Effect of Endothelin-1 on Glomerular Hydraulic Pressure and Renin Release in Dogs

Huabao Lin, Mariem Sangmal, Manis J. Smith Jr., and David B. Young

The present study was designed to analyze quantitatively the effects of a wide range of endothelin-1 levels on renal hemodynamics and renin release in the canine nonfiltering kidney, including their effects on glomerular hydraulic pressure. Intrarenal infusion of endothelin-1 produced dose-dependent reductions in renal blood flow, but it did not affect glomerular hydraulic pressure until the infused dose reached high rates. At the rate of 1.0 ng/kg per minute, endothelin-1 reduced renal blood flow by 23% (p < 0.01), whereas glomerular hydraulic pressure was not significantly changed from 68.1 ±1.3 to 67.4 ±1.2 mm Hg. However, with a higher rate of endothelin-1 infusion (5.0 and 10.0 ng/kg per minute), glomerular hydraulic pressure fell to 59.5 ±1.3 and 51.5 ±1.8 mm Hg (p < 0.01), whereas renal blood flow was reduced from 154.5 ±15 to 83.0 ±9.5 and 53.5 ±9.9 mL/min, respectively. Endothelin-1 infusion also produced an inhibitory effect on renin release. With infusion at 1.0 ng/kg per minute, renin release fell from the control level of 47.9 ±5.6 to 26.6 ±4.9 units/min per gram kidney weight (p < 0.01), and it fell further to 16.1 ±4.3 units/min per gram kidney weight with infusion at 10.0 ng/kg per minute. In summary, endothelin-1 infusion did not affect glomerular hydraulic pressure despite a fall in renal blood flow at low doses, but at high doses it reduced both, suggesting that endothelin-1 exerts separate, dose-dependent effects on preglomerular and postglomerular resistances. In addition, the present study demonstrated that endothelin-1 infusion has an ability to inhibit renin release in vivo when the macula densa-mediated pathway is eliminated.

KEY WORDS • kidney • glomerular filtration rate • renal circulation • endothelins • renin

Endothelin-1 (ET-1) has been found in renal glomerular endothelial cells and other kidney cells, and it has specific binding sites in the glomeruli; therefore, the possibility has been raised that ET-1 may play some functional role in the regulation of renal hemodynamics. In vivo, ET-1 has been reported to have potent renal vasoconstrictor effects, acting on both renal blood flow (RBF) and glomerular filtration rate (GFR) in dogs, rats, and rabbits. Some studies have reported that in low doses the effects of ET-1 on RBF were much greater than those on GFR. In the studies by King et al and Miura et al, ET-1 reduced RBF by as much as 20% and did not significantly alter GFR. These findings suggested that ET-1 had a predominant effect on the efferent arterioles, so that the glomerular capillary hydraulic pressure could be elevated or maintained despite the fall in RBF. King and Brenner also found in rats that the transglomerular capillary pressure gradient was greater in the ET-1 infusion group than that in the control group. However, the direct effect of ET-1 on regulation of glomerular hydraulic pressure (GHP) over the complete dose range has not been fully analyzed.

In addition to its potent renal vasoconstriction, ET-1 may have effects on the renin-angiotension system. Although many in vitro studies have found that ET-1 exerts an inhibitory effect on renin release, its role in control of renin release remains controversial in light of its in vivo effects. Some investigators found that ET-1 increased plasma renin activity, but others reported that a relatively low dose of ET-1 significantly reduced it. Such discrepant effects on the renin-angiotension system are probably due to the complexity of the nature of controlling renin release in vivo. It is most likely that the several control mechanisms known to play important roles in the regulation of renin release are affected by administration of ET-1, such as the macula densa-mediated pathway, the intrarenal baroreceptor, and possibly a direct effect of ET-1 on the juxtaglomerular cells. Furthermore, each control mechanism may have a different threshold for the activation by ET-1, so that the net renin secretory response in vivo varies markedly with the ET-1 doses.

