Determinants of glomerular filtration rate in the dog

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Ott, Cobern E., Gary R. Marchand, Jose A. Diaz-Buxo, and Franklyn G. Knox. Determinants of glomerular filtration rate in the dog. Am. J. Physiol. 231(1): 235-239. 1976. - Micropuncture techniques were used to evaluate the determinants of glomerular filtration in hydropenic dogs. Stop-flow and servo-null techniques were used to estimate the glomerular capillary and proximal tubule hydrostatic pressures. The validity of stop-flow estimates was verified by comparisons with direct puncture of glomerular capillaries in Munich-Wistar rats. Efferent arteriolar oncotic pressure was calculated from the filtration fraction and systemic protein concentrations. This calculation was verified in separate experiments by measurement of the protein concentration in blood collected directly from efferent arterioles. In 14 dogs, estimated glomerular capillary pressure (GCP) averaged 65.8 ± 2.9 mmHg and proximal tubule pressure (PT) averaged 20.5 ± 1.3 mmHg. The net hydrostatic filtration pressure (GCP - PT) of 45.3 ± 2.7 mmHg was significantly higher than the efferent arteriolar oncotic pressure (πEA) of 33.2 ± 2.8 mmHg (P < .001). These findings indicate that filtration dynamics in the dog are characterized by filtration pressure disequilibrium.

Glomerular capillary; glomerular capillary filtration coefficient; effective filtration pressure; filtration pressure disequilibrium; glomerular capillary pressure

Glomerular dynamics in the rat have been characterized by equality of the hydrostatic pressure favoring filtration and the oncotic pressure opposing filtration at the efferent end of the glomerular capillary (3). This phenomenon, termed filtration pressure equilibrium, results in a marked influence of plasma flow on filtration rate. Indeed, renal vasodilatation by plasma volume expansion in the rat results in proportional increases in both renal plasma flow and single nephron filtration (4, 7). However, this relationship has not been found in the dog. Vasodilatation by vasodilator drugs or plasma volume expansion with hyperoncotic albumin markedly increases renal plasma flow but results in only small increases in glomerular filtration rate (9, 14, 15). Three explanations are possible. First, it has been proposed that at least one vasodilator, acetylcholine, decreases the glomerular capillary filtration coefficient (5). The effect of increased plasma flow would be offset by a decreased glomerular capillary filtration coefficient with no change in filtration rate. A second possibility is that efferent arteriole resistance disproportionately decreases. A decreased efferent resistance could result in increased plasma flow because of decreased total renal resistance and a decreased glomerular capillary pressure. The net results would again be no change in glomerular filtration rate. Finally, the dog may not be characterized by filtration pressure equilibrium. If the dog is in filtration pressure disequilibrium, large increases in plasma flow would have only small effects on filtration rate (9).

This latter possibility was examined by defining the determinants of glomerular filtration rate in the dog. By the use of micropuncture techniques, measurements were made of glomerular capillary and proximal tubule hydrostatic pressures and efferent arteriolar protein concentration in hydropenic dogs.

METHODS

Studies were performed on mongrel dogs of either sex weighing 16-28 kg. The animals were anesthetized with 30 mg/kg sodium pentobarbital and prepared for micropuncture as previously described (13). They were allowed free access to water, and food was withheld for 12 h before the experiment. After a 45-min equilibrium period, three 15-min clearance periods were begun and micropuncture measurements were obtained. Blood samples were collected at the midpoint of 15 min urine collections. Plasma and urine concentrations of inulin were determined by the anthrone method, and PAH by the method of Harvey and Brothers (8).

