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)

It was pointed out early on that kallikrein may be related to hypertensive diseases.1 Recently, urinary kallikrein levels were reported to be lower in patients with essential hypertension than in normotensive controls2–5 but were normal in renal artery stenosis and raised in pheochromocytoma and primary aldosteronism.2 Spontaneously hypertensive rats and rats with deoxycorticosterone acetate (DOCA)–salt hypertension showed greater increases in urinary kallikrein excretion than normotensive Wistar control rats from the National Institutes of Health.3 However, the role of the endogenous kallikrein-kinin system in development of hypertension remains unclear.

The kallikrein-kinin system is a system for generating vasodilating peptides, bradykinin, or kallidin, by means of proteolytic enzymes, plasma, or glandular kallikrein, from its own precursor protein, high molecular weight (HMW) or low molecular weight (LMW) kininogen. Brown Norway Katholiek (BN-Ka) rats have been reported to have a congenitally abnormal kallikrein-kinin system6–8 and have been extensively studied and compared with normal rats of the same strain (BN-Kitasato [BN-Ki]).9–19 In a previous study, it was reported that not only HMW but also LMW kininogens were lacking in plasma from mutant BN-Ka rats, and only T-kininogen was present.7–12 Because of the lack of HMW kininogen, mutant BN-Ka strain rats accumulated less inflammatory exudate in experimental pleurisies than did normal BN-Ki rats, and the exudation in the former was not increased by pretreatment with captopril (a kininase II inhibitor).13 The same was true of the degree of swelling in edema of the paw induced in rats by plantar injection of carrageenan.14,15 Furthermore, it is interesting that these mutant BN-Ka rats did not excrete free kinin into the urine collected from the...
ureter. These findings prompted us to study the role of the kallikrein-kinin system in DOCA-salt hypertension using BN-Ki and BN-Ka rats.

**Methods**

**Animals**

BN-Ka rats (Rattus norvegicus, BN/fMai) 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.

**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.

**Measurements of Plasma Prekallikrein and Kininogens**

The residual level of plasma prekallikrein was determined according to a previously reported method. 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.

The residual levels of kininogens were measured by previously reported methods. For HMW kininogen, plasma was incubated with glass powder (Balloctini, Jencons Scientific Ltd., Hemel Hempstead, UK) in the presence of o-phenanthrolone (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-phenanthrolone. 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. 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 (Tirrotori III, Japan Spectroscope 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 blomhoffi), since this kininogenase releases kinin from HMW and LMW kininogens, but not from T-kininogen. 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. 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. Sodium and potassium were determined with coated wire electrodes selective for sodium or potassium.
Measurements of Urinary Kallikreins

The activity of the active kallikrein in the urine collected for 24 hours was measured using a peptidyl fluorogenic substrate selective for glandular kallikrein, Pro-Phe-Arg-methylcoumarinylamide (Peptide Institute). One microliter urine was incubated with 1 ml of 5χ10^-5 M substrate solution in 0.05 M Tris-HCl buffer, containing 0.1 M NaCl and 0.01 M CaCl_2 (pH 8.0). One AU was defined as the amount of urinary kallikrein that released 1 χ 10^-10 mol AMC per 10 min/μl urine, and the activity was expressed in terms of milligrams of creatinine. The amidase activity in urine was inhibited by soya bean trypsin inhibitor (0.5 μg/μl urine) by 12.9±4.8% for BN-Ka rats (n=3) and 15.8±6.5% for BN-Ki rats (n=4) 10 weeks of age. The activity of urinary prokallikrein was determined by the increase in kallikrein activity after treatment with trypsin,26 which was determined by the same method as for urinary active kallikrein.

Measurement of Urinary Kinin

Free kinin in the urine was collected from the ureter of untreated rats of both strains under pentobarbital anesthesia (60 mg/kg s.c.) by insertion of catheters (PE-10, Clay Adams, Parsippany, N.J.) to both ureters. The kinin levels were determined by bradykinin enzyme immunoassay after separation with a Sep-Pak C18 column (Waters Associates, Milford, Mass.).27

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 125I plasma renin activity kits, Baxter Healthcare Corp., Cambridge, Mass.).28 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 differences. When variances were heterogeneous, statistical analyses were performed by the Aspin-Welch type of t 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 within 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). The re-
whereas that of mutant BN-Ka rats rose rapidly to (Figure 1), weeks of age (180±10 mmHg, n=1).

BN-Ki rats increased gradually with time during DOCA-salt treatment and reached a plateau at 18 weeks of age in both strains, showing 130 ±2 mm Hg in BN-Ka rats (rc=1).

SBP reached a plateau at 13 weeks of age in both strains, as Figure 1 shows. The spontaneous increase in SBP with age also did not differ between the strains, as Figure 1 shows. The ordinate shows systolic blood pressure (mm Hg) and abscissa indicates age in weeks. Values show mean±SEM. Pressure changes with age were plotted against the age under no treatment (open circles, BN-Ka and open triangles, BN-Ki). Closed circles (BN-Ka) and closed triangles (BN-Ki) indicate systemic blood pressure changes during DOCA-salt treatment after removal of left kidney at 7 weeks of age (Ope). Rats received 1% NaCl drinking water immediately after the operation and subcutaneous injection of DOCA once a week from the eighth week (DOCA). In experiments for DOCA-salt treatment, two series of experiments were combined, and the numbers of rats for each value varied from seven to 16 for BN-fG rats and from five to 14 for BN-Ka rats. Blood pressure values of BN-Ka rats were compared each week with those of BN-Ki. *p<0.05, **p<0.01.

