Suppression of Rat Deoxycorticosterone-Salt Hypertension by Kallikrein-Kinin System

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Brown Norway kininogen-deficient rats had very low levels of plasma kininogens and lower levels of plasma prekallikrein, compared with those of normal rats of the same strain. Systolic blood pressure, determined by the tail-cuff method, of 5-week-old kininogen-deficient rats (106±0.4 mm Hg, n=7) and the rate of systolic blood pressure increase with age were not different from those in normal rats. Weekly injections of deoxycorticosterone acetate (5 mg/kg s.c.) with 1% sodium chloride solution in drinking water after uninephrectomy at 7 weeks of age caused a gradual increase in the blood pressure of normal rats, reaching a plateau at 18 weeks of age, whereas that of deficient rats rose rapidly to 158±6 mm Hg 2 weeks after the start of treatment and continued to increase significantly higher than normal rats at 8, 9, 10, 11, and 12 weeks of age (p<0.05 or 0.01). The levels of urinary prokallikrein and active kallikrein were slightly higher in deficient rats before deoxycorticosterone acetate-salt treatment but were not significantly increased after this treatment, whereas these levels in normal rats were increased 3.6- and 4.7-fold by this treatment. Urinary free kinin, collected from the ureter in untreated deficient rats, was below the detection limit. The plasma level of low molecular weight kininogen, the substrate of glandular kallikrein, was decreased in normal rats during the treatment. Continuous subcutaneous injection of aprotinin by an osmotic pump to normal rats induced significant increase in blood pressure. These results indicate that glandular kallikrein may play a suppressive role in deoxycorticosterone acetate-salt hypertension. (Hypertension 1991;17:806–813)
Animals

BN-Ka rats (Rattus norvegicus, BN/F(Mai) were obtained from the Katholieke Universiteit of Leuven, Belgium. Normal rats of the same strain were transferred from the Microbiological Association, Frederick, Md. and kept at Kitasato University (BN-Ki). Male rats of both strains (5–20 weeks old) were used. The numbers of animals used for individual experiments are described in the corresponding sections.

Methods

Blood Collection

Under ether anesthesia, blood was collected from the carotid artery of each strain of rats into plastic tubes containing 1/10 volume of 3.8% sodium citrate or in siliconized glass tubes (VT-032NA, TERUMO, Tokyo) containing EDTA (final concentration: 1 mg/ml plasma) and centrifuged at 1,500g for 15 minutes at 25°C to separate plasma.

Systolic Blood Pressure Measurement

Systolic blood pressure (SBP) of unanesthetized BN strain rats was determined weekly using a tail-cuff plethysmography method (model UR-1000, Ueda Seisakusho, Tokyo). Each pressure value was obtained by averaging five to six individual readings.

Deoxycorticosterone Acetate–Salt Hypertension

The left kidney of rats of both strains was removed at 7 weeks of age (150–170 g body wt) while the rat was under light ether anesthesia. After the operation, the rats received 1% NaCl solution ad libitum, and DOCA solution (5 mg/kg, Sigma Chemical Co., St. Louis, Mo.) was injected subcutaneously at the back of the neck once a week, according to an already reported method.17

Blood Collection

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Measurements of Plasma Prekallikrein and Kininogens

The residual level of plasma prekallikrein was determined according to a previously reported method.18 Briefly, plasma was activated by kaolin in the presence of acetone, and plasma kallikrein activity was determined using a peptidyl fluorogenic substrate selective for plasma kallikrein, Z-Phe-Arg-methylcoumarinylamide (Peptide Institute Inc., Osaka, Japan). One arbitrary unit (AU) was defined as the activity of plasma kallikrein that released 1 x 10^-10 mol aminomethylcoumarin (AMC) for 10 min/μl plasma. The difference between the amidolytic activities in the presence of soya bean trypsin inhibitor (Worthington Biochemical Co., Freehold, N.J.) and lima bean trypsin inhibitor (Worthington) was considered to represent the plasma kallikrein activity. The residual levels of plasma prekallikrein were expressed in arbitrary units per milligram plasma protein.18

