Renal Effects of Fab Fragments of Kinin Antibodies on Deoxycorticosterone Acetate-Salt-Treated Rats

Hirofumi Tomiyama, A. Guillermo Scicli, Gloria M. Scicli, and Oscar A. Carretero

The role of the renal kallikrein-kinin system in the regulation of renal function is not completely understood. Intrarenal kinins can influence renal function by acting as paracrine hormones at basolateral, luminal, or both sites in the distal nephron. To examine the role of intrarenal kinins in deoxycorticosterone acetate-salt-treated rats, which have high renal kallikrein, Fab fragments of antibradykinin antibody or DArg[Hyp'Thi3bPhe7]bradykinin, a kinin antagonist, were used to block kinins. At the dose used, the antibody (25 mg) and kinin antagonist (10 μg/min/rat) inhibited the hypotensive effect of intra-arterially injected bradykinin (100 ng) by 70% and 52%, respectively. The antibody appeared in the urine within 30 minutes after administration. Urinary volume was lowered from 9.4±0.2 to 6.7±0.4 μl/min/g kidney wt (p<0.001, paired t test) by the antibody and from 8.5±0.3 to 6.8±0.4 μl/min/g kidney wt (p<0.004, paired t test) by the kinin antagonist. The antibody lowered urine sodium excretion from 1.11±0.04 to 0.88±0.06 μeq/min/g kidney wt (p<0.001, paired t test), whereas the kinin antagonist had no significant effect. Neither altered blood pressure, renal blood flow, or glomerular filtration rate. These data suggest that in deoxycorticosterone acetate-salt-treated rats, excretion of water and sodium is regulated in part by kinins. The antidiuretic effect of the antibody and kinin antagonist might be due to blockade of kinins in the vascular-interstitial space of the kidney, since the kinin antagonist is likely hydrolyzed in the proximal tubule and does not reach the lumen of the distal nephron. The antinatriuretic effect of the antibody on sodium excretion might be due to blockade of kinins in the vascular-interstitial, urinary, or both compartments of the kidney, since the antibody appeared in the urine and since this antidiuretic effect was not observed with the kinin antagonist. (Hypertension 1990;15:761-766)

Kallikrein, a potent kinin-generating enzyme, is known to be synthesized by the connecting cells of the distal nephron and secreted into the tubular lumen. Kinins are released from kininogen in the lumen of the distal nephron and perhaps at a basolateral site where kallikrein has also been found. The kininogen needed for the release of kinins within the distal nephron can be filtered from the plasma or synthesized in the collecting duct. The role of the kallikrein-kinin system in the regulation of renal function is not completely understood. In awake normotensive rats, blocking kinins by using a kinin antagonist caused no significant changes in water or sodium excretion, whereas blocking kinins with Fab fragments of bradykinin (BK) antibodies (BK-Fab) caused a small but significant decrease in diuresis and natriuresis (unpublished results). The differences between BK-Fab and the kinin antagonist DArg[Hyp'Thi3bPhe7]BK (K-ant) might be due to hydrolysis of K-ant in the proximal tubule. Thus, the antagonist might block kinins only at the basolateral site, whereas BK-Fab might block kinins at both basolateral and luminal sites. We hypothesize that intrarenal kinins can regulate water and sodium excretion by acting as paracrine hormones on receptors in basolateral, luminal, or both sites in the distal nephron before they are rapidly destroyed by cell surface peptidases. The present study was conducted 1) to further determine the role of kinins in the regulation of renal function in deoxycorticosterone acetate (DOCA)-salt–treated rats and 2) to compare BK-Fab with K-ant. The DOCA-salt model was used because mineralocorticoids increase urinary kallikrein excretion, suggesting increased renal kallikrein-kinin activity.
Both K-ant and BK-Fab caused urine volume to decrease, but only BK-Fab lowered sodium excretion.

