Perfusion pressure dependency of in vivo renal tubular dynamics

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Rodriguez-Porcel, Martin, Lilach O. Lerman, Patrick F. Sheedy II, and J. Carlos Romero. Perfusion pressure dependency of in vivo renal tubular dynamics. Am. J. Physiol. 273 (Renal Physiol. 42): F667–F673, 1997.—To examine whether changes in renal perfusion pressure (RPP) within the range of autoregulation induce detectable changes in tubular dynamics in an entire nephron population of the intact kidney, we measured, using electron beam computed tomography (EBCT), transit times (TT, s) and intratubular concentration (%) of filterable contrast media in various nephron segments simultaneously with renal regional perfusion. In seven dogs (group A) this was performed at the upper and lower limits of autoregulation (RPP = 130 and 95 mmHg, respectively) while group B (n = 5) served as control. In group A alone, a decrease in RPP led to an increase in TT by 40%, 68%, and 32% in the proximal tubules, ascending limb of Henle’s loop, and distal tubules, respectively, in association with an increase in intratubular concentration (+50%, 80%, and 42%, respectively). Papillary perfusion decreased, whereas perfusion of the adjacent, outlying inner medulla increased. The decrease in papillary perfusion correlated positively with the concurrent change in sodium excretion (R = 0.81). This study demonstrates that changes in RPP within the autoregulatory range elicit changes of tubular sodium reabsorption mainly in proximal, distal, and ascending tubules, in which most of the nephrons participate. These tubular changes are associated with an alteration of perfusion circumscribed to two areas of the inner renal medulla.

METHODS

This study was performed according to institutional animal care and use guidelines. The two groups of dogs studied were an experimental (group A, n = 7, body wt 17–21 kg) and a control (group B, n = 5, body wt 17–20 kg) group.

Animals in group A were anesthetized (pentobarbital sodium, 30 mg/kg iv), intubated, and ventilated (using a Harvard respirator) with room air. One femoral vein was cannulated, and a polyethylene PE-200 catheter was placed for a constant infusion of insulin throughout the experiment (a priming bolus of 20 ml followed by a constant infusion of 1 ml/min for the remainder of the experiment). To maintain anesthesia, pentobarbital sodium (1.5 mg/ml) was included in the insulin infusion. The other femoral vein was cannulated

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for constant infusion of 0.9% saline (1–2 ml/min) throughout the experiment. A femoral artery was cannulated, and a PE-240 catheter was advanced to the level of the renal artery, for continuous monitoring of RPP with a pressure transducer (Statham model P23D; Gould, Hato Rey, PR). For injection of contrast media, a Rodriguez catheter (no. 8 French) was advanced under fluoroscopy via the common carotid artery to the midthoracic descending aorta. Each dog was then placed in dorsal suspension following exposure of the left kidney and its renal artery by a ventrocostal flank incision and placement of a Transonic flow probe around the renal artery for continuous recording of RBF. Both the superior mesenteric and the celiac arteries were ligated to increase blood flow and perfusion pressure to the renal territories. Two plastic vascular clamps were placed on the aorta, one above and the other below the level of the left renal artery, for manipulation of RPP. The corresponding ureter was catheterized with a PE-200 catheter for collection of urine.

Animals in group B were prepared similarly, except for cannulation of the urinary bladder instead of the ureter, and no flow probe was placed around the renal artery.

Following surgical preparation, each animal was transferred and positioned in the EBCT (model C-100; Imatron, South San Francisco, CA) scanning gantry. After a 1-h recovery period, EBCT scans were performed. These were performed in five of the dogs of group A at the upper limit, in two dogs of group A at the lower limit of RPP autoregulation (to avoid bias due to differences in directional changes of RPP), and in group B at the basal RPP level.

Iopamidol (Isovue-370; Squibb Diagnostics, Princeton, NJ) was used as the contrast medium. This is a nonionic, low-osmolar, intravascular contrast medium (mol wt 777). Like most low-osmolar urographic contrast media, it is an inert monomer derivative of triiodinated benzoic acid (16). It is cleared primarily (over 95%) by glomerular filtration, with volume (ml) yields RBF (ml/min) and therefore eliminated the need for laparotomy in group B, which is otherwise necessary for the use of electromagnetic flow measurements of RBF. In group B, on-line measurement of RBF was necessary to define the limits of autoregulation.

