Pressure Natriuresis and Autoregulation of Inner Medullary Blood Flow in Canine Kidney

Dewan S A. Majid, Murrell Godfrey, Sophia A. Omoro

Abstract We have evaluated the responses to changes in arterial pressure on regional blood flows in the renal medulla and sodium excretion simultaneously in denervated kidneys of six anesthetized sodium-replete dogs. Renal regional blood flow responses were determined using laser-Doppler needle flow probes and whole-kidney blood flow was assessed using an electromagnetic flow probe. The responses to stepwise reductions in renal arterial pressure (140 to 70 mm Hg) were examined first with a laser-Doppler needle probe inserted in the outer medulla and then repeated after advancing the same probe in the inner medulla. There were no differences in the control values of total renal cortical flow (0.89±0.7 to 0.94±0.9 mL min⁻¹ g⁻¹), sodium excretion (3.6±0.6 to 3.4±0.5 µmol min⁻¹ g⁻¹), and urinary excretion rate of nitric oxide metabolites (nitrate/nitrite, 1.6±0.2 to 1.5±0.2 µmol min⁻¹ g⁻¹) at the start of both experimental periods. During changes in renal arterial pressure, inner medullary blood flow exhibited efficient autoregulation similar to that in outer medullary blood flow. Usual excretory responses to reductions in renal arterial pressure as well as autoregulation of cortical and whole-kidney blood flows and glomerular filtration rate were observed in these dogs. The slopes of the relationship between arterial pressure and sodium excretion (0.046±0.007 to 0.044±0.009 µmol min⁻¹ g⁻¹ mm Hg⁻¹) or nitrate/nitrite excretion (0.014±0.003 to 0.013±0.003 nmol min⁻¹ g⁻¹ mm Hg⁻¹) were similar in both experimental periods. These data indicate that blood flow to the inner medulla is efficiently autoregulated as in outer medulla and cortex of the kidney in anesthetized dogs and demonstrate further that the arterial pressure-induced natriuretic responses do not require associated changes in medullary blood flow.

Key Words • renal autoregulation • laser-Doppler flowmetry • sodium excretion

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A recent study in anesthetized volume-expanded dogs using LDF with implanted fibers that the autoregulatory behavior of MBF in anesthetized dogs was also reported earlier. However, the influence of arterial pressure on regional blood flow in the renal medulla remains controversial. In our previous studies in dogs, MBF was measured by an LDF needle probe placed near the junction of the inner medulla (white zone) and the inner stripe of the outer medulla (red zone), an area that may not have represented the inner medullary circulation. The present study was therefore designed to examine the possibility of regional variations in the autoregulatory behavior of MBF in anesthetized dogs using LDF needle probes in the kidney. Responses to changes in RAP on sodium excretion as well as blood flow to the renal medulla were examined simultaneously in these dogs to evaluate the relationship between medullary circulation and the phenomenon of pressure natriuresis. The renal responses to stepwise reductions in RAP were examined first with an LDF needle probe inserted in the outer medulla and then repeated after advancing the same probe in the inner medulla. In addition, a routine analysis of urinary excretion rate of NO metabolites, nitrate/nitrite (NO₃⁻/NO₂⁻), in response to changes in RAP was performed in this study as reported previously.
### Methods

Experiments were performed on six mongrel dogs ranging in body weight from 18 to 23 kg. These dogs were given supplemental amounts of sodium chloride (1.5 g/kg body weight per day for 3 days) added to the normal laboratory diet to achieve a sodium-replete state. On the morning of the experiments, the dogs were anesthetized with pentobarbital sodium (30 mg/kg body weight, IV) and artificially ventilated. Surgical anesthesia was maintained throughout the experiment by additional doses of pentobarbital sodium as required. Positive pressure ventilation was provided to the animals with an artificial respirator at a rate of 18 strokes per minute (stroke volume, 15 mL/kg body weight) via auffed endotracheal tube inserted into the trachea. Body temperature was maintained within the range of 99°F to 101°F using an electric heating pad. SAP was measured with a catheter placed in the abdominal aorta inserted via the right femoral artery and recorded on a polygraph (model 7D, Grass Instruments). The left femoral artery was cannulated for collection of blood samples. The femoral and jugular veins were cannulated for administration of saline, mlin solution, and additional doses of pentobarbital sodium as necessary.

