Blockade of renal medullary bradykinin B2 receptors increases tubular sodium reabsorption in rats fed a normal-salt diet

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The tissue kallikrein-kinin-kininase cascade is a complex enzymatic pathway for the generation of kinin peptides (4). Two of these peptides, bradykinin and lys-bradykinin (kallidin), induce local vasodilation and inhibit renal electrolyte reabsorption by acting as paracrine/autocrine ligands for G protein-coupled bradykinin B2 (BKB2) receptors (see reviews in Refs. 4 and 18).

The kallikrein-kinin systems in brain, kidney, and cardiovascular tissue contribute to the regulation of cardiovascular function and blood pressure (3, 5, 18, 22). However, the renal kallikrein-kinin system (through its action to increase electrolyte and water excretion) plays a primary role in the long-term regulation of blood pressure under conditions of hypertensive insult, especially high salt intake (1, 17, 19, 20, 26, 41).

Paradoxically, although renal kinins are natriuretic, high salt intake suppresses the renal kallikrein-kinin system (28, 44). This suppression is rapid. Cortical and medullary interstitial levels of kinins are markedly reduced within 24 h of exposure to a high-salt diet (36). Conversely, cortical interstitial levels of bradykinin and the activity of the renal kallikrein-kinin system are markedly augmented by low salt intake (28, 36, 44). This augmentation of the renal kallikrein-kinin system may modulate the antinatriuretic mechanisms that are activated in response to salt depletion (35). Therefore, by increasing electrolyte and water excretion, the renal kallikrein-kinin system contributes to the defense of normotension during high salt intake and to the regulation of electrolyte and water excretion during salt restriction (17, 25, 35).

We showed previously (13) that renal kinins act on luminal BKB2 receptors in the distal nephron to increase tubular sodium excretion in euvolemic rats fed a normal-salt diet. This finding supports the proposal that renal kinins may tonically decrease electrolyte reabsorption in the terminal segments of the nephron regulating electrolyte excretion under normal or near normal physiological conditions (33).

The mechanism(s) and the site(s) at which kinins act have yet to be fully elucidated. Kinins formed locally in the interstitium or the tubular lumen of the distal nephron appear to inhibit Na+/Cl− transport across medullary and/or cortical collecting ducts (8, 13, 16, 25, 33, 40, 45). However, studies in isolated perfused tubules indicate that bradykinin-dependent inhibition of cortical collecting duct electrolyte transport is not constitutive but appears to be induced by high salt and/or treatment with DOCA (25, 32, 34, 38, 39). In addition to their direct effect on tubular transport, renal kinins appear to also act indirectly to regulate electrolyte and water excretion via increased papillary blood flow (PBF) (9, 23, 24, 31, 41) and increased pressure natriuresis (41). Under conditions in which PBF is either not autoregulated or only poorly regulated (such as acute volume expansion), an increase in PBF would be expected to lead to an increase in renal interstitial pressure that, in turn, would result in a fall in tubular sodium reabsorption (7).

To further explore the role of renal kinins in electrolyte excretion, the present study was performed to test the hypothesis that under normal physiological conditions and/or during augmentation of kinin levels, intrarenal kinins act on medullary BKB2 receptors to acutely increase PBF and therefore sodium excretion. In this study we examined the effect of acute inner medullary interstitial BKB2 receptor blockade on renal hemodynamics and excretory function in anesthetized, euvolemic Sprague-Dawley rats that had been fed either a normal (0.23%)- or a low (0.08%)-NaCl diet.
Animals

The experiments described in this manuscript were conducted with approval of the Medical University of South Carolina Institutional Animal Care and Use Committee and in accordance with the procedures and practices in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats (80–100 g) were obtained from Charles River Laboratories International (Wilmington, MA). Animals were housed in a dedicated room with constant temperature (22°C) and a 12:12-h light-dark cycle. The rats were allowed free access to tap water and fed one of two isocaloric NaCl diets (Teklad, Madison, WI) for at least 2 wk before the study.