Following completion of the studies, each dog was killed with Sleepaway (Fort Dodge Laboratories, Fort Dodge, Iowa). Urinary volume obtained in each of the 10-min control periods was measured in a graduated cylinder, and urinary osmolarity was measured using a Micro Osmometer (Precision Systems). Urinary sodium was measured using a flame photometer.

Data Analysis

The images were reconstructed, and regions of interest were selected from the cross-sectional images as follows. The cortex was defined during the vascular phase as the highly opacified zone at the circumference of the kidney and was arbitrarily further divided into outer and inner cortex. The outer medulla was defined as the anatomical area clearly outlined immediately following the vascular phase, as a ring of contrast that was moving toward the inner medulla. The inner medulla was subdivided in two regions; this was performed due to the known inherent heterogeneity of medullary blood flow, which may vary as a function of distance from the corticomedullary junction (28). Subdivision to only two large regions of interest (rather than a larger number of smaller regions) enables the attainment of a high signal-to-noise ratio and thereby accurate measurements of flow. Two inner medullary regions were anatomically selected as follows: the “deep inner medulla” was identified between adjacent calyces (Fig. 1). The region of interest immediately above the crest, but below the outer medulla, was defined as “outlying inner medulla.”

The computer then generated for each region of interest distinctive time-density curves, describing the change in tissue density consequent to transit of contrast in that region. Perfusion. Perfusion rate, as milliliters of blood per cubic centimeter of tissue per minute, was calculated from the first peak of the time-density curve obtained in each region of interest, representing the passage of contrast through the vascular compartment, using the algorithm (11)

\[
\text{Perfusion} = \left(\frac{h_{\text{peak}}}{A_{0}}\right) \times 60
\]

where \(h_{\text{peak}}\) is the peak height of the tissue curve, and \(A_{0}\) is the area under the aortic curve.

Renal volume. Renal volume (cm\(^3\)) was calculated from each tomographic level by manually tracing the renal con-
RESULTS

As shown in Table 1, a change of $-26.7 \pm 3.1\%$ ($-34.6$ mmHg) in RPP was followed by an efficient hemodynamic autoregulatory response, with no significant changes in either GFR or RBF. As predictable for pressure-induced antinatriuresis, this decrement in RPP was associated with a statistically significant reduction in both urinary flow rate ($-44\%$, $P = 0.043$) and $U_{Na}V$ ($-53.1\%$, $P = 0.025$) (Table 1). This change was not observed in group B, in which RPP remained unaltered ($118.0 \pm 4.6$ vs. $119.4 \pm 6.4$ mmHg, respectively; $P = 0.67$) as did urinary flow rate ($P = 0.1$) and RBF ($P = 0.68$).

In group A, whole kidney perfusion, as well as perfusion of the cortex and outer medulla, did not show significant changes during the modification of RPP (Fig. 3). Furthermore, no redistribution of blood flow was observed between the outer and inner cortex at high [4.98 ± 0.45 and 4.24 ± 0.37 ml·min⁻¹·(cm³ tissue)⁻¹, respectively] vs. low [4.83 ± 0.44 and 4.19 ± 0.39 ml·min⁻¹·(cm³ tissue)⁻¹, respectively] RPP ($P > 0.05$). Although average perfusion of the total inner medulla did not show a significant change ($P = 0.12$), the two components of the inner medulla did show different responses to changes in RPP. The outlying inner medullary perfusion exhibited a significant increase ($+19.1\%$, $P = 0.017$), whereas perfusion in the deep inner medulla decreased significantly ($-36.5\%$, $P = 0.027$) (Fig. 3). This decrease in papillary perfusion was significantly correlated with the decrease in concurrent $U_{Na}V$ (Fig. 4). In the control group, comparison between the two scans revealed no significant changes, either in global or regional perfusion (Table 2).
Determination of changes in intratubular concentration of contrast media (relative to input function) in group A revealed a statistically significant concentration of contrast in the proximal tubule (an increase of 39.3 ± 11.9%, P = 0.009), ascending limb of the loop of Henle (+68.0 ± 26.2%, P = 0.024), and the distal tubule (+32.1 ± 10.8%, P = 0.013), whereas no significant change was observed at the level of the bend of the loop (Fig. 4). However, this reduction in RPP induced a decrease in transit time in the descending limb of Henle's loop (−20.7 ± 6.9%, P = 0.038). Once again, no significant change was observed when this parameter was measured at the level of the bend of the loop (Fig. 6).