**Methods**

**Preparation of BK-Fab Fragments**

Kinin antibodies were produced in rabbits, and Fab fragments were prepared as described previously. Immunoglobulin G (IgG) from antiserum to BK or from nonimmunized rabbits (NRS) was isolated by diethylaminoethyl-affigel blue column (Bio-Rad, Richmond, California) and digested with papaain. Fab fragments were separated from intact IgG, Fc fragments, and other peptides with protein-A gel (Pharmacia, Uppsala, Sweden) and AcA 44 column (IBF Biotechnics, Savage, Maryland). The fraction containing Fab was concentrated by ultrafiltration with an Amicon cell concentrator (Amicon, Danvers, Massachusetts). Protein was determined by Bradford’s method (Bio-Rad). The solution containing BK-Fab had 2.5 times more $^{125}$I-[Tyr]$^8$BK-binding capacity per milliliter than the original rabbit anti-BK serum. The presence of Fab fragments and the absence of intact IgG or Fc fragments were verified by the double immunodiffusion method, using goat anti-rabbit Fab and Fc fragments of antibodies (ICN Biomedicals, Inc., Lisle, Illinois).

**Surgical Preparation**

Male Sprague-Dawley rats weighing 225–250 g (Charles River Labs., Wilmington, Massachusetts) were maintained at constant room temperature with a 12-hour light/dark cycle and given free access to tap water and standard rat chow (Ralston Purina, Lima, Ohio). The rats were anesthetized with sodium pentobarbital (40 mg/kg), and a catheter was implanted in the bladder under ether anesthesia. The experiment consisted of one control and two experimental clearance periods of 30 minutes each. The rats were divided into four groups as follows: 1) BK-Fab was infused into the inferior vena cava at a rate of 30 $\mu$l/min (25 mg/rat) for 11 minutes at the beginning of the first experimental period ($n=8$), 2) rats were infused with NRS-Fab to serve as a control for group 1 ($n=7$), 3) K-ant (10 $\mu$g/min/rat) (Bachem Inc., Torrance, California) was infused throughout the first and second experimental periods by adding it to the 0.15 M NaCl infusion ($n=8$), and 4) controls for group 3 were infused with 0.15 NaCl alone ($n=7$).

Urine collected during each clearance period was used to determine volume, osmolarity, sodium, and $[^{3}H]$inulin. At the midpoint of each period, 70 $\mu$l blood was drawn from the arterial catheter to measure hematocrit and $[^{3}H]$inulin. Urine collected at the end of each period was tested for BK-Fab.

To test whether BK-Fab infusion or K-ant infusion can block the depressor effect of BK, 12 DOCA-treated rats had arterial and venous catheters implanted, as well as an additional catheter in the ascending aorta through the right carotid artery for administration of BK; however, the bladder catheter was omitted. The next day, the unanesthetized rats were placed in plastic restraining cages, and blood pressure was recorded continuously. To increase sensitivity to BK, captopril (9 mg/kg/hr) was administered, and 100 ng BK was injected into the ascending aorta as a bolus (0.1 ml) 30 minutes later. After another 30 minutes, the rats were treated as previously described, and blood pressure response to BK was tested again.

**Analytical Technique**

Urine volume was determined gravimetrically and factored per gram of kidney weight. Urine osmolarity was measured with a freezing-point osmometer (Advanced Instruments, Needham Heights, Massachusetts), sodium and potassium concentrations were measured with a NOVA 1 ion-electrode analyzer (Nova Biochemical, Newton, Massachusetts), and $[^{3}H]$inulin in urine and plasma was measured with a liquid scintillation counter (Packard, Downers Grove, Illinois). Glomerular filtration rate (GFR) was calculated as the urine-to-plasma ratio of inulin multiplied by the urine flow rate; the change in RBF shown by the Doppler flowmeter was expressed as the percentage of change from the control period.
To determine whether BK-Fab was filtered in the glomeruli, urine samples were diluted 1,200 times with 0.1 M Tris-HCl buffer (pH 7.4) and incubated with $^{125}$I-[Tyr]$^8$BK tracer (5,000 cpm) at 37° C for 6 hours. Dextran-coated charcoal was used to separate free from bound tracer.

Statistical Analysis

All data are expressed as mean±SEM. Univariate repeated-measures analysis of variance (ANOVA) with the Greenhouse-Geisser correction was used to evaluate the group-by-time interaction effect in 1) the BK-Fab versus NRS-Fab setting and 2) the K-ant versus vehicle setting. If a statistically significant group-by-time interaction effect was detected, univariate repeated-measures ANOVA with the Greenhouse-Geisser correction was used to evaluate overall change across the three time periods within each separate group. The Bonferroni multiple comparison adjustment was used to reduce the significance level from 0.05 to 0.05/2 (0.025) for the repeated-measures ANOVA result within each group. If a statistically significant overall change across the three time periods was detected within a given group, paired $t$ tests were used to evaluate the three pairwise changes across time. The Bonferroni multiple comparison adjustment was used to reduce the significance level from 0.05 to 0.05/3 (0.017) for each paired $t$ test result.

