Kinin Actions on Renal Papillary Blood Flow and Sodium Excretion


Infusion of bradykinin into the renal medullary interstitium (0.1 μg/min, n=6) significantly increased renal papillary blood flow as measured by laser-Doppler flowmetry to 117±3% of control without altering cortical blood flow or blood pressure in anesthetized Munich-Wistar rats. In animals prepared for clearance studies, renal medullary bradykinin infusion did not alter total renal blood flow, glomerular filtration rate, or renal interstitial hydrostatic pressure but increased urine flow by 100%, sodium excretion by 111%, and fractional sodium excretion by 107%. No changes occurred in mean arterial pressure or contralateral kidney function during the interstitial bradykinin infusion. Blockade of endogenous kinin degradation by interstitial infusion of captopril (1 mg/hr) significantly increased papillary blood flow by 21±5% without altering cortical blood flow. Pretreatment with the nitric oxide inhibitor Nω-nitro-L-arginine-methyl ester (2 μg/min, n=7) eliminated the increase in papillary blood flow associated with either bradykinin or captopril infusion. We conclude that renal medullary interstitial infusion of bradykinin increases sodium and water excretion, which is associated with a selective increase in papillary blood flow by a nitric oxide–dependent mechanism. (Hypertension 1993;21:961–965)

KEY WORDS • renal circulation • laser-Doppler flowmetry • kidney medulla • bradykinin • nitric oxide

Morphological and functional data indicate that kinins may act as paracrine agents in the renal medulla.1-3 Intravenous administration of an inhibitor of neutral endopeptidase, the major kininase in the rat kidney,4 selectively increased renal inner medullary (papillary) blood flow in anesthetized rats.5 This effect was blocked by a kinin receptor antagonist, indicating that an elevation of endogenous kinins can alter medullary blood flow. Similar results have been demonstrated during angiotensin converting enzyme inhibition. Although cortical blood flow increases during inhibition of converting enzyme because of decreased angiotensin II levels, renal medullary blood flow increases because of increased kinins.6,7 These data indicate that kinins have a primary influence on the renal medullary circulation in the rat. In support of these functional data, morphological data indicate that the intrarenal site of kinin formation and release is in the distal connecting tubule and collecting duct.1-3 It is therefore thought that the peptide exerts its physiological effects from the luminal or the interstitial side of the collecting duct in the renal medulla. Previous studies examining the renal hemodynamic and excretory effects of exogenous bradykinin were performed by renal arterial infusion.8-10 This route of infusion may not mimic a physiological route of kinin delivery, because intrarenally formed kinins must reach the vasculature via the interstitium. To address this problem, we have recently developed an infusion technique in our laboratory by which compounds can be infused and localized in the renal medullary interstitium.11,12

The purpose of the present study was to characterize the effects of renal medullary interstitial infusion of bradykinin on the intrarenal distribution of blood flow and on sodium and water excretion in anesthetized Munich-Wistar rats. The participation of kinins in the papillary blood flow response to converting enzyme inhibition was also further examined by determining the blood flow response to interstitially infused bradykinin and the converting enzyme inhibitor captopril in control animals and animals pretreated with Nω-nitro-L-arginine-methyl ester (L-NAME), an inhibitor of nitric oxide.

Methods

General

Experiments were performed on 26 male Munich-Wistar rats (average weight, 259±34 g) purchased from Harlan Laboratories, Madison, Wis. The rats were housed in the Animal Resource Center at the Medical College of Wisconsin with food and water provided ad libitum. All procedures on animals were approved by the Medical College of Wisconsin Animal Care Committee. On the day of the experiment, the rats were anesthetized with an intraperitoneal injection of thiobutabarbital (Inactin) (100 mg/kg) and placed on a heated table to maintain body temperature at 37°C. Cannulas were placed in the carotid and femoral arteries for measurement of arterial pressure and collection of blood. An additional cannula was placed in the jugular vein for intravenous infusion.

Surgical fluid losses were replaced by continuous intravenous infusion of 2% bovine serum albumin (frac-
Arginine-Methyl Ester on Intrarenal Blood Flow Distribution

Infusion of Saline on Intrarenal Blood Flow Distribution

After surgery, 1 hour was allowed for equilibration of the blood flow distribution. On the day of the experiment, rats (n=4) were surgically prepared as described above. In addition, the left kidney was placed in a holder, and the papilla was exposed by excising the ureter. Cortical and papillary blood flows were measured using a laser-Doppler flowmeter (model Pf, Perimed KB, Stockholm) as previously described.13 Blood flow in the renal cortex and papilla was measured every 30 minutes for 3 hours during the interstitial infusion of saline vehicle.