Therefore, in the nephron segments where transit times were prolonged (proximal, ascending, and distal tubules), the contrast medium was proportionally concentrated, whereas in the segment where transit time was shorter at low compared with high RPP (descending limb), contrast medium was found to be less concentrated.

In the control group (group B), no statistically significant changes were observed in either transit times or intratubular contrast concentration (Table 3).

**DISCUSSION**

This study demonstrates that a change in RPP leads to changes in volume reabsorption in the proximal tubule, ascending limb, and distal tubule in the majority of the nephrons of all the kidneys sampled. These alterations occurred in the presence of hemodynamic changes circumscribed to the inner medulla.

The animals of the experimental group experienced a very efficient hemodynamic autoregulation. A change in aortic pressure from 139 to 95 mmHg was attended by no significant change in either RBF or GFR, whereas urine sodium excretion fell by 44%. In the control group, on the other hand, no significant changes were observed in any of the parameters studied. These findings allow us to describe, for the first time, the concurrent intrarenal hemodynamic and tubular changes that are detectable with fast CT within the range of autoregulation.

We succeeded at our goal of developing a method capable of estimating parameters of tubular function (14). We previously demonstrated the capability of the fast CT technique to study the relationship between blood flow distribution and tubular solute handling (14). This technique is based on the fact that radiographic contrast medium (like inulin) is freely filtered by the glomeruli, and its transit time through the tubules can be externally recorded and followed as it appeared and then disappeared from the region of interest. Furthermore, since contrast media are neither reabsorbed nor secreted in the tubules, a change in X-ray density (relative contrast concentration) results from changes in tubular fluid reabsorption within a nephron segment, and vice versa.

We measured tubular transit times (s) and relative contrast (solute) concentration (proportional to aortic

Table 1. Renal hemodynamics and urinary excretion measured in group A (n = 7) at the higher vs. lower limits of autoregulation

<table>
<thead>
<tr>
<th></th>
<th>RPP, mmHg</th>
<th>GFR, ml/min</th>
<th>RBF, ml/min</th>
<th>UV, ml/min</th>
<th>UNaV, meq/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher limit</td>
<td>130.00 ± 5.67</td>
<td>45.66 ± 6.48</td>
<td>177.17 ± 15.20</td>
<td>2.24 ± 0.34</td>
<td>0.256 ± 0.066</td>
</tr>
<tr>
<td>Lower limit</td>
<td>95.43 ± 5.83</td>
<td>42.29 ± 6.49</td>
<td>163.07 ± 20.74</td>
<td>1.00 ± 0.22*</td>
<td>0.120 ± 0.036*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P<0.05 compared with high RPP.

In group A, EBCT-derived measurements of transit times revealed definite and different patterns between the two levels of RPP. A decrease in RPP was associated with a prolongation in transit times through the proximal tubule (an increase of 39.3 ± 22.8%, P = 0.066), ascending limb of the loop of Henle (±68.0 ± 26.2%, P = 0.047), and the distal tubule (±41.8 ± 18.8%, P = 0.047) (Fig. 5), whereas contrast medium was found to be less concentrated. Furthermore, since contrast media are neither reabsorbed nor secreted in the tubules, a change in X-ray density (relative contrast concentration) results from changes in tubular fluid reabsorption within a nephron segment, and vice versa.