Therefore, the present study was designed to analyze quantitatively the effects of a wide range of ET-1 levels on the regulation of renal GHP using a stop-flow technique to determine the effects on preglomerular and postglomerular vascular resistances. In addition, we analyzed the effect of ET-1 on renin release in an acute nonfiltering kidney model so that the effects resulting from the macula densa mechanism could be isolated.

Methods

Experiments were performed on mongrel dogs of either sex obtained from the research animal facilities of...
the University of Mississippi Medical Center (body weight, 19.6±0.6 kg; n=10). Animals were housed in the animal facilities before use and fed a standard laboratory diet. The food was removed from their cages 15 hours before surgery, but the dogs were given free access to water. The animals were initially sedated with 10 mg i.m. acepromazine maleate. Ten minutes later, they were anesthetized with approximately 30 mg/kg i.v. sodium pentobarbital.

**Surgical Procedure and Experimental Measurements**

After anesthesia, a respiratory pump was used to permit artificial ventilation through a trachea tube as needed to maintain normal blood gas values. Both femoral arteries and one femoral vein were cannulated with Tygon (Norton, Akron, Ohio) catheters for measurement of arterial blood pressure above and below the renal artery, sampling of arterial blood, and intravenous infusion. Blood pressure was determined by a pressure transducer (Cobe, Lakewood, Colo.) placed at the same level as the dog's heart and connected to a polygraph (Grass Instrument Co., Quincy, Mass.).

Via a left retroperitoneal flank incision, a portion of the aorta above the left renal artery was gently isolated so that a silicone rubber cuff occluder could be placed around the aorta. The occluder was connected to a servo-control device that was used to maintain arterial pressure below the occluder (i.e., renal arterial pressure) at a constant level. The left renal artery was also isolated, and an electromagnetic flow probe was placed around the renal artery. An electromagnetic flowmeter (model FM-501, Carolina Medical Electronics, Inc., King, N.C.) was used to measure RBF. The left ureter was cannulated with a PE-90 catheter for measurement of urinary pressure by a Cobe pressure transducer. A 22-gauge L-shaped needle attached to a catheter was inserted into the renal vein for sampling of renal venous blood. Finally, a 23-gauge L-shaped needle was also inserted into the left renal artery for an intrarenal infusion.

Blood gas measurement was made with a pH/blood gas analyzer (model 1304, Instrumentation Laboratories, Lexington, Mass.) to adjust the rate of the respirator.

To determine glomerular capillary hydraulic pressure, we used a stop-flow pressure method. In this method, the left kidney was acutely rendered nonfiltering by techniques described previously.14-16 The technique included two steps: first, an osmotic diuresis was established, and second, the ureter was occluded to elevate its pressure until the filtration pressure (PF) was zero. Filtration pressure was determined from the equation

\[ PF = GHP - (POSM + PT) \]

where POSM is plasma colloid osmotic pressure, and PT is proximal tubular hydrostatic pressure. If PT and POSM are increased to a point at which

\[ GHP = POSM + PT \]

then the PF will be equal to zero, and filtration will not occur.

The plasma colloid osmotic pressure was calculated by the method of Navar and Navar17:

\[ POSM = 1.4C + 0.22C^2 + 0.005C^3 \]

where C is plasma protein concentration measured by a refractometer (AO Reichert Scientific Instruments, Buffalo, N.Y.). PT is assumed equal to the stopped-flow urinary pressure (UP) in the present study.18 Therefore, the glomerular hydraulic capillary pressure was determined by the formula

\[ GHP = POSM + UP \]

After completion of surgery, a dose of 300 mL 6.0% mannitol solution was intravenously infused over 10 minutes, followed by a sustaining infusion of 2.0 mL/min. After diuresis had occurred for approximately 10 minutes, the ureteral catheter was clamped and ureteral pressure was measured by a pressure transducer. The pressure reached a plateau in approximately 10 minutes. Absence of filtration was confirmed by measurement of arterial and renal venous

\[ [^{125}I]iothalamate activities. \]

The preglomerular vascular resistance (RVR<sub>pre</sub>) and postglomerular vascular resistance (RVR<sub>post</sub>) were calculated by the equations

\[ RVR_{pre} = \frac{(RAP - GHP)}{RBF} \]

and

\[ RVR_{post} = \frac{GHP}{RBF} \]

where RAP is renal arterial pressure. Resistance is expressed as millimeters of mercury per milliliter per minute.