The left kidney was exposed through a flank incision, and the renal artery was isolated from surrounding tissue. Renal denervation was performed by cutting all the visible nerves projecting to the kidney from the aortocaval ganglion. RBF was measured with an EMF probe placed around the renal artery and connected to a square wave flowmeter (Carolina Medical Electronics). The zero flow for the EMF probe was obtained by occluding the renal artery momentarily at the beginning and end of the experimental periods. An adjustable plastic clamp was placed on the renal artery distal to the EMF probe to allow acute reductions in RAP. A curved 23-gauge needle cannula was inserted into the renal artery distal to the plastic clamp and connected to a pressure transducer to measure RAP. Another catheter was also connected to this needle cannula for continuous infusion of heparinized saline or drug solutions at a rate of 0.4 mL/min. Urine was collected from a catheter placed in the ureter.

A dual-channel LDF (Periflux 4001, Permed) with two needle probes was used to measure relative blood flows in the cortical and medullary regions of the kidney. One of these probes was inserted to 5 mm in depth inside the kidney mass to position the tip in the medulla and the second probe was advanced to locate the tip in the outer medulla for the measurement of CBF. The other probe was first inserted to about 13 mm in depth to localize the tip in the outer medulla region and then advanced the tip to 20 mm in depth to position the tip in the inner medulla region of the kidney for the measurement of outer and inner MBF, respectively. Insertion of the thin probe (500 μm diameter) did not cause any discernible functional impairment in renal hemodynamics and renal function.

The position of the tips of the needle probes was confirmed at the end of each experiment by dissecting the kidney and viewing the needle tract and the regions surrounding the fiber tip. These flow probes were calibrated with a standard calibration device using a motility standard that is a colloid suspension of latex particles (10 μm microspheres). Brownian motion of the latex particles provides the standard value of 250 perfusion units. One perfusion unit is an arbitrary value equal to an analogue output of 10 mV. The output signals from the LDF were recorded on a grass polygraph. Although the changes in absolute voltage signals (PU values) were continuously monitored on the digital screen of the flowmeter, the data are reported as a percent of the basal levels at spontaneous pressures.

To avoid respiratory movement artifacts in the LDF signals, the kidney was kept in a fixed position by placing it on a plastic holder similar to that used for micropuncture experiments. There was no reduction in basal RBF after immobilization of the kidney. The absolute outputs from the probe placed in the cortical tissue were always observed to be substantially greater than (more than three times) the output from that in medulla tissue, indicating the predictable differences in blood flows in the regions. In this study, we have also observed a similar difference in signal output between the probes placed in cortex and in inner medulla. It was also noted that the probe in the inner medulla showed a smaller signal output than that in the outer medulla, indicating regional variations in the basal levels of blood flow to the renal medulla. An example of this difference in signals has been demonstrated in Fig 1. The zero flow recordings of the LDF probe were determined by occluding the renal artery momentarily at the beginning and end of the experiments. It was always observed that the signals recorded from the LDF probe at the inner medulla were substantially higher than the signals obtained at zero flow (36±2 PU, n=6). The cortical probe also showed similar zero flow signals (34±2 PU, n=6). Although we reported earlier that an LDF probe placed in the inner medulla region was able to reflect changes in blood flow induced by various vasoactive agents, we also examined this responsiveness of an LDF probe placed in the inner medulla in some cases. Fig 2 shows an example of such responsiveness of the LDF signals recording inner MBF in response to intra-arterial administration of bolus doses of vasodilators, acetylecholine and vasodilator, angiotensin II.
ner medullary circulation also responded to these vasoactive agents in a manner similar to that observed in midmedullary circulation. After completion of surgery, a 2.5% solution of multih in normal saline was administered into the jugular vein at least 45 minutes before the onset of the experimental protocol. An initial dose of 6 mL/kg body weight was followed by a continuous infusion of 0.3 mL/min kg body weight. At least 45 minutes before the start of the experimental protocol, the left common carotid artery was partially constricted to elevate the basal level of mean arterial pressure to around 140 mm Hg, and the constriction was maintained throughout the experimental period. Initially, the needle probe in the medulla was placed in the outer region (13 mm in depth) to measure outer MBF. The experimental protocol was started with urine collections for two consecutive 10-minute periods at spontaneous RAP. At the midpoint of each collection period, an arterial blood sample (2 mL) was taken to measure plasma multih, sodium, and potassium concentration. RAP was then reduced in two steps (around 105 minutes of each collection period, an arterial blood sample (2 mL) was taken to measure plasma multih, sodium, and potassium concentration. RAP was then reduced in two steps (around 105 and 75 mm Hg) by adjusting the clamp on the renal artery. Five minutes were allowed for stabilization at each level of RAP before a 10-minute urine sample was collected. The average arterial pressure over the 10-minute collection period was taken as the RAP value in each period. After the last reduction in RAP, the clamp was released completely to reestablish control RAP and RBF. Then the medullary probe was advanced to an inner region (20 mm in depth) to measure inner MBF. After stabilization, the protocol was repeated to examine the renal responses to reductions in RAP.

