Hydraulic and oncotic pressure measurements in inner medulla of mammalian kidney

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Departments of Chemical Engineering and Medicine, Stanford University, Stanford 94305, Kidney Research Laboratory, Veterans Administration Hospital, San Francisco 94121, and Departments of Medicine and Physiology and Cardiovascular Research Institute, University of California, San Francisco, California 94143

Sanjana, Veeram M., Paul A. Johnston, William M. Deen, Channing R. Robertson, Barry M. Brenner, and Rex L. Jamison. Hydraulic and oncotic pressure measurements in inner medulla of mammalian kidney. Am. J. Physiol. 228(6): 1921-1926. 1975.—The vasa recta are thought to play an important role in the transfer of water and solutes within the renal medulla. Hydraulic pressures were measured in vasa recta on the surface of the exposed papilla in young Munich Wistar rats, and blood was collected from these microvessels for determination of total protein concentration and calculation of colloid oncotic pressure. In descending vasa recta at the base of the exposed papilla, mean hydraulic pressure was 9.2 ± 0.4 mmHg and plasma protein concentration averaged 7.1 ± 0.4 g/100 ml. Corresponding values in ascending vasa recta at the same level were 7.8 ± 0.4 mmHg and 5.6 ± 0.3 g/100 ml, respectively. The protein concentrations correspond to calculated oncotic pressures of 26 and 18 mmHg in descending and ascending vasa recta, respectively. We interpret these findings as evidence for oncotic pressure differences.

Methods

Young Munich-Wistar rats of either sex ranging in weight from 70 to 120 g, were prepared for micropuncture of the left renal papilla as described previously (10). The Munich Wistar rats were found to possess relatively long extrarenal papillae, averaging 2 mm in length, approximately one-third of the total lengths of the inner medullae (measured after the experiment). Throughout the experiment the animals were infused with normal saline at a rate of 0.015 ml/min.

In initial experiments (9 rats) the papillae were bathed in isotonic saline throughout the experiment. In later experiments (15 rats) mineral oil was used as the bathing medium when tubule fluid and vasa recta blood samples were being collected, after which the oil was replaced by isotonic saline for hydraulic pressure measurements. The use of saline as a bathing medium is mandatory for hydraulic pressure measurements using the servo-nulling technique (4).

Samples of blood were withdrawn from descending and ascending vasa recta, usually as close to the base of the exposed papilla as possible. For descriptive purposes, the “base” of the papilla means the portion of the exposed papilla near the kidney and opposite to the papillary tip. Siliconized micropipettes were used, with tips varying between 10 and 15 μm in external diameter. Oil was ini-
ially injected into the vessel to determine the exact location of the pipette tip. After slight initial suction the flow of blood into the pipette was spontaneous with a continuous stream of red blood cells moving into the pipette, thereby confirming the proper location of the pipette tip during collection of the sample. Blood was collected from as many ascending and descending vessels as possible before hydraulic pressure measurements were made. Hydraulic pressures were determined with the servo-nulling apparatus using pipettes with a tip size of \( \leq 4 \mu m \) and filled with 1.0 M NaCl. Accuracy, frequency response, and stability features of the servo-null device have been described in detail elsewhere (1). The hydraulic pressures in vasa recta, loops of Henle, and collecting tubules were measured randomly and recorded on a Hewlett-Packard recorder (Sanborn 7702 A). All pressures in the vasa recta were measured near the base of the papilla. Those in the collecting ducts were divided into three groups—those measured at the base, at the tip, and in the middle of the papilla. Loops of Henle were punctured randomly over the papillary surface. The location of the pipette with respect to the papillary tip was noted. That the varying composition and osmolality of the fluid in different structures within the renal inner medulla had no effect on the accuracy of the servo-nulling technique is demonstrated by the data shown in Fig. 1. Experiments comparing the pressure measured by the servo-nulling technique with that obtained using a Statham P22AA transducer in a test chamber containing fluid at two different osmolalities and compositions, measured near the base of the papilla, those in the collecting ducts were separated into three groups according to the osmolality of the collecting duct fluid which ranged between 475 and 1,875 mosmol/kg H_2O.

**RESULTS**

The mean arterial blood pressure was 104 mmHg ± 4 SE in 23 rats. The rats were nondiuretic throughout the experiment, as indicated by the osmolality of the collecting duct fluid, which ranged between 475 and 1,875 mosmol/kg H_2O.