An index of renin release was determined from the difference in plasma renin activity between the renal venous and arterial plasma and the renal plasma flow. Blood samples for plasma renin activity measurements were collected in iced sodium EDTA tubes and cold-centrifuged for more than 30 minutes. Plasma (1 mL) was used for the assay using the radioimmunoassay procedure of Haber et al.19 The renin release index (RR) from the left kidney was calculated from the product of the venous minus arterial plasma renin activity (PRA) difference and the renal plasma flow (RPF) divided by the kidney weight. It can be expressed as

\[ RR = \frac{(PRA_v - PRA_a) \times RPF}{g \text{ kidney weight}} \]

where PRA<sub>v</sub> is renin activity in the renal venous sample, and PRA<sub>a</sub> is renin activity in the renal arterial sample. One unit of renin release was taken to be equal to 1 ng angiotensin I/mL per hour, and the rate of renin release is expressed as units per minute per gram of kidney weight.

**Experimental Protocol**

**Within-subject experiment design.** Experiments were begun after ureteral pressure had stabilized for at least 30 minutes. Renal perfusion pressure was maintained at 80 mm Hg during the experiment with the servo-control device.20 The experiment consisted of a 60-minute control period and a 60-minute ET-1 infusion period (n=5). During the control period, 0.9% NaCl solution was infused intrarenally at a rate of 0.5 mL/min. Data were collected every 15 minutes, including 3.0-mL blood samples obtained from the arterial and renal...
TABLE 1. Endothelin-1 Infusion Experiment

<table>
<thead>
<tr>
<th>Systemic arterial pressure (mm Hg)</th>
<th>Control</th>
<th>0.2</th>
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<th>5.0</th>
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<tr>
<td>Mean±SEM</td>
<td>121±7.0</td>
<td>121±7.0</td>
<td>121±6.0</td>
<td>120±6.0</td>
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<td>10</td>
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<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Plasma protein concentration (g%)</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Mean±SEM</td>
<td>6.39±0.20</td>
<td>6.41±0.20</td>
<td>6.41±0.19</td>
<td>6.39±0.17</td>
<td>6.38±0.18</td>
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<td>n</td>
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<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Filtration fraction (%)</td>
<td></td>
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<td>Mean±SEM</td>
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<td>2.8±0.9</td>
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<td>2.9±0.8</td>
<td>1.6±0.9</td>
</tr>
<tr>
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<td>10</td>
<td>10</td>
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<tr>
<td>p</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Hematocrit (%)</td>
<td></td>
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<tr>
<td>Mean±SEM</td>
<td>35.6±1.1</td>
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<td>36.6±1.1</td>
<td>36.6±1.1</td>
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<tr>
<td>p</td>
<td>NS</td>
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<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
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Values of p are for comparison between control groups vs. endothelin-1 infusion groups.

Venous catheters for measurement of hematocrit, plasma protein concentration, plasma renin activity, and [123I]iodoheptodate activity. Three renin samples for each dog were collected during the 60-minute control period. In addition, pH and blood gas were analyzed in the arterial blood samples. In the ET-1 infusion period, four different doses (0.2, 1.0, 5.0, and 10.0 ng/kg per minute) of ET-1 (Sigma Chemical Co., St. Louis, Mo.) solutions were infused intrarenally, and each dose was maintained for approximately 15 minutes. The same measurements were made as in the control period.