At the end of each experiment, the EMF probe was calibrated in situ by collection of timed blood samples into the graduated cylinder at different flow rates from a catheter placed in the renal artery. The kidney was then removed, blotted dry, and weighed so that the calculated parameters could be expressed per gram of kidney weight. The localization of the fiber probes was confirmed by directly observing the track of the needle probe after dissection of the kidneys. Flame photometry (Instrumentation Laboratory) was used to determine the sodium and potassium concentrations in plasma and urine. Multih concentrations in plasma and urine samples were determined by the anthrone colorimetric technique (Gilford Instruments). Urinary concentration of NO$_3^-$ / NO$_2^-$ was measured using the Greiss reaction technique after enzymatic reductions of nitrate to nitrite in the samples. NO$_3^-$/NO$_2^-$ is the metabolic end product of NO, and changes in the urinary excretion rate of NO$_3^-$/NO$_2^-$ are considered to reflect the changes in endogenous NO production.

Values are reported as mean±SEm. Statistical comparisons of differences in the responses were conducted with the use of ANOVA followed by the Newman-Keuls test. Differences in the mean values were deemed significant at $P \leq 0.05$.

**Fig 2** Example of the effect of intrarenal injections of different doses of acetylcholine (Ach, A) and angiotensin II (Ang II, B) on inner MBF recorded by LDF. Ach and Ang II caused dose-dependent vasodilatory and vasoconstrictor responses, respectively, in inner MBF, which corresponded with the responses observed in total RBF recorded by EMF probe. The deflections in RAP tracings are due to injection artifacts.

**Results**

As mentioned earlier in the experimental protocol, renal excretory and regional blood flow responses to changes in RAP in six dogs were evaluated during two experimental periods, first, when an LDF needle probe was positioned at outer medullary region and second, when the same needle probe was advanced and positioned within the inner medullary region. The mean control values of hematocrit, plasma proteins, and colloidal osmotic pressure during the first experimental period in these dogs were 44±2.8%, 5.1±0.3 g/100 mL, and 15.7±0.8 mm Hg, respectively. These values remained the same during the second experimental period (45±2.9%, 4.8±0.25 g/100 mL, and 14.4±0.7 mm Hg).

**Responses Observed During First Experimental Period (Outer MBF Assessment)**

The Table summarizes the results in SAP, total RBF, CBF, GFR, urine flow, and $U_{Na,V}$, obtained during stepwise reductions in RAP from 136±2.3 to 76±13 mm Hg at the first experimental period. The values of $FE_{Na}$, $V_{in}$, and urinary excretion rate of NO$_3^-$/NO$_2^-$ during the control period were 2.8±0.6%, 0.78±0.06 μmol·min$^{-1}$·g$^{-1}$, and 1.6±0.2 nmol·min$^{-1}$·g$^{-1}$, respectively. During stepwise reductions in RAP, RBF, CBF, and $GFR$ demonstrated usual autoregulatory behavior (see the Table). SAP did not change during reductions in RAP with a renal vascular occluder (Table). Spontaneous RAP generally remained slightly lower (5 to 10 mm Hg) than SAP due to slight constriction imposed on the renal artery by the EMF probe and vascular occluder. The outer MBF also maintained autoregulatory efficiency (Fig 3) similar to that of CBF and total RBF. The slope of the relationship between RAP and the outer MBF ($-0.15±0.14$ mm Hg$^{-1}$) was not statistically different from zero. As shown in the Table, $U_{Na,V}$ decreased, as usual, in response to reductions in RAP during the first experimental period. There were also decreases in $FEN_a$, and urinary excretion rate of NO$_3^-$/NO$_2^-$ during reductions in RAP (Fig 4). The slopes of the relationships between RAP and $FEN_a$, and urinary excretion rate of NO$_3^-$/NO$_2^-$ were 0.034±0.006% mm Hg$^{-1}$, $P<.05$ as well as the urinary excretion rate of NO$_3^-$/NO$_2^-$ (0.014±0.003 nmol·min$^{-1}$·g$^{-1}$ mm Hg$^{-1}$, $P<.05$) were significantly different from zero. These responses in renal excretory function to changes in RAP were similar to those previously observed in our laboratory.
Responses Observed During Second Experimental Period (Inner MBF Assessment)