**Hydraulic pressure measurements.** Hydraulic pressure measurements in the inner medulla are shown in Table 1. Note the small difference between the mean hydraulic pressure in descending and ascending vasa recta. There is, however, a decline in the direction expected, and the difference between descending and ascending vasa recta is statistically significant (\( P < 0.025 \)). Hydraulic pressures in the loops of Henle were similar to those in the vasa recta. Pressure measurements in the collecting duct were separated into three groups according to the site of measurement along the length of the papilla. Tip pressures were virtually atmospheric, but there was a substantial pressure drop along the length of the collecting tubule from base to tip of papilla of about 5 mmHg.

A comparison of hydraulic pressures in nephron segments in the papilla and on the surface of the kidney is shown in Fig. 2. Although the tubule segments belong to two separate nephron populations, superficial and juxtamedullary, it is interesting to note that hydraulic pressures tended to decline from proximal to more distal nephron segments, as one would have expected in a single population.

**TABLE 1. Hydraulic pressures in renal papilla**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Pressure, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Descending vasa recta</td>
<td>9.2 ± 0.4 (37)</td>
</tr>
<tr>
<td>Ascending vasa recta</td>
<td>7.8 ± 0.4 (32)</td>
</tr>
<tr>
<td>Loop of Henle</td>
<td>8.1 ± 0.4 (24)</td>
</tr>
<tr>
<td>Collecting duct—base</td>
<td>5.9 ± 0.3 (17)</td>
</tr>
<tr>
<td>Collecting duct—mid</td>
<td>4.1 ± 0.4 (17)</td>
</tr>
<tr>
<td>Collecting duct—tip</td>
<td>0.9 ± 0.3 (18)</td>
</tr>
</tbody>
</table>

All values are means ± SE. Numbers in parentheses denote total number of measurements.
of nephrons. As expected, mean superficial distal tubule hydraulic pressure was higher than mean hydraulic pressure in the most proximal collecting duct in the papilla. However, the decrease in hydraulic pressure along the inaccessible portion of the collecting ducts, between the cortical surface and the exposed papilla, was smaller than the pressure drop along the collecting ducts in the exposed papilla. The hydraulic pressures in the superficial distal tubule are closely bounded by those of the long loops of Henle and the earliest accessible collecting ducts; hence the hydraulic pressure in the juxtamedullary distal tubule should be similar to that in the superficial distal tubule.

**Protein concentration measurements.** The mean concentration of protein in vasa recta plasma samples is shown in Table 2, along with the concentration of protein in femoral artery plasma. The mean protein concentration in vasa recta plasma was much higher than that in systemic plasma. Furthermore, the protein concentration of plasma in the descending vasa recta was greater than that of plasma in the ascending vasa recta by an average of 1.5 g/100 ml.

Figure 3 illustrates paired measurements of protein concentration in ascending and descending vasa recta in 17 rats in which samples were obtained. Vasa recta protein concentrations were normalized with respect to systemic plasma protein concentration. In each of the 17 rats, plasma in descending vessels had a mean concentration of protein higher than or essentially equal to that in plasma in ascending vessels, and in the majority of cases the difference was considerable. These differences were apparent irrespective of whether the papillae were bathed with isotonic saline (dashed lines) or mineral oil (solid lines).

The oncostic pressure of each sample was calculated from the protein concentration using the equation

$$\pi = (1.52 + 0.35 m_s)c + (0.234 + 0.009/m_s)c^2$$

with $m_s = 0.35$ M, where $\pi$ = oncostic pressure (mmHg), $c$ = protein concentration (g/100 ml), and $m_s$ = saline molarity. This equation was empirically obtained from experiments designed to study specifically the effect of elevated saline molarities on the oncostic pressure-protein concentration relationship (unpublished data). There is a small difference between the calculation of oncostic pressure using the Landis-Pappenheimer equation (17), the latter having been empirically fitted for solutions in physiological saline (0.15 M). For instance, for an assumed total protein concentration of 7 g/100 ml (with an albumin:globulin ratio of unity), the Landis-Pappenheimer equation overestimates the value of $\pi$ by 1.2 mmHg, compared with the relation given in equation 1. As is evident from inspection