Results

BK-Fab infusion blocked 70% of the hypotensive effect of 100 ng BK, and K-ant blocked 52% (Figure 1). Urine collected at the end of the first and second experimental periods in rats injected with BK-Fab (1,200 final dilution) bound $^{125}$I-[Tyr]$^8$BK (B/B$_0$ [binding]=20.3±3.4% and 38.5±3.1%, respectively); thus, filtration occurred. Urine from rats injected with NRS-Fab did not bind $^{125}$I-[Tyr]$^8$BK.

Figure 2 shows the changes in urinary sodium volume, urinary osmolarity, and sodium excretion. When testing for a significant group-by-time interaction effect using repeated-measures ANOVA, statistical significance was detected for all three variables in the BK-Fab versus NRS-Fab setting ($p<0.016$ for sodium excretion, $p<0.005$ for urinary volume, and $p<0.005$ for urinary osmolarity). Similarly, for the K-ant versus vehicle setting, a statistically significant group-by-time interaction effect was detected for urinary volume ($p<0.043$) and urinary osmolarity ($p<0.041$) but not for sodium excretion ($p>0.94$).

Given the statistically significant interaction results from the BK-Fab versus NRS-Fab setting, the additional repeated-measures ANOVA results indicate that an overall change across time was detected within the BK-Fab infusion group of rats with regard to urinary volume ($p<0.003$), urinary osmolarity ($p<0.009$), and sodium excretion ($p<0.022$). Subsequent paired $t$ test results within the BK-Fab infusion group of rats indicate that, from the control period to the first experimental period, sodium excretion and urinary volume both decreased ($p<0.001$ in each case) and urinary osmolarity increased ($p<0.006$). During the second experimental period, all three parameters tended to return to approximate control

![Figure 1](image1.png)

**Figure 1.** Bar graph showing effect of BK-Fab and K-ant on the depressor effect (Δ blood pressure) of bradykinin. NRS-Fab, Fab fragments of normal rabbit serum; BK-Fab, Fab fragments of antibradykinin serum; K-ant, kinin antagonist.

![Figure 2](image2.png)

**Figure 2.** Plots of effect of BK-Fab and NRS-Fab (left) and K-ant and its vehicle (right) on urinary sodium ($U_{NaV}$), volume ($UV$), and osmolarity ($U_{osmol}$). *p<0.05, **p<0.01 by repeated-measures analysis of variance (group×time interaction effect), tp<0.017 by paired $t$ test with the Bonferroni multiple comparison adjustment (control vs. first experimental period). NRS-Fab, Fab fragments of normal rabbit serum; BK-Fab, Fab fragments of antibradykinin serum; K-ant, kinin antagonist.
TABLE 1. Effect of Fab Fragments of Antibradykinin Serum, Fab Fragments of Normal Rabbit Serum, Kinin Antagonist, and Sodium Chloride on Renal Hemodynamics

<table>
<thead>
<tr>
<th></th>
<th>BK-Fab (n=8)</th>
<th>NRS-Fab (n=7)</th>
<th>K-ant (n=8)</th>
<th>NaCl (n=7)</th>
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</thead>
<tbody>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C</td>
<td>118±3</td>
<td>116±3</td>
<td>122±4</td>
<td>117±8</td>
</tr>
<tr>
<td>E1</td>
<td>119±4</td>
<td>116±2</td>
<td>121±4</td>
<td>114±8</td>
</tr>
<tr>
<td>E2</td>
<td>121±2</td>
<td>117±3</td>
<td>119±4</td>
<td>114±9</td>
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<tr>
<td>GFR (μl/min/g kidney wt)</td>
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<tr>
<td>C</td>
<td>1.11±0.11</td>
<td>1.25±0.13</td>
<td>1.08±0.14</td>
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<tr>
<td>E1</td>
<td>1.18±0.18</td>
<td>1.31±0.17</td>
<td>1.21±0.16</td>
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<tr>
<td>E2</td>
<td>1.12±0.17</td>
<td>1.25±0.17</td>
<td>1.07±0.08</td>
<td>1.13±0.18</td>
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<td>Renal blood flow (kHz)</td>
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<tr>
<td>C</td>
<td>11.4±0.9</td>
<td>11.5±0.9</td>
<td>11.1±1.4</td>
<td>10.7±1.3</td>
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<tr>
<td>E1</td>
<td>11.4±1.0</td>
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<td>11.2±1.4</td>
<td>10.4±1.4</td>
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<td>E2</td>
<td>11.3±1.0</td>
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<td>11.3±1.5</td>
<td>10.6±1.4</td>
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<td>Renal blood flow (% change)</td>
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<tr>
<td>E1</td>
<td>-0.2±1.4</td>
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<td>0.5±1.2</td>
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<td>5.3±5.6</td>
<td>1.0±0.9</td>
<td>-1.7±3.4</td>
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<td>Urine potassium excretion (μeq/min/g kidney wt)</td>
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<tr>
<td>C</td>
<td>0.51±0.05</td>
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<tr>
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<td>0.54±0.05</td>
<td>0.46±0.05</td>
<td>0.56±0.05</td>
</tr>
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</table>