Table 2. Regional renal perfusion as measured with EBCT in group B (n = 5)

<table>
<thead>
<tr>
<th>Renal Region</th>
<th>Cortex</th>
<th>Outer Medulla</th>
<th>Outlying Inner Medulla</th>
<th>Deep Inner Medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Scan</td>
<td>6.16 ± 0.9</td>
<td>2.81 ± 0.5</td>
<td>0.97 ± 0.2</td>
<td>1.09 ± 0.3</td>
</tr>
<tr>
<td>2nd Scan</td>
<td>6.22 ± 0.5</td>
<td>2.79 ± 0.2</td>
<td>0.81 ± 0.1</td>
<td>1.20 ± 0.3</td>
</tr>
</tbody>
</table>

All values are regional renal perfusion in ml·min⁻¹·(cm³ tissue)⁻¹. EBCT, electron beam computed tomography.
blood), analogous to fluid/plasma ratio. Since our density measurements represented an average across the region of interest, they represent whole populations of different nephron segments contained in these regions. Indeed, our measurements of proximal tubular transit times in the control group (21.8 ± 2.5 s) were highly comparable to those previously measured in normal dogs using Lissamine green, i.e., 26 ± 5.7 s (17) and 21.6 ± 1.3 s (26). Our Henle's loop basal passage time (33.3 ± 4.1 s) was also very similar to previously reported values (26) obtained with Lissamine green (32.3 ± 1.1 s), although our distal tubular transit time (71.5 ± 16 s) was a little longer than the 56 ± 12 s previously reported in the dog (17).

Our results revealed that a reduction in RPP was accompanied by prolongation of transit times in the proximal tubule, ascending limb of Henle's loop, and distal tubule. Since these changes were associated with an increase in relative contrast concentration in the same tubules, it is reasonable to conclude that tubular fluid was being reabsorbed in these segments compared with the condition prevailing during high RPP.

It should be mentioned here that, in the dog, Navar et al. (17) have failed to observe any significant change of sodium reabsorption in proximal tubules of superficial nephrons when RPP was decreased within the range of autoregulation. However, no information was obtained in that study regarding changes of proximal reabsorption in deep nephrons. In the rat, on the other hand, Haas et al. (6) found that changes in proximal tubule sodium reabsorption during pressure natriuresis occurred only in deep nephrons, whereas sodium delivery to distal tubules in superficial nephrons remained unchanged. It is quite possible that a pattern similar to that described for the rat exists in the dog as well. Our results would therefore reflect the integrative nature of our CT methodology, in which measurements of tubular dynamics are derived from a large population of nephrons, rather than being based on those observed in single nephron, like the micropuncture technique.

Our results also showed that, when RPP was decreased, there was an acceleration in the transit of contrast media in the ascending limb of Henle's loop, associated with a lower intratubular contrast concentration. This implies that between its transit in the proximal and descending tubules, the contrast was undergoing less concentration than it did during high RPP. The physiological significance of this phenomenon remains unclear.

Micropuncture studies have demonstrated that changes in RPP induce changes in sodium excretion in the proximal tubule of superficial and deep nephrons, as well as in the thin ascending limb of Henle's loop (6, 20). Sodium reabsorption in the outer medullary thick ascending limb of the loop of Henle is highly load dependent and can prevent changes in sodium excretion when proximal reabsorption is inhibited (7, 9). However, many investigators have observed that chlo-
ride reabsorption in the loop was not changed when proximal delivery was increased by elevations of RPP (3, 10, 17, 20). This means that the major contribution of the thick ascending limb to pressure natriuresis is not confined to altering the increase sodium load that is being delivered from the proximal tubules (19). Consistent with this demonstration, we found that following the decrease in RPP, there was a 70–80% increase of fluid reabsorption in the region of interest corresponding to the thick ascending limb. These observations allow, for the first time, to extend the observations made by micropuncture techniques to the whole nephron segment population.

Our EBCT technique also enabled studying concurrent renal hemodynamics. The hemodynamic changes observed in this study resemble those reported by our group (13) in studies performed under similar experimental conditions but using another fast CT scanner, the dynamic spatial reconstructon. In that study (13), we found that when RPP was decreased, cortical and outer medullary blood flow exerted an efficient hemodynamic autoregulation, whereas papillary (deep inner medulla) blood flow decreased significantly. In the present study we have separately sampled an additional region of interest, which was the more peripheral (outlying) zone of the inner medulla (Fig. 1). We found that, as reported before, changes in RPP were associated with similar directional changes in deep inner medullary blood flow. Opposite directional changes of blood flow, however, were systematically recorded in the outlying inner medullary perfusion.