The residual levels of kininogens were measured by previously reported methods.19,20 For HMW kininogen, plasma was incubated with glass powder (Ballotini, Jencons Scientific Ltd., Hemel Hempstead, UK) in the presence of o-phenanthroline (2 mg/ml plasma, Wako Pure Chemical Co., Osaka, Japan). HMW kininogen was converted to kinin by activation of plasma prekallikrein through activation of coagulation Factor XII. Bradykinin released was assayed by a bradykinin enzyme immunoassay kit (Markit-A, Dainippon Pharmaceutical Co., Osaka, Japan). For LMW kininogen, plasma was incubated with glass powder in the absence of o-phenanthroline. Thus, kinin converted from HMW kininogen by plasma kallikrein through activated Factor XII was destroyed by kininases in plasma. The HMW kininogen-depleted plasma was acidified to pH 2.0 and was incubated with trypsin (twice-crystallized, salt-free, from bovine pancreas, Nutritional Biochemical Co., Cleveland, Ohio) after neutralization. Kinin released from LMW kininogen by trypsin was measured by bradykinin enzyme immunoassay.19,20 The method was slightly modified from the original study, so that the dose of trypsin was reduced to 0.1 mg/ml plasma and the incubation time was prolonged to 120 minutes to avoid the release of T-kinin from T-kininogen in rats. Kinins formed by this determination method were analyzed by reversed-phase, high-performance liquid chromatography (Trirotor III, Japan Spectroscopic Co., Ltd., Tokyo) in combination with enzyme immunoassay of bradykinin. This analysis revealed that the amount of T-kinin formed by this method was less than 5% of bradykinin and the amounts of the former contaminated was below the detection limit for bradykinin enzyme immunoassay in the incubation mixture for LMW kininogen assay. The LMW kininogen levels were also determined in the HMW kininogen-depleted plasma by incubation of plasma with highly purified kininogenase from snake venom (Agkistrodon halys blomhoffii),21 since this kininogenase releases kinin from HMW and LMW kininogens, but not from T-kininogen.12 The LMW kininogen levels were not significantly different from those obtained with the original method (data not shown). The kininogen levels were expressed as the amounts (nanograms) of bradykinin equivalent per milligram protein. The levels of plasma prekallikrein and HMW kininogen in mutant BN-Ka rats was determined after partial replacement (25%, 50%, and 75%) of BN-Ka plasma with normal BN-Ki plasma.22,23

Measurements of Urine Volume, Urinary Creatinine, and Sodium and Potassium in Urine

Urine from individual rats was collected in a metabolic cage for 24 hours and urine volume was measured. The urinary creatinine levels were determined by a kinetic method using the Jaffe's reaction.24 Sodium and potassium were determined with coated wire electrodes selective for sodium or potassium.25
Measurements of Plasma Renin and Plasma Protein

For the renin activity in EDTA-treated plasma, plasma was incubated at 37°C for 90 minutes and the amount of angiotensin I generated was measured by radioimmunoassay kit (Gamma Coat plasma renin activity kits, Baxter Healthcare Corp., Cambridge, Mass.). The plasma renin activity was expressed in terms of nanograms of angiotensin I per milliliter plasma during a 1-hour period.

The total plasma protein was measured by the method of Lowry et al. 29

Continuous Administration of Aprotinin

Six days after the onset of DOCA administration and 1% NaCl solution in drinking water, 11 uninephrectomized rats of the normal BN-Ki strain received continuous subcutaneous administration of aprotinin (a gift of Bayer, Leverkusen, FRG) (10^5 KIU/24 μl/day, dissolved in physiological saline) for 7 days via an osmotic minipump (model 2001, Alza Corp., Palo Alto, Calif.), implanted subcutaneously into the back. For control studies, 10 rats received vehicle physiological saline (24 μl/day) via the same type of osmotic minipump in the back. During the osmotic pump treatment, both rats were treated by weekly injection of DOCA and 1% NaCl drinking solution in the same way as that described above. SBP was determined by tail-cuff method. After the last blood pressure measurement, individual animals were put into the metabolic cages for 24 hours, and urinary active kallikrein was determined by the method described above.