The summarized results of SAP, total RBF, CBF, GFR, urine flow, and UN,V in response to reductions in RAP during the second experimental period are given in the Table. The values of FENO, UN,V, and urinary excretion rate of NO3-/NO2- during the control periods were 2.5±0.4%, 0.83±0.14 μmol min⁻¹ g⁻¹, and 15±0.2 nmol min⁻¹ g⁻¹, respectively. The RBF, CBF, and other renal excretory values during control collections at spontaneous RAP during the second experimental period were not significantly different from the values observed during control collections at spontaneous RAP during the first experimental period. During stepwise reductions in RAP in the second experimental period, the autoregulatory efficiency of RBF, CBF, and GFR remained intact as in the first period (see Table). SAP also remained at steady state during reductions in RAP. Interestingly, the inner MBF also maintained autoregulatory capability similar to that of the outer MBF observed during the first period (Fig 3). The slope of the relationship between RAP and inner MBF (0.28±0.08% mm Hg⁻¹) was not significantly different from the slope of the relationship between RAP and outer MBF (Fig 3). There were no changes in the responses to reductions in RAP on RBF, CBF, GFR, urine flow, UN,V, FENO, and urinary excretion rate of NO3-/NO2- (0.013±0.003 nmol min⁻¹ g⁻¹, mm Hg⁻¹) were not significantly different from the slopes observed during the first experimental period.

Discussion

The technique of using LDF single-fiber probes for the evaluation of renal medullary blood flow has been validated in previous studies conducted in our laboratory. Although LDF does not provide an absolute measure of blood flow, the available data indicate that this technique can provide a good indication of relative changes in regional blood flow within renal tissue. It was demonstrated that the LDF needle probes were able to reflect changes in CBF or MBF during administration of various vasoactive agents, and the changes were seen closely correlated with the changes in total RBF recorded with EMF probes (Fig 2). It may be argued that possible alterations in medullomural condition in these dogs subjected to partial occlusion of common carotid arteries may have influenced the inner MBF response to changes in RAP in this study. This is unlikely, as the LDF probe positioned in the inner medulla was also seen responsive to vasoactive agents (Fig 2) in a manner similar to that observed in the LDF probe inserted into the midmedullary regions. As the kidney was kept denervated and the systemic arterial pressure remained stable, it seems unlikely that subtle fluctuations in release of circulating hormones might influence the inner MBF response to selective changes in RAP elicited with a renal arterial occluder. The LDF needle probe was also able to detect the regional variations in renal blood flows in anesthetized dogs (see Fig 1 and References 20 and 21). These relative differences in LDF signals in the cortex, outer medulla, and inner medulla observed in this study (Fig 1) are in general agreement with the recognized differences in absolute renal regional blood flow, previously measured using techniques such as the rubidium uptake technique (see review article, Reference 27). The blood flows in outer cortical, inner cortical, outer medullary, and inner medullary regions measured by the rubidium uptake technique in canine kidney in different laboratories were averaged to 7.4, 4.8, 2.5, and 1.5 mL min⁻¹ g⁻¹ of kidney tissue, respectively. These values correspond to the relative differences in regional blood flow in the kidney observed with the LDF probe in this study. The changes in

### Table: Responses to Acute Alterations in RAP (n=6)

<table>
<thead>
<tr>
<th>Levels of RAP, mm Hg</th>
<th>First Experimental Period</th>
<th>Second Experimental Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Spontaneous)</td>
<td>Level 136±12.3</td>
<td>Level 106±2.7</td>
</tr>
<tr>
<td>SAP, mm Hg</td>
<td>143±2.0</td>
<td>141±2.3</td>
</tr>
<tr>
<td>Total RBF, mL min⁻¹ g⁻¹</td>
<td>4.4±0.7</td>
<td>4.4±0.6</td>
</tr>
<tr>
<td>CBF, % of control at spontaneous RAP</td>
<td>100</td>
<td>130±2.3</td>
</tr>
<tr>
<td>GFR, mL min⁻¹ g⁻¹</td>
<td>0.89±0.07</td>
<td>0.85±0.05</td>
</tr>
<tr>
<td>Urine flow, μL min⁻¹ g⁻¹</td>
<td>22.5±6.0</td>
<td>12.8±2.3</td>
</tr>
<tr>
<td>UN,V, μmol min⁻¹ g⁻¹</td>
<td>3.6±0.6</td>
<td>2.5±0.4</td>
</tr>
</tbody>
</table>