Between-subject experiment design. Experiments were performed in two groups of dogs: control dogs and ET-1-infused dogs (n=10). Experiments lasted 60-75 minutes. In the control group, 0.9% NaCl solution was infused, and in the ET-1-infused group, four different doses of ET-1 were infused intrarenally as described above. Measurements in the two groups of dogs were similar to those described above.

After completion of the experiment, the ureteral clamp was released, and 45 minutes were allowed to permit fluid accumulated during ureteral occlusion to be removed before the kidney was weighed.

Data Analysis

Group means and SEM are presented in the text and figures. Statistical comparisons of all data were performed with a single-factor analysis of variance. The values in each group of control and ET-1 infusion were compared. First, analysis of variance was used to determine whether there were differences between the population means and group means with between-subject or within-subject design. Second, when the analysis of variance led to a rejection of the null hypothesis, Dunnett's test was used to determine the statistical probability of differences between the groups. A probability value of <5% was accepted as statistically significant. If a probability value was <1%, it was also indicated in the results.

Results

In the present study, renal perfusion pressure was maintained throughout the experiment at 80 mm Hg by the servo-control device. The systemic arterial pressure (Table 1) was 121±6 mm Hg and remained stable during the intrarenal infusion of ET-1. Table 1 also shows filtration fraction, which demonstrated that the left kidney was in a nonfiltering state. Plasma protein concentrations were not affected by the ET-1 infusion in the experiment, whereas hematocrit in the ET-1 infusion groups was slightly greater than in the control group, but increases were no more than 4%.

Within-Subject Design

Figure 1 shows that RBF and GHP in the saline infusion period remained stable during the 60 minutes of observation; GHP was virtually unchanged throughout this period. RBF was 163±25 mL/min at the end of the 60 minutes compared with 148±17 mL/min at the beginning (p<0.05). GHP was 67.3±2.0 mm Hg at the beginning versus 67.3±2.1 mm Hg at the end of the observation. However, there were significant changes during ET-1 infusion. RBF was reduced approximately 70%, from 148±22 to 48±15 mL/min (p<0.01), and GHP fell from 67.5±2.2 to 52.9±2.4 mm Hg (p<0.01).

Between-Subject Design

The experiment was performed in two different groups of dogs. Figure 2 shows that RBF and GHP in the ET-1-infused dogs were significantly different compared with values in the control animals. RBF in the control dogs was not different during the 60 minutes of observation, whereas in the ET-1-infused animals, it fell from 136±17 to 59±14 mL/min (p<0.01). GHP was reduced 28%, from 69.1±2.1 to 50.0±2.7 mm Hg in the ET-1-infused group (p<0.01), whereas there was no difference in the control group during the observation. The relations between ET-1 infusion rate and RBF and GHP from the two experi-
Experimental designs are presented in Figure 3. The relations were independent of study design and therefore of time.

**Endothelin-1 Dose-Response Relations**

Figure 4 presents the combined data from the two study designs plotted as ET-1 infusion rate versus RBF and GHP values as percent of the control level. The present study showed that ET-1 infusion could significantly alter renal hemodynamics, including RBF and GHP. However, the response of RBF to ET-1 was quite different from that of GHP. RBF was altered with the ET-1 infusion starting at 0.2 ng/kg per minute; it was reduced approximately 10% after 15 minutes of ET-1 infusion. With the ET-1 infusion rate of 1.0 ng/kg per minute, RBF was reduced from 154.5±15.0 to 119±11.4 mL/min, a 23% reduction from the level during saline infusion (p<0.01). However, GHP was not significantly changed until the ET-1 infusion rate reached 5.0 ng/kg per minute. At this infusion rate, GHP was reduced approximately 12%, from 68.1±1.4 to 59.5±1.3 mm Hg (p<0.01), whereas RBF was decreased more than 45%. With a higher dose of ET-1 (10.0 ng/kg per minute), RBF was reduced further to 53.5±9.9 mL/min, and GHP was reduced to 51.5±1.8 mm Hg.