### Table 2. Protein concentrations and oncostic pressures in vasa recta

<table>
<thead>
<tr>
<th>Structure</th>
<th>Protein Concn g/100 ml</th>
<th>Conc. Ratio, VR/P</th>
<th>Oncostic Pressure, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Descending vasa recta</td>
<td>7.1±0.4</td>
<td>1.76±0.10</td>
<td>26.0±2.3</td>
</tr>
<tr>
<td>Ascending vasa recta</td>
<td>3.6±0.3*</td>
<td>1.38±0.06*</td>
<td>18.1±1.4*</td>
</tr>
<tr>
<td>Femoral artery</td>
<td>4.1±0.3</td>
<td></td>
<td>14.9±0.7</td>
</tr>
</tbody>
</table>

All values are means ± SE and are based on 17 rats. Where more than one ascending or descending vasa recta was sampled in a single rat, the mean protein concentration was used. * Difference between value for the descending vasa recta and that for the ascending vasa recta is statistically significant, P < 0.001.

| Vasectomy to femoral artery (systemic) plasma protein concentration ratio. |
of Tables 1 and 2, oncotic pressures of vasa recta plasma substantially exceed hydraulic pressures everywhere along the vasa recta.

**DISCUSSION**

The small difference in hydraulic pressures between descending and ascending vasa recta is difficult to reconcile with previous estimates of the axial hydraulic pressure drop along the vasa recta. Thurau and others (25), using the Landis technique, estimated a decrease in pressure of 6 mmHg/mm length along the vasa recta in golden hamsters. However, measurements of hydraulic pressures using the Landis technique and the servo-nulling method have been found to yield different results in the hands of the same investigators (13). In any case, no evaluation of the accuracy of the method used by Thurau et al. is possible because it was not described in their abstract and a detailed description was not subsequently published. While no accurate estimate can be made of the pressure drop from a theoretical standpoint because of the complex two-phase flow of erythrocytes and plasma, an approximate calculation indicates that the measured decrease in hydraulic pressure of 1.5 mmHg would be more than sufficient to account for fluid velocities similar to those that exist in vivo (see APPENDIX).

The drop in collecting duct hydraulic pressure is much greater than that in the vasa recta. This could be the result of either a greater average fluid velocity in the collecting duct, a considerably greater fluid viscosity, or changes in geometry of the collecting tubule system.

The values for the concentrations of protein in the vasa recta plasma agree with the range of values noted previously. Gottschalk et al. (7) found vasa recta plasma/systemic plasma ratios of protein concentration to average 1.6 in nondiuretic hamsters. No attempt was made to distinguish between values in descending and ascending vessels.

The concentration of plasma protein in the vascular system can change as a consequence of two processes: net transmural water flux or net transmural protein flux. Thus the consistent decline in protein concentration from descending to ascending vessels could be the result either of net water entry or of net protein loss from the capillaries. To our knowledge no unequivocal evidence for the existence of a lymphatic system in the inner medulla has been reported. Kriz and Dieterich (15), in their electron and light microscopic examination of the ultrastructure of the inner medulla, failed to observe any structures resembling lymphatics. In the absence of an extravascular route, the difference in protein concentration of plasma in descending and ascending vasa recta observed in the present study is taken as evidence for fluid uptake by the vascular system in the inner medulla.

The mechanism by which net fluid reabsorption occurs in capillaries in the inner medulla has not previously been examined. Fluid movement across a membrane can be described, according to the principles of linear nonequilibrium thermodynamics, by the following equation (12):

$$J_i = L_i (A_i - \sum \sigma_i \Delta \pi_i)$$

(2)

where

- $J_i$ = transmural water flux in volume/time per unit surface area
- $L_i$ = membrane hydraulic permeability
- $\sigma_i$ = solute reflection coefficient of the membrane to solute $i$
- $\Delta p_i$ = transmural hydraulic pressure difference
- $\Delta \pi_i$ = transmural osmotic pressure difference due to solute $i$

Thus water movement occurs in response to a net imbalance between transmural hydraulic pressure driving forces and opposing effective osmotic pressure driving forces. The degree to which the osmotic pressure of a given solute is effective in producing a volume flux is determined by its reflection coefficient, which normally varies from zero to unity.