Surgical Preparation

On the day of study, the rats were anesthetized with pentobarbital (50 mg/kg ip) and placed on a thermostatically controlled, heated table to maintain body temperature at 37°C. After tracheotomy, a triple-lumen polyethylene catheter was inserted into the right jugular vein for the infusion of fluids [1% bovine serum albumin (BSA) in 0.9% NaCl, 10% polyfructosan, and 1% BSA in 0.9% NaCl] and supplemental doses of anesthetic. During the surgical preparation the total infusion rate was 1.2 ml/h. The left femoral artery was cannulated for direct measurement of blood pressure with a Transpac pressure transducer and a Transonic T206 flowmeter with a blood pressure analyzer (Transonic Systems, Ithaca, NY). The urinary bladder was exposed through an abdominal incision and cannulated with PE-50 for collection of urine from the right kidney. The left kidney, exposed through a flank incision, was carefully freed from the perirenal fat, supported in a Lucite cup, and covered with cotton moistened with 0.9% NaCl. A PE-10 catheter was placed into the left ureter for collection of urine. Mean arterial pressure (MAP) data obtained with the Transonic T206 flowmeter were recorded on a Dell PC with WinDaq Lite data acquisition software (DataQ, Akron, OH). Waveform analyses were performed by using the WinDaq Waveform Browser (DataQ) to obtain values for MAP for each clearance period. A 23-gauge needle was used to puncture the capsule at two sites above the midregion of the outer convex surface of the kidney. One of the interstitial catheters (described below) was inserted into the site closest to the midcurvature of the kidney. With the aid of a micro-manipulator, the tip of the catheter was tunneled to a depth of ~5 mm, i.e., the catheter tip was located within the inner medulla. A dark tissue fiber attached to a Perimed masterprobe 418-2 laser Doppler flow probe was then tunneled with a micromanipulator into the second site so that the tip was at the same depth in the inner medullary interstitium as the tip of the interstitial catheter. The masterprobe was connected to a Periflux 4001 laser Doppler system (Perimed, Jarfalla, Sweden) for the measurement of PBF. A Perimed 407 probe with adhesive miniholder was placed on the surface of the kidney and connected to the Periflux 4001 for the measurement of cortical blood flow (CBF). Both probes were calibrated to 250 perfusion units (PU) with the Perimed motility standard. Data were recorded on a Dell PC with PersiSoft data acquisition software. Mean values were calculated for each clearance period. The measurement of tissue blood flow by the laser Doppler method is dependent on both the number of red blood cells (RBCs) in motion within the measurement area and the velocity of the cells. Therefore, a change in perfusion detected with the Perimed device may be due to a change in RBC velocity, a change in the total number of RBCs in the measure area, or a combination of both.

After completion of the surgery, the animals received a 1.8-ml bolus of 1% BSA in 0.9% NaCl over 10 min, and then the infusion rate was reset and maintained at 1.2 ml/h through the rest of the experiment. The animals were allowed 1 h to achieve a steady state before the measurement of renal hemodynamics and excretory function.

Interstitial Catheterization

Interstitial catheters (60- to 80-μM OD) were prepared by manually pulling heated PE-10 (Clay Adams, Parsippany, NJ). Each catheter was cemented into the lumen at one end of a 5-mm Y connector. Two PE-50 catheters were cemented into the other end of the Y connector and connected via a three-way stopcock to syringes attached to two separate Braun infusion pumps. Immediately after insertion of the catheter into the inner medullary interstitium, an infusion of 0.9% NaCl was commenced via one syringe to maintain the patency of the catheter. The infusion rate was set to ensure a catheter tip flow of 2.6 μl/min (156 μl/h).

Protocol 1: Effect of Interstitial Infusion of HOE-140 on Renal Hemodynamics and Excretory Function

Salt diets. Two groups of rats (groups 1 and 2) were fed a normal-NaCl diet (0.23%, Teklad, Madison, WI) and two groups (groups 3 and 4) were fed a low-NaCl diet (0.05%, Teklad) for at least 2 wk before study.

