Autoregulation of Single Nephron Filtration Rate in the Presence and the Absence of Flow to the Macula Densa

By Franklyn G. Knox, Cobern Ott, Jean-Louis Cuche, Josianne Gasser, and John Haas

ABSTRACT
Flow of tubule fluid to the macula densa is part of a proposed feedback loop for autoregulation of glomerular filtration rate. In the present study, autoregulation of single nephron filtration rate was tested in the presence and the absence of flow to the macula densa in the rat. Filtration rates were measured at elevated arterial blood pressures caused by carotid occlusion and vagal section and at reduced renal perfusion pressures caused by partial aortic constriction. Measurements of single nephron filtration rate in the presence and the absence of flow to the macula densa were obtained by complete volume collections from the distal nephron beyond the macula densa and from the proximal tubule, respectively. Mean blood pressure was 130 ± 4 (SE) mm Hg for the initial collections, and renal perfusion pressure was 100 ± 1 mm Hg for the repeat collections (nine rats). Distal single nephron filtration rate was 42 ± 1 nliters/min at elevated perfusion pressure and 41 ± 1 nliters/min at reduced perfusion pressure; proximal single nephron filtration rate was 41 ± 1 nliters/min at elevated perfusion pressure and 41 ± 1 nliters/min at reduced perfusion pressure. Similarly, glomerular filtration rate was 4.6 ± 0.4 ml/min kg⁻¹ body weight and 4.7 ± 0.3 ml/min kg⁻¹ body weight at elevated and reduced perfusion pressures, respectively. Additional studies in seven dogs showed good correlation between autoregulation of single nephrons in the absence of flow to the macula densa and autoregulation of the micropunctured kidney. It is concluded that autoregulation of single nephron filtration rate is unaltered by interruption of tubule fluid flow to the macula densa.

KEY WORDS
proximal tubule perfusion pressure glomerular filtration in rats and dogs micropuncture distal tubule juxtaglomerular feedback

Although it has been well established that renal blood flow and glomerular filtration rate are autoregulated over a wide range of renal perfusion pressures, the mechanism of this response has not been clearly established. One proposed mechanism for the autoregulation of glomerular filtration rate involves the flow of tubule fluid constituents to the macula densa region of the distal nephron (1-5). According to this thesis, a decrease in the delivery of tubule fluid constituents results in an increase in the glomerular filtration rate. Although many studies have attempted to evaluate feedback mechanisms for regulation of glomerular filtration rate (6), autoregulation of single nephrons has not been specifically tested. Thus, previous studies in rats, in which evidence for a macula densa feedback mechanism has not been found, have not critically tested this hypothesis for autoregulation of glomerular filtration rate (7-9). A recent report of experiments in dogs with autoregulating kidneys indicates that collections from proximal tubules result in measurements of single nephron filtration rates higher than those determined from collections from distal tubules; these higher rates are consistent with the proposed feedback mechanism for regulation of glomerular filtration rate (10).

In the present experiments, the macula densa feedback hypothesis was tested at the single nephron level in rat kidneys that demonstrated good autoregulation for the renal microcirculation and the kidney as a whole. Since interruption of tubule fluid flow to the macula densa did not alter autoregulation of single nephron filtration rate in the rat, additional studies were performed in dogs. As in the rat, interruption of tubule fluid flow to the macula densa in the dog did not interfere with autoregulation of single nephron filtration rate.

Methods
Four experimental protocols were used. In group 1, the autoregulation of single nephron filtration rate was tested in the presence and the absence of flow to the macula densa in the rat (Fig. 1). Measurements of single...
nephron filtration rate in the presence of flow to the macula densa were obtained by complete volume collections from the distal nephron beyond the macula densa. To evaluate the independent variables of tubule flow rate and the ratio of tubule fluid inulin concentration to plasma inulin concentration, these collections were made from both early distal tubule segments and from late distal tubule segments. Measurements of single nephron filtration rate in the absence of flow to the macula densa were obtained by complete volume collections from the proximal tubule. (In the absence of antegrade flow in the loop of Henle, a small retrograde flow of tubule fluid might be established at the macula densa.) Filtration rates from these three tubule sites and from the kidney as a whole were obtained at elevated perfusion pressures caused by bilateral carotid occlusion and bilateral vasa recta occlusion and at reduced renal perfusion pressures caused by partial aortic constriction. In group 2, as a measure of autoregulation in the superficial microcirculation of the dog, hydrostatic pressures in peritubule capillaries were measured in the presence of both elevated perfusion pressure and reduced renal perfusion pressure following a protocol identical to that used in group 1 experiments. In group 3, autoregulation of single nephron filtration rate was tested in the absence of flow to the macula densa in the dog. Measurements of single nephron filtration rate in the absence of flow to the macula densa were obtained by complete volume collections from the proximal tubule. Single nephron filtration rates and whole kidney filtration rates were obtained at elevated perfusion pressure caused by occlusion of one carotid artery and, if necessary, partial occlusion of the remaining carotid artery, and at reduced renal perfusion pressure caused by partial aortic constriction. In group 4, to test for autoregulation in the superficial microcirculation of the dog kidney, hydrostatic pressures in peritubule capillaries were measured at both elevated and reduced perfusion pressures following a protocol identical to that used in group 3 experiments.