Group 1: Effect of Renal Medullary Interstitial Infusion of Saline on Intrarenal Blood Flow Distribution

Experiments were performed in this group of rats (n=6) to describe the effects of renal medullary interstitial bradykinin and captopril infusion on intrarenal blood flow distribution. On the day of the experiment, rats were surgically prepared as described above. Cortical and papillary blood flows are expressed as a percentage of the respective laser-Doppler flow signal measured during the control period.

In this group of rats, blood flow in the renal cortex and papilla was measured by laser-Doppler flowmetry during the final 15 minutes of four successive 30-minute periods in which different compounds were infused directly into the renal medullary interstitium. Isotonic saline was infused as a vehicle control (0.5 mL/hr). Blood flow in the renal medullary interstitium (0.5 mL/hr) and two 30-minute periods during the renal medullary interstitial infusion of bradykinin (0.1 µg/min) in saline (0.5 mL/hr).

Analytical Methods

Urine flow was determined gravimetrically. Sodium concentrations of the samples were determined with a flame photometer (model 143, Instrumentation Laboratories, Lexington, Mass.). Urine osmolality was determined by freezing point depression with an osmometer (model 5004, Precision Instruments, Sudbury, Mass.). Systemic arterial protein concentration was determined with a refractometer.3 H]Inulin concentration of the samples was determined with a liquid scintillation counter (model 2450, Packard Instrument Co., Inc., Downers Grove, Ill.). Glomerular filtration rate was calculated by multiplying the ratio of urine to plasma [3H]Inulin counts per minute by the urine flow rate.

Statistical Analysis

Data are presented as mean±SEM. The significance of differences in measured values was evaluated with an analysis of variance for repeated measures and a Duncan multiple-range test. A value of p<0.05 was considered significant.

Results

Group 1: Effect of Renal Medullary Interstitial Infusion of Saline on Intrarenal Blood Flow Distribution

Infusion of isotonic saline produced no significant changes in renal cortical or papillary blood flow from the first measurement period over the time course of these experiments (n=4, data not shown). Mean arterial pressure and hematocrit were unaltered from control values of 125±6 mm Hg and 51±1% throughout the time control experiment.

Group 2: Effect of Renal Medullary Interstitial Infusion of Bradykinin and Captopril on Intrarenal Blood Flow Distribution

Renal medullary interstitial infusion of bradykinin (0.1 µg/min) significantly increased papillary blood flow.
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Distribution After Interstitial Bradykinin and Captopril Infusion

**Group 3: Effects of Pretreatment with N\textsuperscript{G}-Nitro-L-Arginine-Methyl Ester on Intrarenal Blood Flow Distribution After Interstitial Bradykinin and Captopril Infusion**

Renal medullary interstitial infusion of L-NAME (2 \(\mu\)g/min) significantly decreased papillary blood flow to 78±3% of control without altering superficial cortical blood flow (n=7, Figure 2). During L-NAME infusion, interstitial bradykinin had no significant effect on papillary blood flow. Coadministration of captopril with L-NAME also failed to alter either cortical or papillary blood flow. Hematocrit was not altered from the control value of 48±1% during this protocol. Mean arterial pressure significantly increased from the control level of 116±6 to 130±11 mm Hg after L-NAME infusion and remained at that level through the remainder of the experiment.

**Group 4: Effects of Renal Medullary Interstitial Bradykinin Infusion on Sodium and Water Excretion**

In the animals prepared for clearance experiments (n=6), the plasma sodium concentration was 151±4 mEq/L, plasma osmolality was 305±5 mOsm/L, and plasma protein concentration was 4.2±0.1 g/dL. Clearance data for the kidney infused with bradykinin are summarized in Table 1. Urine flow rate, urinary sodium excretion, and fractional sodium excretion all doubled after the interstitial infusion of bradykinin, and urine osmolality fell from approximately 1,300 to 650 mOsm/L. However, mean arterial pressure, glomerular filtration rate, renal blood flow, and renal interstitial hydrostatic pressure were unaltered after interstitial bradykinin infusion. In the contralateral kidney, during the initial control period, urine flow was 4.4±1.9 \(\mu\)L/min per gram kidney weight, urinary sodium excretion was 1.1±0.6 \(\mu\)Eq/min per gram kidney weight, glomerular filtration rate was 0.86±0.08 mL/min per gram kidney weight, and urine osmolality was 1,900±189 mOsm/L. None of these parameters was significantly altered during any collection period in this protocol.

