular arteriolar resistance increases to a level above the control level whereas afferent arteriolar resistance increases back towards 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 proximal tubular flow rate decreases steadily during the 30 min of reduced RPP (Fig. 2 and 5). $Q_{\text{LP}}$ 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]$_{\text{ED}}$ 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 following 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 preglomerular and postglomerular resistance.

We have calculated the preglomerular and postglomerular resistances using the equations from Hall et al. (8):

\[ R_{\text{pre}} = \frac{(\text{RPP} - \text{PGC})}{\text{RBF}}, \quad (1) \]

\[ R_{\text{post}} = \frac{\text{PGC} - \text{PC}}{\text{RBF} - \text{GFR}}. \quad (2) \]

Assuming that the kidney in Sprague Dawley rats is composed of 25,000 nephrons (1; 44) the glomerular capillary pressure (PGC) used in equation 1 and 2 was estimated from the equation:

\[ \text{PGC} = \left( \frac{\text{snGFR}}{\text{kf}} \right) + \text{P}_{\text{prox}} + \Pi_G, \quad (3) \]

where single nephron GFR was calculated from GFR by dividing by 25,000. The ultrafiltration coefficient (kf) was assumed to be 0.08 nl/(sec · mm Hg) (6), and P_{prox} was measured directly in the single nephron.

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

\[ \Pi_G = \frac{\Pi_A + \Pi_E}{2}. \quad (4) \]

\(\Pi_A\) and \(\Pi_E\) were calculated from an assumed arterial protein concentration (CP_A) of 5.5g/dl in the rat (28), and an efferent arteriolar protein concentration (CP_E) calculated as:

\[ \text{CP}_E = \frac{\text{CP}_A}{(1 - \text{FF})}. \]

\(\Pi_A\) and \(\Pi_E\) used in equation 4 to was found from the following relation (28):

\[ \Pi = 1.825 \cdot \text{CP} + 0.164 \cdot \text{CP}^2 + 0.012 \cdot \text{CP}^3, \quad (5) \]

where CP are 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:

$$P_C = P_{prox} - 4 \text{ mm Hg}.$$  \hspace{1cm} (7)

Single nephron proximal reabsorption rate (snAPR) was calculated as:

$$\text{snGFR} - Q_{LP},$$ \hspace{1cm} (8)

where $Q_{LP}$ is the measured late proximal flow rate.
Acknowledgements

The present study was supported by grants from the Danish Heart Association (no. 22028), the Novo Nordisk Foundation, Ruth I. E. König-Petersen Foundation, the Danish Medical Research Council and the Foundation to the Advancement of Medical Science. The technical assistance of Mrs. Anni Salomonsson, Mr. Ian Godfrey, Mrs. Mette Fredenslund and Mrs. Inge Andersen is gratefully acknowledged.
References


Legends to figures.

Figure 1.
The effect on plasma renin concentration (A) and renal blood flow (B) when RPP is reduced to 88 mm Hg for 30 min (●). Control rats (■) remained at the spontaneous RPP for 30 min. Values are mean ± SE. *, P < 0.05 vs. control-value. †, P < 0.05 vs. value after 5 min.

Figure 2.
The effect on late proximal tubular flow rate of reducing RPP to 88 mm Hg for 30 min (●). Control rats (■) remained at the spontaneous Q_{LP} for 30 min. Values are mean ± SE. *, P < 0.05 vs. control-value. #, P < 0.01 vs. control-value.

Figure 3.
The percent-wise change in RBF, GFR, P_{prox}, Q_{LP}, [NaCl]_{ED} and PRC from 5 min of reduced RPP to 30 min of reduced RPP. Please note break on Y-axis. Values are mean ± SE. †, P < 0.05. #, P < 0.01.

Figure 4.
Changes over time in estimated preglomerular resistance (■) and postglomerular resistance (●). †, P < 0.05 vs. value at 5 min.
Table 1. Physiological status of rats used in series I.