Studies were performed on 14 Sprague-Dawley rats weighing 150-250 g. Rats were allowed free access to water, and food was removed 24 hours prior to the experiment. Rats were anesthetized with sodium pentobarbital (60 mg/kg, iv). Catheters were inserted into the jugular vein for infusions and the femoral and carotid arteries for withdrawal of blood and for blood pressure monitoring. A tracheotomy was performed. A small left subcostal incision was made, and the left kidney was placed in a holder. The left ureter was catheterized with PE10 tubing near the renal pelvis, and urine from the right kidney was collected via a bladder catheter. Body temperature was monitored and maintained with a thermal-regulated micropuncture table. The kidney surface was illuminated with a fiberoptic light source, and the kidney was bathed with mineral oil for tubule fluid collections and with isotonic saline solution for hydrostatic pressure measurements. Rats were primed with 0.5 ml of 10% inulin in saline solution; plasma inulin concentration was maintained with a constant inulin infusion rate of 0.02 ml/min. Two late proximal tubule segments, two early distal tubule segments, and two late distal tubule segments were identified with six injections of 0.005 ml of lissamine green dye (pH adjusted to 7.4) 1 hour before the first micropuncture samples were obtained. Tubules were punctured with sharpened pipettes, and tubule fluid samples were collected at spontaneous tubule pressure. Great care was taken to maintain an injected oil column in a constant position and to avoid leakage of tubule fluid (9, 11). Samples were recollected from the previously punctured segments following reduction in renal perfusion pressure.

Eighteen dogs were fed a standard pellet diet that provided approximately 30 mg/kg sodium/day; they were allowed free access to water, and food was withheld on the day of the experiment. The dogs were anesthetized with sodium pentobarbital (60 mg/kg, iv), and a tracheotomy was performed. Cannulas were placed in jugular veins for infusions, in a femoral vein and a left renal vein (through the ovarian or testicular vein) for blood sampling, and in the carotid and femoral arteries for blood pressure recordings. Dogs were prepared for micropuncture, and surface segments of proximal tubules were micropunctured using the recollection micropuncture technique as it has been previously described (12).

Hydrostatic pressures in tubules and peritubule capillaries (5 μ in diameter) of both rats and dogs were measured with a servonull device as previously described for use in rats by Falchuk and Berliner (13) and for use in dogs by Knox et al. (14). In both rats and dogs, the volume of the tubule fluid samples was measured with a micropipette that had been calibrated with a radioactive tracer. The single nephron filtration rate was calculated from the expression \( V_f = V_a \times TF/P_a \), where \( V_a \) is the volume collected per minute and \( TF/P_a \) is the ratio of tubule fluid inulin concentration to plasma inulin concentration. Inulin concentration in tubule fluid samples was determined in duplicate by the microfluorometric method (15). Blood samples for clearance determinations were collected at the midpoint of 15-minute urine collections. Inulin concentration in plasma and urine of rats and dogs was measured by the anthrone method (16). Renal blood flow in dogs was calculated from the clearance, the extraction of para-aminomuhippuric acid (PAH), and the hematocrit; it was also measured with an electromagnetic flowmeter.

The data for each variable were averaged for statistical comparison in each rat and dog. Student's t-test for
paired and unpaired comparisons was used for statistical analysis.

**Results**

The results of group 1 experiments in which autoregulation of single nephron filtration rate was tested in the presence and the absence of flow to the macula densa in the rat are shown in Table 1 and summarized in Figure 2. Reduction in perfusion pressure to the kidney from 130 ± 4 mm Hg to 100 ± 1 mm Hg did not change single nephron or whole kidney glomerular filtration rates. Single nephron filtration rate measured in proximal tubules from nine rats averaged 40.9 ± 0.9 nliters/min before and 40.5 ± 0.9 nliters/min after reduction in perfusion pressure. Thus, in the absence of flow to the macula densa, there was good autoregulation of single nephron filtration rate. Single nephron filtration rate measured from early and late distal tubule segments was not significantly different from that measured from proximal tubule collections. There were no significant changes in single nephron filtration rate collected from either the proximal or the distal tubules following reduction in renal perfusion pressure.