**Discussion**

The present study evaluated the effects of increasing bradykinin levels in the renal medulla on intrarenal blood flow distribution and sodium and water excretion. These data reveal a possible physiological role of the kallikrein-kinin system on renal function. Renal medullary interstitial infusion of bradykinin increased renal papillary blood flow and sodium and water excretion without changing cortical blood flow, glomerular filtration rate, renal blood flow, or renal interstitial hydrostatic pressure. In addition, systemic blood pressure and renal function in the contralateral kidney were unaltered during the medullary interstitial infusion of bradykinin, indicating that the effects of bradykinin in the present experiments were not due to recirculation of the peptide. These data demonstrate that infusion of bradykinin into the renal medulla selectively increases renal papillary blood flow, which is associated with increased sodium and water excretion.

The present results indicate that papillary blood flow may be regulated by the local effects of bradykinin on the vasculature of the renal medulla. We have previously demonstrated that the elevation of papillary blood flow during intravenous administration of captopril is blocked by pretreatment with a kinin receptor antagonist,\(^\text{a,7}\) and the present studies demonstrate that infusion of captopril directly into the medullary interstitial space increases blood flow. The present experiments also show that bradykinin can increase papillary blood flow when administered into the medullary interstitial space. Taken together, these data indicate that both infused bradykinin and endogenous elevations of kinin levels result in elevations of papillary blood flow. There

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**FIGURE 1.** Line graph shows effect of renal medullary interstitial infusion of bradykinin (0.1 \(\mu\)g/min) and captopril (1 mg/hr) on cortical and papillary blood flow. *Significant difference (p<0.05) from previous point; †significant difference (p<0.05) from control.

**FIGURE 2.** Line graph shows effect of pretreatment with renalin medullary interstitial infusion of N\textsuperscript{G}-nitro-L-arginine-methyl ester (L-NAME)(2 \(\mu\)g/min) on renal cortical and papillary blood flow response to interstitial bradykinin (0.1 \(\mu\)g/min) or captopril (1 mg/hr) infusion. *Significant difference (p<0.05) from previous point; †significant difference (p<0.05) from control.
were no significant changes in glomerular filtration rate, renal blood flow, or cortical blood flow during medullary interstitial bradykinin infusion, whereas papillary blood flow significantly increased and filtration fraction fell. These data indicate that bradykinin in the renal medullary interstitium may selectively alter medullary blood flow by postglomerular dilation. This is supported by the data of Edwards,14 which demonstrated selective effects of bradykinin to dilate norepinephrine-preconstricted rabbit efferent arterioles.

Experimental evidence indicates that the vasodilator effects of bradykinin are mediated by nitric oxide.15 To further test the role of kinins in the medullary blood flow response to captopril, we pretreated a group of rats with the nitric oxide inhibitor L-NAME before captopril administration. Interstitial L-NAME infusion, which blocks the acetylcholine-induced increase in renal cortical and papillary blood flow,12 blocked the medullary vasodilator effects of interstitial bradykinin or captopril infusion. We have previously demonstrated that the renal medullary vasodilation induced by captopril is not blocked by pretreatment with meclofenamate, which reduced the baseline level of papillary blood flow by 30%; therefore, it appears that L-NAME blockade of bradykinin and captopril-induced vasodilation is due to nitric oxide inhibition rather than nonspecific vasoconstriction. These data lend further support to the view that converting enzyme inhibition increases inner medullary blood flow by a kinin-dependent mechanism in rats. The present finding that renal medullary interstitial infusion of L-NAME decreases inner medullary blood flow without altering superficial cortical blood flow is consistent with our previous report in which we demonstrated a lack of systemic spillover during interstitial infusion of L-NAME at the same dose.12 It is important to note that the selective decrease in papillary blood flow after interstitial L-NAME infusion in the present study is probably not solely due to blockade of kinin actions, although a kinin component may be an important part of the overall renal vascular response to L-NAME treatment.