Protocol. The experimental protocol consisted of baseline and experimental periods, each of 1-h duration. During the baseline period, the infusion of 0.9% NaCl into the papillary interstitium was maintained at 2.6 μl/min. Two 30-min urine collections were obtained from the left and right kidneys for the determination of renal excretory function. During these clearance periods, measurements of left kidney CBF and PBF were also obtained.

At the completion of the baseline period, rats were subjected to one of two treatments. In groups 1 and 3 (HOE-140 treated, n = 11), the medullary interstitial infusion was switched from the vehicle (0.9% NaCl) to the second line containing HOE-140 (100 μg·kg⁻¹·h⁻¹). Vehicle-treated animals [groups 2 (n = 10) and 4 (n = 9)] served as time controls where the medullary infusion was switched to a second line containing 0.9% NaCl. The infusion rate for both groups was maintained at 2.6 μl/min. A 30-min period was allowed for equilibration before commencement of the experimental period. Two 30-min urine collections were again obtained from the left and right kidneys for the determination of renal excretory function. During these clearance periods, measurements of left kidney CBF and PBF were also obtained. Blood samples (400 μl) were taken between the pairs of clearance periods.

At the end of the experiment, both kidneys were removed, blotted gently, and weighed. The left kidney was sectioned sagittally, and the placement of the catheter and the blood flow probe within the papilla was confirmed.

Protocol 2: Effect of Interstitial Infusion of nitro-L-arginine methyl ester on Renal Hemodynamics and Excretory Function

Another group of rats (group 5, n = 4) were fed a normal-NaCl diet (0.23%, Teklad) for at least 2 wk before study. As with protocol 1, the experimental protocol consisted of baseline and experimental periods, each of 1-h duration. During the baseline period, the infusion of 0.9% NaCl into the papillary interstitium was maintained at 2.6 μl/min. Two 30-min urine collections were obtained from the left and right kidneys for the determination of renal excretory function. During these clearance periods, measurements of left kidney CBF and PBF were also obtained.

At the completion of the baseline period, the medullary interstitial infusion was switched from the vehicle (0.9% NaCl) to nitro-L-arginine methyl ester (L-NAME; 120 μg/h). A 30-min period was allowed for equilibration before commencement of the experimental period. Two 30-min urine collections were obtained from the left and right kidneys for the determination of renal excretory function. During these clearance periods, the parameters of left kidney
hemodynamics were again measured. Blood samples (400 µl) were taken between the pairs of clearance periods.

At the end of the experiment, both kidneys were removed, blotted gently, and weighed. The left kidney was then sectioned sagitally to verify the placement of the catheter and probe within the papilla.

Analytical Procedures

Urine volumes were determined gravimetrically. Sodium concentrations in plasma and urine were determined by flame photometry (model 2655-10 Dual Channel Flame Photometer, Cole Palmer, Vernon Hills, IL). An internal lithium standard was used for the standardization of sodium measurements. Polyfructosan concentration was determined colorimetrically with a method modified after Fuhr et al. (15). Glomerular filtration rate (GFR), urine flow rate (Uv), urinary sodium excretion rate (UNaV), and fractional sodium excretion (FENa) were calculated with standard formulas. Renal excretory data are expressed per gram of kidney weight (kwt).

Data Treatment and Statistical Analysis

For each animal, the blood flow data were averaged over each 30-min clearance period. Values for each parameter were averaged over the two clearance periods that constituted the baseline period and over the two clearance periods that constituted the experimental period. All normally distributed data are expressed as means ± SE. Nonnormally distributed data are expressed as medians (range). Differences between means were tested with one-sample t-tests with the Bonferroni modification. Within-group differences in UNaV were tested with the Wilcoxon signed rank test. A significant difference was accepted when P < 0.05.