The results of micropressure measurements at elevated and reduced perfusion pressures for five rats are shown in Table 2. Reduction in perfusion pressure to the kidney from 135 ± 10 mm Hg to 101 ± 3 mm Hg did not cause significant changes in...
pressures measured in proximal tubules, distal tubules, or capillaries. Similarly, glomerular filtration rate was not significantly changed; the mean difference was $-0.36 \pm 0.56$ ml/min/kg$^{-1}$ body weight.

The effects of reduction in renal perfusion pressure in the dog from 142 ± 2.8 mm Hg to 103 ± 0.6 mm Hg on renal hemodynamics are summarized in Table 3. Following the 28% reduction in renal perfusion pressure, renal blood flow measured with an electromagnetic flowmeter decreased 10%. The changes in renal blood flow measured with the electromagnetic flowmeter were closely paralleled by the changes in renal blood flow measured by the clearance and extraction of PAH and by glomerular filtration rate (Table 3). Since the autoregulatory response of the dog kidney was somewhat more variable than that observed in the rat kidney, the single nephron and whole kidney glomerular filtration rates at elevated and reduced perfusion pressures are detailed for each experiment in Table 4. In dog 1 there was good autoregulation of both single nephron filtration rate and glomerular filtration rate following reduction in perfusion pressure. In dog 2 there were modest decreases in both single nephron filtration rate and glomerular filtration rate. In dog 3 there were modest increases in both single nephron filtration rate and glomerular filtration rate following reduction in perfusion pressure. In dog 4 there were marked reductions in single nephron filtration rate and glomerular filtration rate, indicating very poor autoregulation in this experiment. In dog 5 there was good autoregulation of both single nephron filtration rate and glomerular filtration rate. In dog 6 there were modest reductions in both single nephron filtration rate and glomerular filtration rate following reduction in perfusion pressure. Finally, dog 7 showed somewhat greater reductions in both single nephron filtration rate and glomerular filtration rate.

The effects of changes in renal perfusion pressure on peritubule capillary hydrostatic pressures in the dog are shown in Figure 4. There were no significant changes in peritubule capillary pressures following reduction in renal perfusion pressure. Again, the responses from dog to dog varied, but the results generally support autoregulation of the microcirculation of the superficial cortex.

### TABLE 2

Microvascular Pressures at Elevated and Reduced Perfusion Pressure in Five Rats

<table>
<thead>
<tr>
<th></th>
<th>Elevated pressure</th>
<th>Reduced pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PP (mm Hg)</td>
<td>Prox (mm Hg)</td>
</tr>
<tr>
<td>MEAN</td>
<td>135 ± 10</td>
<td>93.8 ± 1.6</td>
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<tr>
<td></td>
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</tbody>
</table>

PP = perfusion pressure to the kidney. Prox = proximal tubules, Dist = distal tubules. Cap = peritubule capillaries. GFR = glomerular filtration rate. Hydrostatic pressures in proximal tubules, distal tubules, and peritubule capillaries were measured with a servonull device.

* Following carotid occlusion and vagal section.
† Following partial aortic constriction.
Renal Hemodynamics at Elevated and Reduced Perfusion Pressures in the Dog

<table>
<thead>
<tr>
<th>Perfusion pressure (mm Hg)</th>
<th>GFR (ml/min g⁻¹)</th>
<th>RPF* (ml/min g⁻¹)</th>
<th>FF</th>
<th>Hematocrit</th>
<th>From RPF and hematocrit</th>
<th>From electromagnetic flowmeter recordings</th>
</tr>
</thead>
<tbody>
<tr>
<td>142 ± 2.8</td>
<td>0.64 ± 0.05</td>
<td>2.2 ± 0.3</td>
<td>0.29 ± 0.01</td>
<td>0.50 ± 0.02</td>
<td>4.4 ± 0.6</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>103 ± 6</td>
<td>0.54 ± 0.07</td>
<td>1.8 ± 0.3</td>
<td>0.30 ± 0.01</td>
<td>0.51 ± 0.02</td>
<td>3.8 ± 0.6</td>
<td>3.7 ± 0.5</td>
</tr>
</tbody>
</table>

Values are expressed per gram kidney weight and represent means ± SE for seven dogs. GFR = glomerular filtration rate, RPF = renal plasma flow, FF = filtration fraction, and RBF = renal blood flow.

* Renal plasma flow was calculated from clearance and extraction of PAH.