Since the dog does not possess surface glomeruli which allow for direct measurement of glomerular capillary pressure, estimates were obtained by the stop-flow method. To validate the stop-flow method, a comparison of this method with the direct-puncture method was performed in five hydropenic Munich-Wistar rats, which possess surface glomeruli. Studies were performed on rats weighing 181-223 g. Animals were allowed free access to water and food was withhold for 12 h prior to the experiment. The animals were anesthetized with 50 mg/kg sodium pentobarbital and a tracheotomy was performed. Catheters were placed in the jugular veins for infusion of saline and withdrawal of blood. The carotid artery was catheterized for blood pressure monitoring. The animals were given an initial injection of 0.5 ml saline and a continuous infusion of 0.02 ml/min for the duration of the experiment. The left kidney was isolated through a left flank incision and gently freed of surrounding tissue. The kidney was placed in a Lucite holder and bathed with warm saline solution. Body temperature was monitored and maintained with a

1 Breeding stock kindly provided by Dr. B. M. Brenner, San Francisco, Calif.
thermally regulated micropuncture table. Pipettes were sharpened to 1–3 μm OD and filled with 1.0 M NaCl containing 5% lissamine green. Direct measurements of glomerular capillary pressures were obtained with the servo-null device from 1 to 4 surface glomeruli per animal. Glomerular capillary pressures were obtained from 1 to 4 separate tubules in the same kidney using stop-flow techniques. A small droplet of mineral oil stained with Sudan black was injected into an early-proximal tubule and allowed to flow in order to determine the direction of tubular flow. The tubule was filled with oil and stop-flow pressure measured with the servo-null device at least two tubule convolutions proximal to the site of oil injection. As an additional safeguard, the pipette that was used to measure pressures was filled with 5% lissamine green in hypertonic saline solution. Any tubule seen to leak lissamine green around the puncture site was discarded. Glomerular capillary pressure (GCP) was estimated from stop-flow pressure (SFP) and the oncotic pressure determined from the protein concentration in simultaneously obtained systemic plasma (Pv), GCP = SFP + PT. Glomerular capillary pressure (GCP) was estimated from stop-flow pressure (SFP) and the oncotic pressure determined from the protein concentration. The criteria used to prevent this contamination have been published in detail elsewhere (10). Protein concentration in systemic plasma and in efferent arteriolar plasma was determined in duplicate with an ultramicrocolorimeter (10). Oncotic pressure (π) was calculated from the empiric relationship between protein concentration (C) and oncotic pressure: π = 2πC + .16C² + .008C³ (11). Protein concentration in directly collected efferent arteriolar plasma averaged 8.4 ± 0.3 g/100 ml (Table 2). This value was not significantly different from the value 8.4 ± 0.4 g/100 ml obtained by using whole-kidney filtration fraction and systemic protein concentration: Δ = 0.0 ± 0.3 g/100 ml. These results agree with a previous study from a smaller group of animals in which no difference was found between directly and indirectly determined efferent arteriolar protein concentrations (10). Similarly, the calculated efferent oncotic pressures were not significantly different: 34.8 ± 2.3 mmHg vs. 34.3 ± 3.0 mmHg, A = 0.5 ± 1.9 mmHg.

Statistical analysis was performed with the Student t-test for paired data within groups and for unpaired data between groups. Statistical significance was considered to be P < .05.

RESULTS

Table 3 shows the renal hemodynamic and single-nephron data for the determinants of glomerular filtration rate. None of the whole-kidney or single-nephron data in this group are significantly different from the group of animals (Table 2) in which efferent protein concentrations were directly determined. Free-flow proximal tubule pressure averaged 20.5 ± 0.1 mmHg. Stop-flow pressure averaged 45.6 ± 2.3 mmHg and calculated glomerular capillary pressure averaged 66.8 ± 2.9 mmHg. The net hydrostatic pressure favoring filtration (GCP – PT) averaged 45.3 ± 2.7 mmHg. This value was significantly greater than either the indirectly determined efferent oncotic pressure of 33.3 ± 2.8 mmHg or the directly determined value of 34.8 ± 2.3 mmHg. The indirectly determined value for efferent protein concentration of 8.1 ± 0.4 g/100 ml was not different from the directly determined value of 8.4 ± 0.3 g/100 ml. Although there was variability in systemic protein concentration, filtration fraction, and hence efferent protein concentration, on the average the net hydrostatic pressure favoring filtration at the efferent end of the
TABLE 2. Comparison of efferent arteriole protein concentrations determined directly and indirectly