Statistical Analyses

All data are expressed as mean±SEM. Student’s t test was used to evaluate the significance of difference. When variances were heterogeneous, statistical analyses were performed by the Aspin-Welch method or the Wilcoxon’s rank sum test.

Results

The kallikrein-kinin system in both strains of rats is shown in Table 1. The plasma level of HMW kininogen in mutant BN-Ka rats 10 weeks of age was below the detection limit; that of LMW kininogen was also very low in mutant BN-Ka rats (0.06±0.01 ng bradykinin equivalent/mg protein, n=5), whereas the plasma concentrations of HMW and LMW kininogens in BN-Ki rats (15.9±0.7 and 8.7±0.5 ng bradykinin equivalent/mg protein [n=5]), respectively) were approximately the same as those in Sprague-Dawley strain rats (12.0±1.2 and 9.7±1.4 ng bradykinin equivalent/mg protein, respectively. 18) The re-
whereas that of mutant BN-Ka rats rose rapidly to 180±10 mmHg, 12 weeks old. The SBPs of mutant BN-Ka rats at 8, 9, 10, 11, and 12 weeks old (130±5, 158±6, 163±5, 171±6, and 181±7 mmHg) were significantly (p<0.05 or 0.01) higher than those in normal BN-Ki rats (120±3, 134±5, 144±4, 148±4, and 153±6 mmHg, respectively). Plasma renin levels did not differ between the two strains in untreated animals at 10 weeks of age (Table 1). These activities, however, were markedly reduced by the DOCA-salt treatment, but again they were not different in the two strains.

Table 2 shows the urine volume, creatinine amounts, and sodium and potassium excretion of nontreated BN-Ka rats and BN-Ki rats at 10 weeks of age in urine collected over 24 hours. The values were virtually the same in the two strains. As shown in Figure 2, the mean values of prokallikrein in the urine of untreated, kininogen-deficient BN-Ka rats of 10 weeks of age, expressed in terms of amounts of creatinine, were slightly higher than those in BN-Ki rats, but the differences were not statistically significant. The level of free kinin in the urine of normal BN-Ki rats was 113.2±50.3 ng/24 hr (n=4), whereas it was below the detection limit (less than 4.8 ng/24 hr, n=4) in kininogen-deficient BN-Ka rats, despite the presence of urinary kallikrein.

During DOCA-salt treatment, the 24-hour urine volumes for normal BN-Ki rats were increased markedly (8.6-fold) (Table 2), but the values were dispersed (8, 10, 16, 190, and 250 ml/24 hr) during the treatment, compared with those (9, 10, 11, 11, 11, 11, and 14 ml/24 hr) without the treatment, and the differences were not statistically significant. The urine volumes for BN-Ka rats were slightly increased (2.5-fold) and dispersed (7, 8, 17, 12, and 67 ml/24 hr during the treatment versus 6, 8, 9, 10, 13, and 14 ml/24 hr without the treatment). The creatinine amounts were decreased in both strains during the treatment (Table 2), and the reduction was significant in normal BN-Ki rats (p<0.05). Sodium excretion was significantly increased in both strains of rats, but the mutant BN-Ka rats excreted less amounts. Potassium excretion was changed slightly during DOCA-salt treatment, but the differences were not significant.