Table 4

<table>
<thead>
<tr>
<th>Dog</th>
<th>No. of tubules</th>
<th>Elevated pressure</th>
<th>Reduced pressure</th>
<th>GFR (ml/min g⁻¹)</th>
<th>Snfr (n liters/min/kg wt)</th>
<th>Mean ± SE</th>
<th>% Δ GFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7</td>
<td>152</td>
<td>102</td>
<td>0.69</td>
<td>0.69</td>
<td>±2.8</td>
<td>0.05</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>131</td>
<td>103</td>
<td>0.84</td>
<td>0.77</td>
<td>±11</td>
<td>0.05</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>139</td>
<td>100</td>
<td>0.43</td>
<td>0.53</td>
<td>±11</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>145</td>
<td>103</td>
<td>0.51</td>
<td>0.25</td>
<td>±11</td>
<td>0.05</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>147</td>
<td>104</td>
<td>0.67</td>
<td>0.62</td>
<td>±11</td>
<td>0.05</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>147</td>
<td>105</td>
<td>0.71</td>
<td>0.50</td>
<td>±11</td>
<td>0.05</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>135</td>
<td>102</td>
<td>0.62</td>
<td>0.40</td>
<td>±11</td>
<td>0.05</td>
</tr>
<tr>
<td>Mean</td>
<td>142</td>
<td>88</td>
<td>103</td>
<td>0.64</td>
<td>0.54</td>
<td>±11</td>
<td>0.05</td>
</tr>
</tbody>
</table>

See legend to Table 1 for definitions.

Discussion

The macula densa feedback theory which was tested in the present study states that a decrease in the flow of tubule fluid to the macula densa region of the distal nephron results in an increase in the glomerular filtration rate. Two consequences of interruption of flow to the macula densa can be predicted from this thesis. First, collections from proximal tubules will result in higher single nephron filtration rates than do collections from distal tubules. Second, although distal tubules should show good autoregulation of single nephron filtration rate, proximal tubules should show no autoregulatory response. Neither of these predictions was found in the present study. Single nephron filtration rates determined from collections from distal tubules were not significantly different from those determined from proximal tubules in rats.

Correlation between changes in single nephron filtration rate and whole kidney filtration rate following reduction in perfusion pressure in dogs.

Autoregulation of peritubule capillary pressures in the renal microcirculation of the dog for changes in renal perfusion pressures.

This observation was true for collections at both elevated perfusion pressures and reduced perfusion pressures. Also, autoregulation of single nephrons...
The autoregulation of nephron filtration rate was observed for collections from both proximal tubules and distal tubules.

Leakage from distal tubule micropuncture sites has been documented by Bartoli and Earley (9) as a major potential pitfall and a cause for underestimation of single nephron filtration rates determined from collections from distal tubules. Since undetected leakage of tubule fluid affects the measurement of tubule fluid flow rate but probably not the ratio of tubule fluid inulin concentration to plasma inulin concentration, punctures were made at both early and late distal tubule sites with significantly different flow rates. Values for single nephron filtration rates from early distal tubule micropuncture sites with relatively high tubule fluid flow rates and relatively low ratios of tubule fluid inulin concentration to plasma inulin concentration were not different from values obtained from late distal tubule micropuncture sites with relatively low flow rates and relatively higher ratios of tubule fluid inulin concentration to plasma inulin concentration. This finding coupled with the finding of similar values for glomerular filtration rate from proximal tubules and distal tubules in the rat.

The present study extends previous observations by demonstrating autoregulation of single nephrons in rat kidneys in which good autoregulatory responses were demonstrated for the microcirculation and for the kidney as a whole. The demonstration of autoregulation by the micropunctured kidneys was facilitated by increasing the perfusion pressure to the kidneys by activation of the carotid baroreceptors. Although this maneuver facilitated the demonstration of the autoregulatory response, these experiments were conducted against a background of increased sympathetic tone. The role of increased sympathetic tone on the macula densa feedback hypothesis has not been specifically studied; however, conditions associated with increased renin activity might be expected to facilitate the detection of a feedback mechanism. Since interruption of tubule fluid flow to the macula densa did not interfere with autoregulation of single nephron filtration rate in rats, additional studies were performed in dogs.

In those dogs in which there was good autoregulation of glomerular filtration rate of the micropunctured kidney, there was also good autoregulation of single nephron filtration rate. In some dogs there was relatively poor autoregulation of single nephron filtration rate; however, in the same experiments glomerular filtration rate for the kidney as a whole did not show good autoregulation. As shown in Figure 3, changes in single nephron filtration rate measured in the absence of flow to the macula densa correlated well with changes in filtration rate of the kidney as a whole but did not correlate with changes in perfusion pressure.

Results from microperfusion experiments in which flow to the macula densa was increased are conflicting. In separate studies, increased perfusion rate to the macula densa has been reported to decrease filtration rate (17) and stop flow pressures (taken as representative of glomerular capillary pressures) (18). In contrast, Morgan (7) found no effect of increased perfusion to the macula densa on proximal flow rate; Blantz et al. (8) found no effect of proximal blockade on glomerular capillary pressures directly measured by micropuncture and a servonull pressure-measuring system. Although the present study does not rule out a feedback system which responds to increased flow to the macula densa, it provides strong evidence that autoregulation of single nephron filtration rate is unaltered by interruption of tubule fluid flow to the macula densa.

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References


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