Although medullary interstitial bradykinin infusion significantly increased papillary blood flow, the mechanism of the natriuresis and diuresis is unclear. Because renal medullary interstitial bradykinin infusion increased sodium and water excretion but did not change glomerular filtration rate or renal blood flow, the significant increase in fractional sodium excretion indicates that the natriuresis and diuresis during bradykinin were due to direct or indirect effects of bradykinin to alter tubular transport. Micropuncture studies indicate no direct effect of bradykinin to alter proximal tubular sodium and water reabsorption,10 although direct infusion of bradykinin into the distal nephron appears to inhibit the distal nephron reabsorption of radiolabeled sodium.16 In addition, bradykinin has been demonstrated to oppose the hydroosmotic effects of vasopressin in the rabbit cortical collecting duct17 and to inhibit sodium reabsorption in the rat cortical collecting duct.18 A direct influence of bradykinin on tubular sodium and water transport is a possible mediator of the diuresis and natriuresis in the present experiments. Alternatively, increases of vasa recta blood flow in the renal medulla may indirectly alter tubular reabsorption of sodium and water by altering renal interstitial hydrostatic pressure or washing out the medullary concentration gradient.19 Interstitial bradykinin infusion did not alter renal interstitial pressure in the present experiments despite increases in medullary blood flow. Previous data demonstrated that an elevation of endogenous kinins increases medullary blood flow but decreases vasa recta hydrostatic pressure.7 During interstitial infusion of exogenous bradykinin, there may be dilatory effects on both the pre- and post-vasa recta circulation, leading to increased medullary blood flow without changing interstitial pressure. Because renal interstitial hydrostatic pressure was unaltered by bradykinin infusion in the present experiments, the natriuretic mechanism of action of bradykinin may have been mediated by washout of the medullary concentrating gradient. Reduced medullary tonicity may have reduced loop of Henle sodium and water absorption and played a role in the natriuretic effects of bradykinin infusion.

In summary, the present studies demonstrate a possible mechanism whereby the kallikrein-kinin system may participate in the regulation of sodium and water homeostasis by acting as a paracrine factor in the renal medulla. Elevation of renal medullary interstitial bradykinin levels by infusion selectively increased papillary blood flow without altering cortical blood flow, renal blood flow, glomerular filtration rate, or renal interstitial hydrostatic pressure. This selective increase in pap-

### Table 1. Effect of Renal Medullary Interstitial Infusion of Bradykinin (0.1 μg/min) on Renal Function

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control 1</th>
<th>Control 2</th>
<th>Bradykinin 1</th>
<th>Bradykinin 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV (μL/min/g kwt)</td>
<td>5.3±1.2</td>
<td>4.9±0.9</td>
<td>8.4±1.1†</td>
<td>9.8±1.2†</td>
</tr>
<tr>
<td>Uo,V (μg/min/g kwt)</td>
<td>0.66±0.16</td>
<td>0.67±0.12</td>
<td>1.08±0.20†</td>
<td>1.37±0.20†</td>
</tr>
<tr>
<td>FM (μg/min/g kwt)</td>
<td>0.61±0.19</td>
<td>0.65±0.17</td>
<td>1.0±0.16</td>
<td>1.35±0.14†</td>
</tr>
<tr>
<td>Uo,s (mOsm/kg H2O)</td>
<td>1,308±259</td>
<td>1,324±254</td>
<td>691±50†</td>
<td>654±46†</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>119±5</td>
<td>116±5</td>
<td>116±5</td>
<td>116±5</td>
</tr>
<tr>
<td>GFR (mL/min/g kwt)</td>
<td>0.78±0.05</td>
<td>0.76±0.05</td>
<td>0.70±0.04</td>
<td>0.65±0.06</td>
</tr>
<tr>
<td>RBF (mL/min/g kwt)</td>
<td>6.6±0.6</td>
<td>6.8±0.7</td>
<td>6.9±0.7</td>
<td>7.1±0.7</td>
</tr>
<tr>
<td>FF (%)</td>
<td>27±3</td>
<td>26±3</td>
<td>23±2</td>
<td>20±2*</td>
</tr>
<tr>
<td>RIHP (mm Hg)</td>
<td>7.8±0.5</td>
<td>7.1±0.8</td>
<td>7.6±0.7</td>
<td>7.1±1.0</td>
</tr>
</tbody>
</table>

UV, urine flow rate; kwt, kidney weight; Uo,V, sodium excretion; FM, fractional sodium excretion; Uo,s, urine osmolality; MAP, mean arterial pressure; GFR, glomerular filtration rate; RBF, renal blood flow; FF, filtration fraction; RIHP, renal interstitial hydrostatic pressure. Values are mean±SEM.

*P<0.05 compared with Control 1.
†P<0.05 compared with Control 2.
illary blood flow was associated with increased sodium and water excretion and decreased urine osmolality. The intrarenal kinin system may regulate sodium and water excretion by direct tubular effects, modulation of renal papillary blood flow, or both.

References
Kinin actions on renal papillary blood flow and sodium excretion.
D L Mattson and A W Cowley, Jr

Hypertension. 1993;21:961-965
doi: 10.1161/01.HYP.21.6.961

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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