As shown in Figure 2, the urinary levels of prokallikrein (0.7±0.2 AU/µg creatinine) and active kallikrein (1.4±0.7 AU/µg creatinine) in normal 10-week-old BN-Ki rats were increased by DOCA-salt treatment to 2.5±0.4 AU/µg creatinine (3.6-fold) and 6.6±3.1 AU/µg creatinine (4.7-fold), respectively. In contrast, the urinary levels of prokallikrein (1.1±0.2 AU/µg creatinine) and active kallikrein (2.4±0.4 AU/µg creatinine) in mutant BN-Ka rats were not significantly increased (1.9±0.3 and 4.2±1.4 AU/µg creatinine, respectively). Thus, it can be seen that urinary prokallikrein and active kallikrein were less responsive to DOCA-salt treatment in BN-Ka rats than in the normal BN-Ki rats at a time of maximal blood pressure increase and retarded sodium excretion.
TABLE 2. Urine Volumes, Creatinine Amounts, and Sodium and Potassium Excretion in Nontreated and Deoxycorticosterone Acetate-Salt Treated Rats (10 Weeks Old)

<table>
<thead>
<tr>
<th>Rats/treatment</th>
<th>Urine volume (ml/day)</th>
<th>Creatinine (mg/day)</th>
<th>Sodium/Cr (mg/mg Cr/day)</th>
<th>Potassium/Cr (mg/mg Cr/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN-Ka (mutant)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (n=7)</td>
<td>9.7±1.0</td>
<td>10.2±2.2</td>
<td>2.0±0.5</td>
<td>6.4±1.6</td>
</tr>
<tr>
<td>DOCA-salt treated (n=5)</td>
<td>24.2±11.1</td>
<td>8.1±0.5</td>
<td>14.4±6.1*</td>
<td>4.6±0.2*</td>
</tr>
<tr>
<td>BN-Ki (normal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated (n=6)</td>
<td>11.0±0.7</td>
<td>13.7±2.1</td>
<td>2.0±0.4</td>
<td>6.1±1.8</td>
</tr>
<tr>
<td>DOCA-salt treated (n=5)</td>
<td>94.8±52.0</td>
<td>6.0±0.5*</td>
<td>62.7±34.4*</td>
<td>8.5±1.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM of the numbers (n) of animals indicated. Values in the deoxycorticosterone acetate (DOCA)-salt treated groups were compared with those in the nontreated groups. Numbers in parentheses indicate percent values of DOCA-salt-treated rats, compared with those in nontreated animals (100%). Sodium and potassium concentrations are expressed in terms of creatinine excretion (Cr) for 24 hours. BN-Ka, Brown Norway Katholiek; BN-Ki, Brown Norway Kitasato.

![Bar graphs showing excretion of urinary prokallikrein and active kallikrein in Brown Norway Katholiek (BN-Ka) and Brown Norway Kitasato (BN-Ki) (10 weeks old) under no treatment and during deoxycorticosterone acetate (DOCA)-salt treatment.](http://hyper.ahajournals.org/)

**Discussion**

Mutant BN-Ka rats were reported to be congenitally abnormal in kallikrein-kinin systems.6-12 Very low levels of HMW and LMW kininogens in plasma were confirmed in the present experiments, and the plasma prekallikrein level in mutant BN-Ka rats was only one third of that of normal BN-Ki rats, as shown in Table 1. The low plasma prekallikrein level was also observed in patients deficient in HMW kininogen,22-30-33 as this kininogen acts as a carrier protein of plasma prekallikrein.34 Urinary prokallikrein and active kallikrein levels in BN-Ka rats were slightly higher than those in normal BN-Ki rats, but the free kinin in the urine of the former strain was below the...
Subcutaneous administration of aprotinin (10^5 KIU/day) by an osmotic minipump caused a significant elevation of the systolic blood pressure in BN-Ki rats (closed circles, n=11), compared with that of the control BN-Ki rats, which received physiological saline (24 μl/day) (open circles, n=10). All rats were treated with DOCA and salt. The following two observations may provide further strong support for the suppressive role of glandular kallikrein-kinin system in the initial increase of DOCA-salt hypertension of normal BN-Ki rats in vivo: First, constant infusion of aprotinin (an inhibitor of plasma and glandular kallikreins) for 7 days by a subcutaneous osmotic minipump implanted in normal uninephrectomized BN-Ki rats caused a significant rise of SBP during DOCA-salt treatment (Figure 3).