Figure 5 shows the preglomerular and postglomerular resistances in response to the different doses of ET-1. With a low dose of ET-1, both the preglomerular and postglomerular resistances were increased at a similar pace. However, with higher doses (5 and 10 ng/kg per minute), ET-1 infusion raised preglomerular resistance much more than postglomerular resistance (70% versus 23% and 175% versus 775%, respectively; p<0.05).
Effects of Endothelin-1 on Renin Release

Figure 6 presents renin release data in response to saline and ET-1 infusions as renal perfusion pressure was constantly maintained at 80 mm Hg by a servocontrol system. In the nonfiltering kidney preparation, the rate of renin release in control dogs did not change during the 60 minutes of observation (Figure 6, panel A). The renin release rate was 29.1±2.5, 31.0±4.1, and 34.6±3.3 units/min per gram kidney weight after 15, 30, and 60 minutes of observation, respectively (p>0.05). However, in animals infused with ET-1, renin release decreased progressively as the infusion rate was raised. These data are presented in Figure 6B, with renin release rate expressed as percent of the control value. With infusion at 1.0 ng/kg per minute, renin release fell from the control level of 47.9±5.6 to 26.6±4.9 units/min per gram kidney weight (p<0.01), whereas GHP remained unchanged (Figure 4). Yet, with a higher rate of ET-1 infusion at 10.0 ng/kg per minute, renin release fell further to 16.1±4.3 units/min per gram kidney weight, 34% of the control level, despite the fall in GHP.

Discussion

The present study has demonstrated that ET-1 had profound effects on renal hemodynamics and renin release in the nonfiltering kidney preparation. The results also indicated that ET-1 infusion can cause nonparallel effects on RBF and GHP under these conditions. Previous studies showed that with a low dose of ET-1, renal plasma flow was reduced by more than 20%, whereas GFR was unchanged in rats and dogs.6,8 Furthermore, King and Brenner5 and King et al8 also reported that low doses of ET-1 increased the mean...
transglomerular capillary hydraulic pressure gradient in rats, suggesting that the increase in GHP is probably the reason that GFR was maintained despite reductions in RBF and filtration coefficient in rats. However, direct effects of ET-1 on the glomerular hydraulic capillary pressure in dogs in vivo have not been reported. Other investigators have found that with an infusion rate of 1.15 ng/kg per minute, ET-1 caused parallel reductions in RBF and GHP without affecting the filtration fraction.5

To determine GHP in response to various doses of ET-1, we used an in vivo stop-flow pressure method in the present study, which had been used in previous studies.15,21 The method was based on a model in which the kidney was rendered nonfiltering. The glomerular capillary hydraulic pressure was expected to be elevated due to activation of the tubuloglomerular feedback mechanism. In the present study, renal perfusion pressure was controlled at the level of 80 mm Hg by a servo-control device, which could attenuate the atypical increased GHP. The estimated GHP in the experiment with a dose of 0.14 ng/kg per minute was 65.8±2.9 mm Hg obtained by Otte et al22 with a micropuncture technique in dogs. During the control experiments, GHP was stable throughout the 60-minute experiment (see Figure 1), suggesting that the experimental control was not a factor affecting GHP in this preparation. ET-1 infusion produced dose-dependent changes in GFR, but it did not affect GHP until the dose reached 5.0 ng/kg per minute, at which point RBF was already reduced by 45%. Therefore, low doses of ET-1 did not alter GHP despite the fall in RBF. This discrepant effect on RBF and GHP by ET-1 reflects the fact that ET-1 may affect the precapillary and postglomerular resistances in parallel, thereby tending to maintain GHP while decreasing RBF.

With higher doses, ET-1 increased precapillary resistance 300-400% more than postglomerular resistance. Therefore, in the upper dose range, GHP decreased concomitantly with RBF. Such changes in RBF and GHP can result in severe decreases in GFR, such as those reported by Miura et al.8 In their study, GFR fell by as much as 75% with a dose of 5.0 ng/kg per minute.