<table>
<thead>
<tr>
<th></th>
<th>Reduced RPP</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 17, BW = 266 ± 7 g</td>
<td>N = 10, BW = 267 ± 7 g</td>
</tr>
<tr>
<td></td>
<td>KW = 1.2 ± 0.0 g</td>
<td>KW = 1.2 ± 0.0 g</td>
</tr>
<tr>
<td>RPP, mm Hg</td>
<td>102 ± 2</td>
<td>103 ± 1</td>
</tr>
<tr>
<td>RBB, ml/min</td>
<td>6.1 ± 0.6</td>
<td>6.0 ± 0.3</td>
</tr>
<tr>
<td>GFR, µl/min x g kw</td>
<td>934 ± 85</td>
<td>895 ± 110</td>
</tr>
<tr>
<td>VU, µl/min x g kw</td>
<td>12.5 ± 1.2</td>
<td>11.4 ± 1.1</td>
</tr>
<tr>
<td>UNa+, µmol/min/g kw</td>
<td>2.4 ± 0.2</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>UK+, µmol/min/g kw</td>
<td>1.9 ± 0.2 1.6 ± 0.2 *</td>
<td>2.0 ± 0.2 1.9 ± 0.2</td>
</tr>
<tr>
<td>PNa+, mM</td>
<td>145 ± 1</td>
<td>142 ± 2</td>
</tr>
<tr>
<td>PK+, mM</td>
<td>4.2 ± 0.1</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>PRC, 10⁻⁵ GU/ml</td>
<td>34.5 ± 9.0 65.2 ± 16.4*</td>
<td>30.6 ± 6.0 24.3 ± 7.0</td>
</tr>
</tbody>
</table>

*, P < 0.05 vs. baseline. #, P < 0.01 vs. baseline. BW, body weight. KW, kidney weight. VU, urine flow rate. UNa+, urinary sodium excretion rate. UK+, urinary potassium excretion rate. PNa+, plasma sodium concentration. PK+, plasma potassium concentration. GU, Goldblatt Units.
**Table 2.** Physiological status of rats used in series II.

<table>
<thead>
<tr>
<th></th>
<th>Reduced RPP</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 13</td>
<td>N = 12</td>
</tr>
<tr>
<td></td>
<td>BW = 277 ± 4 g</td>
<td>BW = 289 ± 4 g</td>
</tr>
<tr>
<td>Baseline</td>
<td>After 30 min</td>
<td>Baseline</td>
</tr>
<tr>
<td>After 30 min</td>
<td></td>
<td>After 30 min</td>
</tr>
<tr>
<td>RPP, mm Hg</td>
<td>103 ± 2</td>
<td>100 ± 1</td>
</tr>
<tr>
<td></td>
<td>88</td>
<td>101 ± 1</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>6.6 ± 0.5</td>
<td>6.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>5.6 ± 0.5 *</td>
<td>6.1 ± 0.4</td>
</tr>
<tr>
<td>P_{prox}, mm Hg</td>
<td>14.5 ± 0.9</td>
<td>15.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>13.0 ± 1.0 #</td>
<td>15.0 ± 0.8</td>
</tr>
<tr>
<td>Q_{LP}, nl/min</td>
<td>17.6 ± 0.7</td>
<td>16.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>15.2 ± 1.0 #</td>
<td>16.6 ± 0.9</td>
</tr>
<tr>
<td>PRC, 10^{-5} GU/ml</td>
<td>50.8 ± 16.1</td>
<td>32.0 ± 8.1</td>
</tr>
<tr>
<td></td>
<td>107 ± 31.7 *</td>
<td>39.6 ± 7.4</td>
</tr>
</tbody>
</table>

*, P < 0.05 vs. baseline. #, P < 0.01 vs. baseline. BW, body weight. GU, Goldblatt Units.
Table 3. Physiological status of rats used in series III.

<table>
<thead>
<tr>
<th></th>
<th>Reduced RPP</th>
<th></th>
<th>Control</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 18</td>
<td>BW = 275 ± 5 g</td>
<td>N = 9</td>
<td>BW = 278 ± 9 g</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>After 30 min</td>
<td>Baseline</td>
<td>After 30 min</td>
</tr>
<tr>
<td>RPP, mm Hg</td>
<td>105 ± 1</td>
<td>88</td>
<td>101 ± 2</td>
<td>99 ± 2</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>7.0 ± 0.4</td>
<td>5.1 ± 0.4 *</td>
<td>6.3 ± 0.5</td>
<td>5.9 ± 0.3 *</td>
</tr>
<tr>
<td>P_prox, mm Hg</td>
<td>15.4 ± 0.7</td>
<td>14.1 ± 0.8 #</td>
<td>15.0 ± 0.7</td>
<td>14.1 ± 0.9</td>
</tr>
<tr>
<td>[NaCl]_{ED}, mM</td>
<td>43.9 ± 1.9</td>
<td>37.9 ± 2.4 #</td>
<td>43.9 ± 2.8</td>
<td>43.8 ± 2.5</td>
</tr>
<tr>
<td>PRC, 10^{-5} GU/ml</td>
<td>31.4 ± 7.5</td>
<td>98.1 ± 30.4 *</td>
<td>31.1 ± 6.3</td>
<td>42.5 ± 7.4</td>
</tr>
</tbody>
</table>

*, P < 0.05 vs. baseline. #, P < 0.01 vs. baseline. BW, body weight. GU, Goldblatt Units.
**Table 4.** Physiological status of rats used in series IV.