Values are mean±SEM. BK-Fab, Fab fragments of antibradykinin serum; NRS-Fab, Fab fragments of normal rabbit serum; K-ant, kinin antagonist; NaCl, sodium chloride; C, control period; E1 and E2, first and second experimental periods; GFR, glomerular filtration rate.

Given the statistically significant interaction results from the K-ant versus vehicle setting, the additional repeated-measures ANOVA results indicate that within the K-ant infusion group of rats, only urinary volume exhibited an overall change across time (p<0.022). Subsequent paired t test results within the K-ant infusion group show that urinary volume decreased from the control period to the first experimental period (p<0.004). No statistically significant changes across time were detected in the vehicle group.

Neither BK-Fab infusion nor K-Ant infusion had a significant effect on the other variables studied (Table 1). In the BK-Fab versus NRS-Fab setting, the repeated-measures ANOVA results did not show a significant group-by-time interaction for mean blood pressure (p<0.77), GFR (p<0.99), RBF kHz (p<0.93), RBF percent change (p<0.40), potassium excretion (p<0.70), or urinary pH (p<0.83). Furthermore, in the K-ant versus vehicle setting, the repeated-measures ANOVA results did not show a significant group-by-time interaction for mean blood pressure (p<0.74), GFR (p<0.40), RBF kHz (p<0.57), RBF percent change (p<0.81), potassium excretion (p<0.75), or urinary pH (p<0.93).

Discussion

Both BK-Fab infusion and K-ant infusion partially blocked the vasodepressor effect of a high dose of BK (100 ng); BK-Fab appeared in the urine within 30 minutes. We used BK-Fab fragments instead of the intact antibodies because BK-Fab is rapidly distributed in the extracellular fluid and is filtered and excreted by the kidney10,11; thus, it can block kinins not only in the vascular-interstitial space but also the nephron lumen. Fab fragments do not form high-molecular-weight complexes or activate complement and other proteolytic systems in plasma, possibly altering blood pressure and renal function.

K-ant is a small peptide analogue of BK that antagonizes the renal vascular effect of kinins by competing with them for their receptors. While it is distributed rapidly in the extracellular space and filtered by the kidney, it does not reach the lumen of the distal nephron (where kinins are released). This might be due to hydrolysis by cell surface peptidases, probably in the proximal tubule.2-12 We have administered radioactive K-ant to normal rats and found no intact tracer in the urine (unpublished results). Thus, a major difference between these two pharmacological tools is that BK-Fab might block kinins in the lumen of the distal nephron, whereas K-ant does not.

We used mineralocorticoid-treated and salt-treated rats because of their pronounced urinary kallikrein excretion, suggesting high activity of the kallikrein-kinin system.34 Furthermore, previous studies have suggested that the kallikrein-kinin system might participate in the regulation of water and sodium excretion under such conditions.13-15 This model also exhibits high antidiuretic hormone, which is known to stimulate release of kinin in the nephron.16,17 The studies were performed 2 weeks after DOCA-salt pretreatment, before hypertension developed, to avoid secondary renal damage that can
alter the integrity of the renal kallikrein-kinin system and thus water and sodium excretion; additionally, rats were unanesthetized to avoid any untoward effect on water and sodium excretion.