The physiological importance of endothelin in regulating renal hemodynamics is still uncertain, but it appears that the effect of ET-1 is different from that of other vasoconstrictors, such as angiotensin II and norepinephrine. Several studies have indicated that angiotensin II selectively constricted the efferent arterioles.21,22 In a preparation similar to the one in the present study, Hall and Granger22 reported that angiotensin II infusion raised GHP despite a fall in RBF, suggesting that angiotensin II mainly affected the postglomerular vessels. In contrast, we found (unpublished data) that norepinephrine infusion could concomitantly reduce RBF and GHP. This finding suggests that norepinephrine may preferentially affect precapillary vessels. A similar finding was also reported by others with norepinephrine infusion.21 Unlike angiotensin II and norepinephrine, ET-1 has a parallel effect on the precapillary and postglomerular vessels at a low dose.

There has been some controversy regarding the effect of endothelin on renin release, particularly in vivo. Miller et al12 found that intravenously infused ET-1 (50.0 ng/kg per minute) in anesthetized dogs produced an increase in plasma renin activity, and they believed the increase was due to activation of the intrarenal baroreceptor and macula densa pathway. However, in the study by Cavero et al,13 the intravenously infused ET-1 (5.0 ng/kg per minute) suppressed plasma renin activity in dogs. Such discrepant effects on renin secretion in vivo might be ascribed to dose dependence. Because a low dose of ET-1 had little effect on afferent arteriolar pressure and a relatively small effect on GFR, the direct inhibitory effect of ET-1 on renin release was revealed. With a higher dose, ET-1 may severely reduce GFR and perfusion pressure at the level of the juxtaglomerular cells, thereby stimulating renin release and overcoming the direct inhibitory effect by the ET-1.

We found that ET-1 infusion produced an inhibitory effect on renin release in vivo in the nonfiltering kidney. This finding is consistent with those from in vitro studies,9-11 which indicated that ET-1 had a direct inhibitory effect on renin secretion. The present study also showed that ET-1 inhibited renin release not only at the low dose but also at higher doses. This result does not agree with the previous in vivo study by Miller et al,12 probably because the macula densa-mediated pathway was blocked in our experiment, so renin release could be affected only by the intrarenal baroreceptor mechanism and by direct effects on the juxtaglomerular cells. Data from a previous study in this laboratory using nonfiltering kidney24 suggested that the intrarenal baroreceptor mechanism still played an active role in controlling renin release. At the low dose, ET-1 did not alter GHP and, presumptively, did not change the perfusion pressure at the juxtaglomerular cells; therefore, the intrarenal baroreceptor mechanism was not activated to stimulate renin release. However, with higher doses of ET-1, GHP was also reduced by as much as 25%, suggesting that the intrarenal baroreceptor mechanism may have participated in stimulating renin release. But the results of the present study indicate that this stimulation was not strong enough to offset the direct inhibitory effect of ET-1 on the juxtaglomerular cells. This finding suggests that if a higher dose of ET-1 could cause an increase in renin release in the intact kidney under normal conditions, its effect is most likely dependent on indirect effects mediated by the macula densa-mediated pathway.

In summary, the present study demonstrated that in the nonfiltering kidney ET-1 decreased RBF in a dose-dependent fashion. The effects of ET-1 on precapillary and postglomerular resistances were quantitatively similar at low doses, whereas they were much greater on precapillary resistance at a high dose. As a result, GHP remained nearly unchanged at low doses even though RBF fell, whereas at higher doses GHP decreased sharply. These findings suggest that endothelin may play an important role in regulating GFR and renal hemodynamics as a potential local factor. ET-1 significantly inhibited renin release at both low and high infusion rates. This inhibition was independent of changes in GHP, suggesting that the direct effect of ET-1 on renin release may have been stronger than the intrarenal baroreceptor mechanism when tubuloglomerular feedback was eliminated.

References
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