*P<0.05 vs 1st control, †P<0.05 vs 2nd control
autoregulatory efficiency of CBF or MBF could also be detected by this technique when they actually occurred during calcium channel blockade23 or when RAP was reduced below the usual autoregulatory range in anesthetized dogs20 Thus, it is likely that the signals from the LDF needle probes in the present study provided a satisfactory assessment of regional blood flows in the renal medulla. It may be possible that a substantial decrease in RBF during reductions in RAP below autoregulatory range can provoke a change in renal volume and thus may displace the LDF probe tip inside the kidney. However, it should be emphasized that when the total RBF remained unchanged during alterations in RAP within autoregulatory range in our canine kidney preparations, the net renal volume did not change perceptibly. Thus, it was unlikely that the LDF probe in the inner medulla changed its position during changes in RAP within autoregulatory range in this present study.

The results of the present investigation demonstrate that the blood flow to the inner region of the medulla exhibits efficient autoregulatory behavior similar to that seen in the outer medullary region of the kidney in anesthetized dogs (Fig 3). In our previous studies,20,21 we have also observed similar autoregulatory capacity in the medullary region of the kidney. These observations in anesthetized dogs are in agreement with findings of a recent study in dogs22 which demonstrated that blood flows to both the juxta-medullary and papillary regions of the kidney maintained autoregulatory efficiency similar to that in cortex. Both inner and outer MBF in hydropenic rats also showed an efficient autoregulation, as reported in a previous study24. Although acute saline loading in rats showed an impairment of autoregulation in MBF,13,24 such response was not seen in dogs under similar condition21,22. Apart from the species differences, the exact reason for such discrepancy between the findings in dogs and those in rats during volume expansion is not yet clear. However, in the present study, the usual arterial pressure-induced changes in urine flow and sodium excretion were observed in the presence of efficient autoregulation of blood flow to the inner as well as the outer medullary region. These results confirm our earlier observation21 that pressure-natriuretic responses are not dependent on the changes in MBF in anesthetized dogs. However, the study by Lerman et al23 using a technique based on a fast computed tomography imaging indicated a diminished efficiency in autoregulatory behavior for blood flow to the inner but not the outer medulla in canine kidney, which is at variance with the findings in the present study. The reason for this discrepancy in the findings is not clear, except that a different methodological approach was adopted in that study. The assessment of renal regional blood flow responses to changes in aortic pressure was made in that study23 using the dynamic spatial reconstructor, run for about 30 seconds. On the other hand, laser-Doppler flowmetry provides a continuous blood flow monitoring over the whole period of the experimental protocol. However, a direct correlation between renal excretory function and regional blood flows was not assessed in most of the previous studies attempting to evaluate the autoregulatory behavior of the medullary circulation.13,22-24 In the present investigation as well as our earlier studies,21 simultaneous observation of the intrarenal blood flows and the urinary excretory responses to changes in RAP allowed a more complete characterization of the relationship between MBF and the pressure-natriuretic phenomenon.

The mechanism involved in mediating the pressure-natriuretic response remains unresolved. Recent studies have suggested that changes in intrarenal NO activity during changes in RAP may be involved in mediating the pressure-natriuretic response.7,11 Significant correlation between RAP and the urinary excretion rate of nitrate/nitrite (metabolites of NO) have been demonstrated in our earlier studies in anesthetized dogs.7,21 A similar linear relationship between RAP and nitrate/nitrite excretion rate was also observed in the present study (Fig 4). These data, along with the observations in some in vitro studies showing direct effects of NO on tubular reabsorptive function,28-30 support the hypothesis that increases in intrarenal NO production rate in response to increases in RAP could serve as an important mediator of the arterial pressure-induced changes in urine flow and sodium excretion. Increases in the release of NO during increases in RAP could influence tubular transport either directly28-30 or by altering intrarenal hemodynamic environment such as increases in RIKP12,14,15,21. Selective inhibition of NO in the rat renal medulla,19 as well as the administration of the NO precursor L-arginine in Dahl salt-sensitive rats,31 has been shown to influence RIKP. However, further studies would be needed to clarify the possible link between intrarenal NO and the RIKP responses to changes in RAP.

![Graph](http://hyper.ahajournals.org/)
In conclusion, the results of the present investigation demonstrate that the autoregulatory efficiency of the blood flow to the inner medulla is similar to that in the outer medulla or the cortex in canine kidney. These data provide further evidence that changes in medullary blood flow are not an essential components in the mechanism of pressure natriuresis.

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