The reasons for these reciprocal changes of flow in the inner medulla are not known. Nonetheless, they were reproducible from animal to animal and always occurred in a reciprocal fashion whether RPP was initially increased (5 dogs) or decreased (2 dogs). In brief, this observation emphasizes the fact that significant changes in tubular sodium reabsorption are associated with constant and reproducible changes and localized to a relatively small area of the inner renal medulla.

Several techniques, mainly radioactive microspheres and more recently laser-Doppler flowmetry, have attempted to study the hemodynamic renal response to alterations in RPP. However, radioactive microspheres have a preferential regional distribution within the renal cortex, and do not reach the medulla, therefore providing unreliable and incomplete blood flow measurements of these regions (28). Moreover, it is conceivable that the papillary region could be perfused by vessels regulated independently from those that provide circulation to the more outlying inner medulla or that the increase in outlying inner medullary perfusion observed with a decrease in RPP is at expenses of blood flow to the deep inner medulla and vice versa. These alterations may be difficult to detect using laser-Doppler techniques, since it requires one to position the laser detector in a very specific area of the renal medulla.

On the other hand, it is quite possible that placement of the laser-Doppler probe in different anatomical regions of the inner medulla may explain the contradictory results obtained by different investigators. Using laser-Doppler flowmetry, some investigators (21) hypothesized that changes in medullary circulation play a central role in regulating sodium excretion by producing parallel alterations in renal interstitial pressure. In a dog model, Majid et al. (15) could not detect changes in medullary circulation during changes in RPP within the range of autoregulation, whereas Strick et al. (27) found good medullary autoregulation only on the upper part of the autoregulatory curve. From our study it is difficult to determine whether the observed changes in papillary flow were sufficiently significant to alter renal interstitial pressure and/or release renal autacoids. However, this is conceivable since there was a significant positive correlation between $\text{U}_{\text{Na}}$ and percent change in papillary (deep inner medullary) flow. Furthermore, the slope of this correlation (Fig. 4) indicates an amplification of this relationship, so for any increase in papillary perfusion there was more than a twofold increase in sodium excretion. This may suggest a causal relationship between the two. However, our experimental protocol and resolution do not allow us to determine whether this is indeed the case or whether the observed concomitant change in medullary flow is just an epiphenomenon or the consequence of changes in the tubular pressure.

In conclusion, we found that a decrease in RPP was associated with hemodynamic changes in inner medullary perfusion, as well as with tubular functional changes (increase in volume and probably sodium reabsorption). Most of the nephron population sampled played a role in the regulation of sodium excretion, but the specific mechanisms underlying this regulation remain unclear. EBCT provides a unique opportunity to study hemodynamics and tubular function in an integrated and noninvasive fashion.

## Table 3. Renal tubular dynamics (transit times and relative contrast concentration) as measured with EBCT in group B (n = 5)

<table>
<thead>
<tr>
<th>Tubular Segment</th>
<th>Proximal Tubule</th>
<th>Descending Limb</th>
<th>Henle's Loop</th>
<th>Ascending Limb</th>
<th>Distal Tubule</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transit time, s</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Scan</td>
<td>21.8 ± 2.5</td>
<td>25.9 ± 2.4</td>
<td>33.3 ± 4.1</td>
<td>23.9 ± 4.3</td>
<td>71.5 ± 1.6</td>
</tr>
<tr>
<td>2nd Scan</td>
<td>21.6 ± 3.2</td>
<td>35.3 ± 8.0</td>
<td>36.5 ± 1.3</td>
<td>17.5 ± 3.5</td>
<td>68.5 ± 2.5</td>
</tr>
<tr>
<td>Relative contrast conc. %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st Scan</td>
<td>0.72 ± 0.06</td>
<td>1.24 ± 0.13</td>
<td>2.26 ± 0.29</td>
<td>1.28 ± 0.29</td>
<td>2.31 ± 0.12</td>
</tr>
<tr>
<td>2nd Scan</td>
<td>0.90 ± 0.22</td>
<td>2.02 ± 0.59</td>
<td>2.58 ± 0.39</td>
<td>0.83 ± 0.23</td>
<td>1.91 ± 0.53</td>
</tr>
</tbody>
</table>

Values are means ± SE.
REFERENCES