Consider the “small” solutes in the inner medulla (solutes other than proteins, in this case principally NaCl and urea). For tubule epithelium in the inner medulla, osmotic gradients appear to be the principal driving forces for water movement, far exceeding hydraulic pressure differences. This may not be the case, however, for capillary endothelia. If the permeability characteristics of the endothelia of vasa recta are similar to those of extrarenal capillary endothelia, then they have very low reflection coefficients for small solutes. The driving force for water movement exerted by small solutes is outward (lumen to interstitium) as long as solute concentration of the interstitium exceeds that of the vasa recta plasma. As the descending vasa recta penetrate the inner medulla into regions of increasing interstitial concentrations of solutes, this condition prevails. In the limit of extremely rapid solute diffusion, the transcapillary concentration gradient for small solutes is reduced to zero, thereby eliminating the osmotic driving force for water provided by small solutes. To the extent that such diffusion equilibrium is not achieved, an outward driving force for water due to small solutes will persist, not only along the entire descending vasa recta, but also along initial segments, however brief, of ascending vasa recta. At some point in the ascending vessels, the transcapillary concentration gradient for small solutes will be reversed, as the hyperosmotic plasma courses up through a progressively less concentrated interstitium. At that point the force for water movement due to small solutes is now directed into the ascending capillaries.

Therefore, to the extent that equilibrium between the concentrations of small solutes in vasa recta and in the interstitium is not achieved, the forces favoring outward water movement will exceed those for reabsorption. It follows that unless the endothelium of descending vasa recta is less permeable to water than that of ascending vasa recta, there can be no “net” influx of water along the entire capillary network in the inner medulla due to small solute driving forces alone. Net water reabsorption by the vascular system therefore appears to be governed by the same combination of transcapillary forces that influence transcapillary fluid movement in the renal cortex, namely hydraulic and oncotic pressure differences (5). Small solutes, however, may affect fluid movement in the descending and
ascending vasa recta separately. In the descending vasa recta the osmotic pressure exerted by small solutes would oppose capillary oncotic pressure while in the ascending vasa recta the two forces would be additive. Because the concentration of protein in vasa recta plasma is low compared to that of small solutes, the former has heretofore been ignored in a consideration of driving forces influencing water movement across vasa recta (2, 8). Nonetheless, the plasma proteins appear to be the only solutes for which the capillary membrane has a reflection coefficient significantly greater than zero.

Transcapillary hydraulic pressure differences could not be measured directly because it was impossible to measure the hydraulic pressure of the interstitium. Hydraulic pressure in loops of Henle, however, did not differ from vasa recta values by more than 1 mmHg (Table 1). The mean oncotic pressure in the descending vasa recta was 26.0 mmHg and declined to a mean of 18.1 mmHg in the ascending vasa recta. Thus, unless interstitial colloid oncotic pressure greatly exceeds interstitial hydraulic pressure, the transcapillary oncotic pressure difference is greater than the opposing transcapillary hydraulic pressure difference. If the interstitial spaces were at atmospheric pressure (assuming the absence of noncommunicating interstitial spaces within the renal papilla, it appears unlikely that subatmospheric (negative) pressures exist in the interstitium), then even if the interstitial oncotic pressure were as high as 12 mmHg, a net inward driving force for water reabsorption by the vasa recta would still result.

A more difficult question is whether under normal physiological conditions or otherwise, uptake of water from tubular segments in the inner medulla is limited by driving forces across the vascular endothelium. If vascular mem- brane processes are rate limiting, then the interstitium and fluid within the tubule lumen will be close to osmotic equilibrium. Conversely, if tubule epithelial processes are rate limiting, then the interstitium will equilibrate osmotically with the vascular system. Until this question is resolved it is unjustified to assume that the interstitium and vasa recta represent a single fluid “compartment” (14, 22).

In summary, these experiments demonstrate net fluid uptake by the vasa recta in the renal papilla. The net inward driving force for fluid reabsorption can be explained as the result of the transcapillary oncotic pressure difference exceeding the opposing hydraulic pressure difference.

APPENDIX

Prigson’s equation is used to calculate the pressure drop for a given set of flow parameters. Thus

\[ \Delta P = \frac{32 \mu L V}{D^2} \]

where \( \Delta P \) = pressure drop, dyn/cm²
\( \mu \) = viscosity, P
\( L \) = length, cm
\( V \) = velocity, cm/s
\( D \) = diam, cm

The viscosity of blood has been found to vary with flow rate, hemato- crit, and size of vessel. In particular there is a dramatic decrease in viscosity in very small vessels and an independent decrease with the decreasing hematocrit (6). Estimating the viscosity to be 0.025 P, the diameter to be 15 μ, the average length between points of measurement to be about 2 mm, and the measured \( \Delta P \) of 1.5 mmHg, the computed velocity is 0.3 mm/s. Whereas no accurate measure of the velocity of flow has been made, visually this velocity appears to be reasonable (unpublished observations). This value also agrees with those reported by Marsh and Segel (19).

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REFERENCES