| Dog No. | BP, mmHg | GFR, ml/min | RBF, ml/min | Hct, % | RPF, ml/min | FF | SP, g/100 ml | EA, mmHg | EAP, g/100 ml | EA+, mmHg | EAP*, mmHg | EA*, mmHg | N or Samples |
|---------|----------|-------------|-------------|--------|-------------|----|-------------|---------|-------------|---------|-------------|---------|------------|--------|
| 1       | 127      | 33.1        | 302         | 49     | 154         | 22 | 6.8         | 24.6    | 7.9         | 8.7     | 31.0        | 36.3    | 4          |
| 2       | 120      | 26.3        | 194         | 49     | 99          | 26 | 5.3         | 21.3    | 6.8         | 10.7    | 33.6        | 56.7    | 3          |
| 3       | 140      | 27.0        | 171         | 48     | 87          | 31 | 9.2         | 20.1    | 6.7         | 24.0    | 29.7        | 27.9    | 2          |
| 4       | 118      | 23.6        | 206         | 50     | 103         | 23 | 8.3         | 33.6    | 10.3        | 10.6    | 48.0        | 52.7    | 3          |
| 5       | 140      | 15.0        | 267         | 57     | 115         | 13 | 6.9         | 25.1    | 7.9         | 7.9     | 31.0        | 31.0    | 4          |
| 6       | 129      | 26.3        | 197         | 47     | 116         | 23 | 5.1         | 16.1    | 6.7         | 7.4     | 32.9        | 28.5    | 1          |
| 7       | 156      | 66.1        | 650         | 50     | 338         | 20 | 5.9         | 19.8    | 8.6         | 7.4     | 35.6        | 27.9    | 3          |
| 8       | 136      | 35.7        | 276         | 49     | 141         | 25 | 4.9         | 15.2    | 9.0         | 6.5     | 38.4        | 22.9    | 1          |
| 9       | 137      | 22.5        | 125         | 46     | 68          | 33 | 6.3         | 21.8    | 7.7         | 9.4     | 28.9        | 41.4    | 4          |
| 10      | 126      | 40.5        | 298         | 44     | 167         | 24 | 8.8         | 24.5    | 8.6         | 8.9     | 35.6        | 37.7    | 3          |
| 11      | 113      | 26.3        | 319         | 37     | 201         | 26 | 6.8         | 24.5    | 8.9         | 9.2     | 37.0        | 39.9    | 3          |
| 12      | 152      | 15.0        | 160         | 44     | 95          | 33 | 6.2         | 21.3    | 8.8         | 9.3     | 37.0        | 31.0    | 2          |
| 13      | 132      | 13.0        | 306         | 34     | 202         | 16 | 4.5         | 13.5    | 6.1         | 5.4     | 20.8        | 17.4    | 2          |

Mean 128 34.1 268 45 148 .24 6.3 22.3 8.4 8.4 34.8 34.3

See footnote to Table 1 for abbreviations. * EAP and EA determined from FF and SP.

TABLE 3. Determinants of glomerular filtration in the dog

| Dog No. | BP, mmHg | GFR, ml/min | RBF, ml/min | Hct, % | RPF, ml/min | FF | SP, g/100 ml | EA, mmHg | EAP, g/100 ml | EA+, mmHg | EAP*, mmHg | EA*, mmHg | N or Samples |
|---------|----------|-------------|-------------|--------|-------------|----|-------------|---------|-------------|---------|-------------|---------|------------|--------|
| 14      | 130      | 31.0        | 239         | 46     | 129         | 22 | 6.8         | 24.6    | 7.9         | 8.7     | 31.0        | 36.3    | 4          |
| 15      | 131      | 34.1        | 268         | 45     | 148         | 24 | 6.3         | 22.3    | 8.4         | 8.4     | 34.8        | 34.3    | 3          |

Mean 130 34.1 268 45 148 .24 6.3 22.3 8.4 8.4 34.8 34.3

See footnote to Table 1 for abbreviations. * RPF determined from PAH clearance and extraction. † EAP and EA determined from FF and SP.