RESULTS

Effect of Interstitial Infusion of HOE-140 on Renal Function of Rats Fed a Normal-Salt Diet

Although both groups of rats remained euvoletic, there was a small but significant drop in MAP in both HOE-140- and vehicle-infused rats over the duration of the experiment (Table 1). Infusion of HOE-140 into the medullary interstitium did not alter GFR or regional blood flow (Table 1). There was a significant decrease in Uv from the left kidney during HOE-140 infusion, but this occurred in the absence of a significant change in fractional water excretion (Table 1). In the time control rats, the interstitial infusion of 0.9% NaCl did not alter Uv, CBF, or PBF (Table 1). In both groups of rats, CBF and inner medullary blood flow remained stable during the control and experimental clearance periods.

Infusion of HOE-140 into the medullary interstitium decreased urinary sodium excretion (Figs. 1 and 2). The median UNaV for the baseline and HOE-140 infusion periods was 270 (53–2,125) and 126 (24–720) nmol·min⁻¹·g kwt⁻¹, respectively (P < 0.05). In contrast, infusion of vehicle into the interstitium did not alter UNaV (Figs. 1 and 2). For the time control rats, median UNaV for the baseline and vehicle

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HOE-140 (n = 11)</th>
<th>Vehicle (n = 10)</th>
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<tbody>
<tr>
<td>MAP, mmHg</td>
<td>113 ± 2</td>
<td>109 ± 2†</td>
</tr>
<tr>
<td>Hct, %</td>
<td>47 ± 1</td>
<td>47 ± 1</td>
</tr>
<tr>
<td>Uv, μl·min⁻¹·g kwt⁻¹</td>
<td>5.2 ± 0.4</td>
<td>4.5 ± 0.4*</td>
</tr>
<tr>
<td>GFR, ml·min⁻¹·g kwt⁻¹</td>
<td>1.23 ± 0.13</td>
<td>1.09 ± 0.08</td>
</tr>
<tr>
<td>P/Uin, %</td>
<td>0.44 ± 0.04</td>
<td>0.41 ± 0.04</td>
</tr>
<tr>
<td>CBF, perfusion units</td>
<td>435 ± 17</td>
<td>436 ± 13</td>
</tr>
<tr>
<td>PBF, perfusion units</td>
<td>44 ± 2</td>
<td>45 ± 2</td>
</tr>
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</table>

Values are means ± SE for n rats. MAP, mean arterial pressure; Hct, hematocrit; Uv, urine flow rate; GFR, glomerular filtration rate; P/Uin, fractional water excretion; CBF, cortical blood flow; PBF, papillary blood flow; kwt, kidney weight. *P < 0.05, †P < 0.01 compared with Baseline.
an increase in tubular Na$^+$ decreases in absolute and fractional excretion indicate that more, FENa was significantly lower ($P < 0.01$) during the HOE-140 infusion was significantly with the baseline period [0.19% (0.04– 0.75)] (Fig. 3). This represented a 40% decrease from baseline ($P < 0.01$). The decreases in absolute and fractional excretion indicate that there was an increase in tubular Na$^+$ absorption during the HOE-140 treatment. Because of technical problems with the polyfructosan, values for GFR were obtained for only a subset of the time control animals, and therefore GFR data are not reported for this group.

Infusion of HOE-140 into the left kidney did not alter excretory function of the right kidney (Table 2).

**Effect of Interstitial Infusion of HOE-140 on Renal Function of Rats Fed a Low-Salt Diet**

The MAP of both groups of rats remained unchanged throughout the baseline and experimental periods (Table 3). In vehicle-treated rats, no significant changes in left kidney hemodynamics or excretory function were observed between the baseline and experimental periods (Table 3, Figs. 4 and 5). Infusion of HOE-140 into the medullary interstitium did not alter GFR, Uv, CBF, or PBF (Table 3). In contrast to the rats fed a normal-salt diet, HOE-140 treatment did not significantly alter $U_{NaV}$ ($P = 0.062$) in the rats fed low salt. For the low-salt rats, the median $U_{NaV}$ was 98 (17– 409) nmol·min$^{-1}$·g kwt$^{-1}$ during the baseline period and 100 (11–303) nmol·min$^{-1}$·g kwt$^{-1}$ during infusion of HOE-140 (Fig. 4). Furthermore, the percent change in $U_{NaV}$ from baseline induced by HOE-140 ($–15 ± 14%$) was not different from zero ($P = 0.062$, Fig. 5).