Both BK-Fab infusion and K-ant infusion decreased urinary volume and increased urine osmolality; BK-Fab infusion decreased urinary sodium as well. These results support the hypothesis that, in this volume-expanded model, kinins have diuretic and natriuretic capability. These effects were not secondary to changes in blood pressure, total RBF, or GFR because they did not change after the administration of either blocker. It is possible, however, that the changes in water and sodium excretion were mediated by altered renal blood distribution. Roman et al.18 have reported that, although K-ant infusion does not affect flow in the outer cortex, it significantly decreases papillary blood flow. In their study, changes in urinary volume and sodium were not very consistent; however, their experimental conditions were different in that the rats were not treated with mineralocorticoids, and the experiments were performed under anesthesia, which decreases water and sodium excretion. The effect of kinins on renal function could be mediated by prostaglandins, endothelium-derived relaxing factor, or both, since BK infusion has been shown to stimulate their release.14,19

Because both BK-Fab infusion and K-ant infusion decrease urinary volume and increase urinary osmolality, this suggests that the kinin receptors that mediate their diuretic effect are localized to a basolateral site in the nephron. This agrees with Schuster,20 who reported that, in the isolated perfused rabbit cortical collecting tube, addition of lysyl-BK to the basolateral but not the luminal surface inhibits the hydro-osmotic response to antidiuretic hormone in a prostaglandin-mediated manner.

It could be that kinins have a diuretic effect by stimulating the release of prostaglandin, which in turn antagonizes antidiuretic hormone in the basolateral surface of the nephron where its receptors are localized.20

The fact that BK-Fab infusion decreased natriuresis whereas K-ant infusion did not could be explained if BK-Fab infusion blocks kinins in both the vascular-interstitial space and the lumen of the nephron but K-ant infusion does not. Kaufer21 has shown that kinins administered into the late proximal nephron double the excretion of Na that was simultaneously perfused into the tubule; thus, kinins acting in the luminal receptors could cause natriuresis. On the other hand, Tomita et al.22 using isolated perfused tubules, reported that kinins inhibit sodium transport in DOCA-salt-treated rats only when applied to the basolateral surface. Another possibility is that BK-Fab infusion is more effective than K-ant infusion because it blocks kinins at both the basolateral and luminal surfaces of the nephron or because K-ant infusion has some residual agonistic effect that can mask its antinatriuretic effect. Independent of the site of action, the effect of BK-Fab infusion suggests that endogenous kinins have a natriuretic effect.

The effect of BK-Fab infusion on water and sodium excretion was of short duration, being observed only during the first experimental period (between 0 and 30 minutes). Previously, we found that in normal rats the maximum antidiuretic and antinatriuretic effect of BK-Fab infusion was between 40 and 80 minutes; however, in that study, the rats were not DOCA-salt–treated. It might be that volume expansion alters the duration of the BK-Fab infusion effect. The limited effect of BK-Fab infusion could be due to decreased plasma concentration because renal clearance started immediately after infusion; however, it cannot be due to decreased concentration in the lumen of the nephron, since its concentration in the urine was higher during the second than during the first experimental period. Other factors that control water and sodium excretion compensate for lack of kinins.

Aprotinin, a serine protease inhibitor that also inhibits renal kallikrein, has been reported to decrease RBF, urinary sodium, water, and prostaglandin excretion and renin secretion.14,23-25 These results, however, are not universal26 and should be interpreted with caution because aprotinin is a nonspecific inhibitor of kallikrein and has been reported to inhibit myocardial contractility. Also, aprotinin is a highly cationic peptide that at high doses can exert charge-related effects such as increased capillary permeability.27 Recently, Nakagawa et al.28 have shown that neither aprotinin nor K-ant infusion alters water and sodium excretion in DOCA-treated rats; however, in their study the rats received water instead of saline and were under anesthesia during the study. Experimental differences that affect intrarenal kinin release and sodium and water excretion might explain the discrepancy.

In summary, in DOCA-salt–treated rats, K-ant infusion and BK-Fab infusion decreased urine flow and increased osmolality; BK-Fab infusion also lowered sodium excretion. These changes were not secondary to changes in blood pressure, total RBF, or GFR. Thus, renal kinins acting as paracrine hormones might participate in the control of water and sodium excretion in rats given mineralocorticoids and salt.

References

KEY WORDS • kallikrein • antibodies • bradykinin • kinins • water balance • sodium excretion • rat studies
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