<table>
<thead>
<tr>
<th></th>
<th>Reduced RPP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 8</td>
</tr>
<tr>
<td></td>
<td>BW = 283 ± 8 g</td>
</tr>
<tr>
<td>Baseline</td>
<td>After 30 min</td>
</tr>
<tr>
<td>RPP, mm Hg</td>
<td>102 ± 2</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>4.7 ± 0.6 #</td>
</tr>
<tr>
<td>PRC, 10^{-5} GU/ml</td>
<td>38.2 ± 11.0</td>
</tr>
<tr>
<td></td>
<td>158.5 ± 42.9 *</td>
</tr>
</tbody>
</table>

*, P < 0.05 vs. baseline. #, P < 0.01 vs. baseline. BW, body weight. GU, Goldblatt Units.
**Table 5.** Changes in RBF, GFR $P_{\text{prox}}$, $Q_{\text{LP}}$, $[\text{NaCl}]_{\text{ED}}$ and PRC 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>RBF, ml/min (N = 56)</td>
<td>6.5 ± 0.3</td>
<td>6.0 ± 0.3</td>
<td>5.2 ± 0.3 *</td>
</tr>
<tr>
<td>GFR µl/min/g kw (N = 17)</td>
<td>934 ± 85</td>
<td>764 ± 67 #</td>
<td>745 ± 78 #</td>
</tr>
<tr>
<td>$P_{\text{prox}}$ mm Hg (N = 31)</td>
<td>15.1 ± 0.5</td>
<td>13.8 ± 0.6 #</td>
<td>13.5 ± 0.6 #</td>
</tr>
<tr>
<td>$Q_{\text{LP}}$, nl/min (N = 13)</td>
<td>17.6 ± 0.7</td>
<td>16.3 ± 0.9</td>
<td>15.2 ± 1.0 #</td>
</tr>
<tr>
<td>$[\text{NaCl}]_{\text{ED}}$ mM (N = 18)</td>
<td>43.9 ± 1.9</td>
<td>37.0 ± 2.7 #</td>
<td>37.9 ± 2.4 #</td>
</tr>
<tr>
<td>PRC, x 10^{-5} GU/ml (N = 56)</td>
<td>38.2 ± 11.0</td>
<td>87.1 ± 25.1 *</td>
<td>158.5 ± 42.9 #</td>
</tr>
</tbody>
</table>

*, P < 0.05 vs. baseline. #, P < 0.01 vs. baseline. KW, kidney weight. GU, Glodblatt Units.
### Table 6. Changes in estimated snAPR, Πₐ, PGC, Rₚₚₑₛ, Rₚₒₛₚₑₚ 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>PGC mm Hg</td>
<td>47.2 ± 2.0 ‡</td>
<td>45.6 ± 1.8 *</td>
<td>44.8 ± 1.9 *</td>
</tr>
<tr>
<td>Rₚₑₛ mm Hg/nl/min</td>
<td>0.234 ± 0.02 ‡</td>
<td>0.184 ± 0.02 *</td>
<td>0.205 ± 0.02 *‡</td>
</tr>
<tr>
<td>Rₚₒₛₚₑₚ mm Hg/nl/min</td>
<td>0.185 ± 0.02</td>
<td>0.177 ± 0.01</td>
<td>0.196 ± 0.02 *‡</td>
</tr>
<tr>
<td>RVR mm Hg/ml/min</td>
<td>17.6 ± 1.2 ‡</td>
<td>15.6 ± 1.0 *</td>
<td>17.3 ± 1.1 ‡</td>
</tr>
</tbody>
</table>

snAPR, single nephron proximal reabsorption rate. RVR, renal vascular resistance. *, P < 0.05 vs. baseline ‡, P < 0.05 vs. value after 5 min.
Figure 1.
Figure 2.
Figure 4.

Control  5 min  10 min  15 min  20 min  25 min  30 min

Resistance (mm Hg/ml/min)