Infusion of HOE-140 or vehicle into the left kidney did not alter right kidney Uv or GFR (Table 4). However, $U_{NaV}$ decreased between the baseline and experimental periods in both HOE-140- and vehicle-infused rats.

**Effect of Interstitial Infusion of L-NAME on Left Kidney Function of Rats Fed a Normal-Salt Diet**

Our findings indicate that in rats fed a normal-salt diet medullary BKB$2$ receptor blockade decreased sodium excretion in the absence of a change in inner medullary blood flow. The absence of an effect of intrarenal kinins on PBF contrasts with findings obtained by other investigators (9, 23, 24, 31, 41). Therefore, as a positive control we determined the effect of interstitial infusion of L-NAME on $U_{NaV}$, CBF, and PBF.

Table 2. Renal excretory function of right kidney before and during medullary interstitial infusion of HOE-140 (group 1) or vehicle (group 2) in rats fed normal-salt diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HOE-140 (n = 11)</th>
<th>Vehicle (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$U_{NaV}$, nmol·min$^{-1}$·g kwt$^{-1}$</td>
<td>45 (14–772)</td>
<td>30 (8–229)</td>
</tr>
<tr>
<td>$\Delta U_{NaV}$, %</td>
<td>–4 ± 21</td>
<td>–10 ± 20</td>
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Values are means ± SE or medians (range) for n rats. $U_{NaV}$, urinary sodium excretion rate.
Medullary Kinins Decrease Sodium Excretion

left kidney [74 (36–107) to 36 (27–57) nmol·min⁻¹·g kwt⁻¹, for baseline and L-NAME periods, respectively; \( P < 0.05 \)]. Furthermore, L-NAME infusion also induced a decrease in PBF (64 ± 6 vs. 43 ± 4 PU, for baseline and L-NAME periods, respectively; \( P < 0.05 \)). Therefore, concomitant with the 42 ± 7% decrease (\( P < 0.01 \)) in UNaV there was a 31 ± 6% decrease (\( P < 0.01 \)) in PBF (Fig. 6). In contrast, CBF was not altered by L-NAME (375 ± 16 vs. 364 ± 10 PU, for baseline and L-NAME periods, respectively; \( P > 0.05 \)).

Discussion

Renal kinins are vasodilatory and natriuretic paracrine/auto-crine factors that act via BKB₂ receptors to increase electrolyte transport. Therefore, we examined the effect of infusion of the BKB₂ receptor antagonist HOE-140 into the medullary interstitium of rats fed a normal-salt diet. Blockade of medullary nitric oxide synthase decreased UNaV and PBF but did not alter CBF. \( \dagger P < 0.01 \) compared with Baseline.

Table 4. Renal excretory function of right kidney before and during medullary interstitial infusion of HOE-140 (group 3) or vehicle (group 4) in rats fed low-salt diet

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HOE-140 (n = 11)</th>
<th>Vehicle (n = 10)</th>
</tr>
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<tbody>
<tr>
<td>( U_v, \mu l \cdot min^{-1} \cdot g \cdot kwt^{-1} )</td>
<td>4.4 ± 0.2</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>( GFR, ml \cdot min^{-1} \cdot g \cdot kwt^{-1} )</td>
<td>1.08 ± 0.07</td>
<td>1.01 ± 0.10</td>
</tr>
<tr>
<td>( UNaV, nmol \cdot min^{-1} \cdot g \cdot kwt^{-1} )</td>
<td>10 (7–99)</td>
<td>8 (4–76)</td>
</tr>
<tr>
<td>( \Delta UNaV, % )</td>
<td>-28 ± 4</td>
<td>-25 ± 7</td>
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Values are means ± SE or medians (range) for \( n \) rats. \( * P < 0.05 \), \( \dagger P < 0.01 \) compared with Baseline.
renal kinins act via medullary BKB2 receptors to tonically inhibit sodium reabsorption in euvoletic, anesthetized rats. In this earlier study, injection of HOE-140 into the lumen of distal tubules increased sodium absorption by 22%. The blockade of electrolyte reabsorption by renal kinins appears to be due either to a direct effect of the kinins on membrane transport processes or to kinin-mediated changes in prostaglandin and/or NO, which in turn, inhibit electrolyte transport (33).