Second, the significant reduction of LMW kininogen in the plasma during DOCA-salt treatment was observed without change in the levels of plasma protein or hematocrit, or even in the HMW kininogen level. The plasma kallikrein-HMW kininogen system and the glandular kallikrein-LMW kininogen system operate separately in the body, and the reduction of the level of either kininogen can be interpreted as a consumption of that kininogen and the involvement of the corresponding kallikrein-kinin system, since kininogen, particularly HMW kininogen, did not recover in rats to the previous level before 72 hours after the depletion. Thus, the reduced plasma level of LMW kininogen may be attributed to increased activity of glandular kallikrein. In fact, the urinary kallikrein level was elevated during DOCA-salt treatment so that the enhanced activity of glandular kallikrein in BN-Ki rats can be reflected by the increased excretion of urinary kallikrein.

In normal BN-Ki rats 10 weeks old, when the SBP of mutant BN-Ka rats had almost reached a plateau, urinary excretion of prokallikrein and active kallikrein in normal BN-Ki rats increased to reach 3.6 and 4.7 times, respectively, as much as the values in rats of the same age without the treatment. These significant increases of urinary prokallikrein and kallikrein were accompanied with large increases in urine volume and urinary sodium excretion, but not in potassium excretion. The raised excretion of urinary kallikrein in normal BN-Ki rats during DOCA-salt treatment in the present experiment confirms the finding of a previous report, that rats with DOCA-salt hypertension excrete more kallikrein in the urine than control rats, although the values of the previous report were not corrected by the amounts of creatinine.

Thus, it is plausible that the lack of a hypotensive system may be causative of increase in SBP in mutant BN-Ka rats.

**Graphs showing effect of continuous administration of aprotinin on systolic blood pressure of normal Brown Norway Kitasato (BN-Ki) rats under deoxycorticosterone acetate (DOCA)-salt treatment.** Values indicate mean±SEM of from 10 to 11 animals. Subcutaneous administration of aprotinin was reduced markedly during DOCA-salt treatment at the same degree in both strains of rats (Table 1).
DOCA-salt hypertension

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BN-Ki</th>
<th>BN-Ka</th>
</tr>
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<tbody>
<tr>
<td>Kinogens</td>
<td>LMW</td>
<td>-</td>
</tr>
<tr>
<td>Urinary kallikrein</td>
<td>↑ ↑</td>
<td>↑</td>
</tr>
<tr>
<td>Active-kallikrein</td>
<td>↑ ↑</td>
<td>↑</td>
</tr>
<tr>
<td>Prokallikrein</td>
<td>↑ ↑</td>
<td>↑</td>
</tr>
<tr>
<td>Sodium excretion</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Early SBP increase</td>
<td>↑</td>
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</tr>
</tbody>
</table>


It has been reported that urinary kallikrein is excreted from the distal tubules of the nephrons and the collecting tubules of the renal cortex and medulla show a high affinity for the binding of [3H]bradykinin in rabbits. Kinin may inhibit the water reabsorption stimulated by the antidiuretic hormone through the formation of prostaglandins, or the reabsorption of sodium and chloride under the stimulus of mineral corticoids or the antidiuretic hormone.

From the present experiments, it is reasonable to conclude that increased production of glandular kallikrein, possibly urinary kallikrein, may contribute to suppression of SBP in the initial development of DOCA-salt hypertension.

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References


KEY WORDS • kallikrein • kinins • deoxycorticosterone • kininogens • kininogen-deficient rats
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