DISCUSSION

The present studies were designed to investigate the determinants of glomerular filtration rate in the dog. Although there was variability in efferent oncotic pressure whether determined directly or indirectly, the net hydrostatic pressure favoring filtration was significantly greater than the oncotic pressure opposing filtration at the efferent end of the glomerular capillary. This was true whether the net hydrostatic pressure was compared to the efferent oncotic pressure indirectly measured in the same kidney or to the efferent oncotic pressure directly measured in different kidneys. This finding indicates that, under normal conditions, the dynamics of glomerular filtration in the dog are characterized by filtration-pressure disequilibrium. However, consideration of the methods for determining the respective pressures is necessary for evaluating the results. Proximal tubule pressures can be measured by direct puncture of the tubule with the servo-null device. Afferent and efferent oncotic pressure can be determined directly from the protein concentration of systemic and efferent arteriolar plasma, respectively. Since the dog kidney does not possess surface glomeruli to allow for direct puncture of glomerular capillaries, measurements of glomerular capillary pressure were indirectly determined from single-nephron stop-flow pressures and afferent oncotic pressure.

A comparison of 13 direct punctures and 12 stop-flow measurements in five rats indicated that the two techniques agree reasonably well. However, as emphasized by Lassiter (12), both techniques have limitations. The stop-flow technique is indirect and could potentially underestimate or overestimate the true glomerular capillary pressure. If the oil block used to stop filtration is not sufficiently close to the glomerulus, a small amount of continued filtration and subsequent reabsorption would occur. The measured glomerular capillary pressure would then be an underestimation of true glomerular capillary pressure. However, consideration of the methods for determining the respective pressures is necessary for evaluating the results. Proximal tubule pressures can be measured by direct puncture of the tubule with the servo-null device. Afferent and efferent oncotic pressure can be determined directly from the protein concentration of systemic and efferent arteriolar plasma, respectively. Since the dog kidney does not possess surface glomeruli to allow for direct puncture of glomerular capillaries, measurements of glomerular capillary pressure were indirectly determined from single-nephron stop-flow pressures and afferent oncotic pressure.
An application of the stop-flow technique to the dog showed that the net hydrostatic pressure favoring filtration at the efferent end of the glomerular capillary was 12.1 mmHg higher than the oncotic pressure opposing filtration. Thus, the present study presents evidence that the dynamics of glomerular filtration in the dog are characterized by filtration pressure disequilibrium.

As a consequence of this finding, some pertinent characteristics of the dog glomerulus can be uniquely determined. A mass-balance model of ours (9) and a sophisticated computer-based model of Deen et al. (6) indicate that the oncotic pressure profile along the length of the glomerular capillary is essentially linear from afferent to efferent arteriole when efferent oncotic pressure is less than 80% of the effective hydrostatic filtration pressure. Because of this linearity, a reasonable estimation of the effective glomerular capillary oncotic pressure (πGC) can be determined from afferent oncotic pressure (πA) and efferent oncotic pressure (πEA):

\[ πGC = \frac{(πA + πEA)}{2} \]

\[ πGC = \frac{(20.2 + 33.2)}{2} = 26.7 \text{ mmHg} \]

The ratio of single-nephron filtration rate (SNGFR) to EFP yields the glomerular capillary filtration coefficient (Kf). In a previous study, SNGFR in 33 animals averaged 59.2 ± 3.4 nl/min (13). In six additional dogs in this study, SNGFR averaged 57.7 ± 5.4 nl/min. Using the data from the much larger series:

\[ Kf = \frac{(59.2 \text{ nl/min})}{18.6 \text{ mmHg}} = 3.2 \text{ nl/(min·mmHg)} \]

For comparison, the filtration coefficient determined in rats averaged 4.8 nl/(min·mmHg) and the calculated net effective filtration pressure averaged from 4.3 to 5.8 mmHg (7). An important additional consequence of filtration pressure disequilibrium in the dog is that increased renal plasma flow per se would have little effect on glomerular filtration rate. The present studies can thus explain the independence of glomerular filtration rate and renal plasma flow found in the dog.

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4. The very-near linearity of the oncotic pressure profile in this range is the result of two offsetting, nonlinear functions. As flow proceeds down the glomerular capillary, the rate of protein concentration due to filtration decreases. However, because of the nonlinearity of the protein concentration-oncotic pressure relationship, the increase in oncotic pressure per unit increase in protein concentration is increasing. The result is offsetting, so that the oncotic pressure profile is almost exactly linear.