The increase in tubular sodium absorption induced by medullary BKB2 receptor blockade was not accompanied by a decrease in PBF, indicating that under euvoletic conditions kinins do not act on medullary BKB2 receptors to increase PBF. This finding contrasts with the effect of blockade of renal BKB2 receptors by systemic or intrarenal arterial delivery of BKB2 receptor antagonists combined with maneuvers that increase medullary kinin levels. Previous studies have shown that acute blockade of BKB2 receptors decreased basal PBF in volume-expanded rats (9, 31), blocked the increase in PBF induced by converting enzyme inhibition (24) or volume expansion (9), and established PBF autoregulation after volume expansion (9, 27, 41). Furthermore, enhancement of medullary kinin levels by direct medullary infusion of bradykinin or the converting enzyme inhibitor captopril increased PBF and renal excretory function (23).

The effect of renal kinins on PBF is mediated by NO (23, 41). To determine whether the lack of effect of blockade of medullary BKB2 receptors on PBF in the present study was due to an altered level of NO, we examined the effect on renal regional blood flows and excretory function of a medullary infusion of the nitric oxide synthase inhibitor L-NAME. Infusion of L-NAME into the medullary interstitium induced a 42 ± 7% decrease in UNaV and a 31 ± 6% decrease in PBF. The magnitudes of the changes in sodium excretion and PBF were similar to those reported for blockade of nitric oxide synthase in previous studies (23, 41). Our findings suggest that there was no impairment of NO production in the euvoletic rats. Therefore, the lack of an effect of medullary BKB2 receptor blockade on PBF does not appear to be due to an impairment of NO activity.

Together these findings suggest that under near normal conditions renal kinins act on medullary BKB2 receptors to inhibit electrolyte transport across medullary collecting ducts. In contrast, during volume expansion (when there is a need to further decrease sodium reabsorption) or after inhibition of degradation of intramedullary kinins, at least one further mechanism is activated, i.e., kinins increase PBF, altering the pressure-natriuretic relationship and thereby indirectly inhibiting tubular electrolyte reabsorption.

Acute blockade of renal BKB2 receptors by the renal arterial delivery of BKB2 receptor antagonists during salt restriction has been shown to induce a marked decrease in Na+ and Cl− excretion (35). Furthermore, we previously reported (25) that in salt-restricted rats blockade of renal BKB2 receptors by systemic delivery of HOE-140 markedly increased Cl− and water reabsorption along the inner medullary collecting ducts (IMCD). We concluded that during salt restriction renal kinins directly and/or indirectly inhibit electrolyte reabsorption in the IMCD (25). However, in the present study, acute intramedullary interstitial infusion of HOE-140 did not alter urinary Na+ excretion in rats fed a low-salt diet. This latter finding indicates that in salt-restricted rats the effect of BKB2 receptor blockade on electrolyte reabsorption across the IMCD does not appear to be mediated through the direct interaction of renal kinins with medullary BKB2 receptors. Taken together, our findings suggest that during salt restriction, renal kinins inhibit IMCD electrolyte reabsorption indirectly by acting on BKB2 receptors located at sites proximal to the inner medulla. The mechanism(s) by which renal kinins act indirectly, upstream of the medulla, to regulate medullary collecting duct reabsorption remains to be determined. However, it is possible that the kinins may act on postglomerular vascular receptors to shunt blood flow from the cortex to the medulla, increasing PBF and/or altering pressure natriuresis, thereby indirectly inhibiting electrolyte reabsorption in the IMCD.

In summary, the findings of the present study add further support to the proposal that in the absence of perturbations in salt intake or volume status renal kinins act via medullary BKB2 receptors to tonically regulate sodium reabsorption by inhibiting electrolyte transport across medullary collecting ducts.

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