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, 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.

FIGURE 1. Line graph showing changes with age in systolic blood pressure of normal Brown Norway Kitasato (BN-Ki) and mutant Brown Norway Katholiek (BN-Ka) rats under no treatment and during deoxycorticosterone acetate (DOCA)-salt treatment. Ordinate shows systolic blood pressure (mm Hg) and abscissa indicates age in weeks. Values show mean±SEM. Pressure changes with age were plotted against the age under no treatment (open circles, BN-Ka and open triangles, BN-Ki). Closed circles (BN-Ka) and closed triangles (BN-Ki) indicate systemic blood pressure changes during DOCA-salt treatment after removal of left kidney at 7 weeks of age (Ope). Rats received 1% NaCl drinking water immediately after the operation and subcutaneous injection of DOCA once a week from the eighth week (DOCA). In experiments for DOCA-salt treatment, two series of experiments were combined, and the numbers of rats for each value varied from seven to 16 for BN-Ka rats and from five to 14 for BN-Ka rats. Blood pressure values of BN-Ka rats were compared each week with those of BN-Ki. *p<0.05, **p<0.01.

Residual prekallikrein level in the plasma of BN-Ka rats was also lower than in BN-Ki rats. These results clearly indicate that BN-Ka rats have abnormal levels in the components of the kallikrein-kinin system in plasma.

SBP of mutant BN-Ka rats 5 weeks old was 106±0.4 mm Hg (n=7), which was not different from that of BN-Ki rats (104±1.0 mm Hg, n=4). The rate of the spontaneous increase in SBP with age also did not differ between the strains, as Figure 1 shows. The SBP reached a plateau at 13 weeks of age in both strains, showing 130±2 mm Hg in BN-Ka rats (n=7) and 131±3 mm Hg in BN-Ki rats (n=4).

For hypertension experiments, SBP of normal BN-Ki rats increased gradually with time during DOCA-salt treatment and reached a plateau at 18 weeks of age (180±10 mm Hg, n=7) (Figure 1), whereas that of mutant BN-Ka rats rose rapidly to 158±6 mm Hg (n=10) 2 weeks after the onset of the treatment and then continued to increase at a slower rate to reach a plateau (181±7 mm Hg, 12 weeks old). The SBPs of mutant BN-Ka rats at 8, 9, 10, 11, and 12 weeks old (130±3, 158±6, 163±5, 171±6, and 181±7 mm Hg) 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 mm Hg, 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 and kallikrein in the plasma 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.

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In addition, plasma level of LMW kininogen in BN-Ki rats 10 weeks of age was decreased by 31.0% during DOCA-salt treatment (Table 1). The reduced level of LMW kininogen in the plasma was not accompanied by lowered levels of HMW kininogen and plasma prekallikrein nor with decrease in plasma protein concentrations. Hematocrit values in the blood of both strains of rats were also not changed by DOCA-salt treatment (44.7 ±0.7% without treatment versus 42.0 ±1.1% during DOCA-salt treatment in BN-Ka rats, and 44.7 ±0.7% without treatment versus 43.0 ±0% during DOCA-salt treatment in BN-Ki rats; n=3). Thus, the reduction of the LMW kininogen level in plasma might represent consumption of this kininogen by glandular kallikrein, and it was associated with an attenuated SBP rise and accentuated sodium excretion in the BN-Ki rats.

Furthermore, as shown in Figure 3, continuous administration of aprotinin by an osmotic minipump to normal BN-Ki rats for 7 days induced significant increase of SBP compared with that in saline-treated control rats. The SBPs at 9 weeks of age (158.6±3.7 mm Hg) and at the end of this experiment (9 weeks±3 days) (167.9±4.1 mm Hg) were significantly higher (\( p<0.01 \) and \( p<0.05 \)), compared with the SBPs at the same periods during the saline treatment (140.3±1.9 and 149.4±4.7 mm Hg, respectively). The active kallikrein in urine, collected for 24 hours at the end of the aprotinin injection, was markedly inhibited from 3.7±0.7 to 0.6±0.2 AU/\( \mu \)g creatinine (\( p<0.001 \)) by aprotinin treatment, as shown in the inserted panel of Figure 3.

### Discussion

Mutant BN-Ka rats were reported to be congenitally abnormal in kallikrein-kinin systems.5–12 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

### 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>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td></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>(249)</td>
<td>(79)</td>
<td>(720)</td>
<td>(72)</td>
<td></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>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td>(100)</td>
<td></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>
<tr>
<td>(861)</td>
<td>(44)</td>
<td>(3,135)</td>
<td>(139)</td>
<td></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.

\*\( p<0.05 \).
Subcutaneous administration of aprotinin (10^6 KIU/day) by an osmotic minipump caused a rapid early increase in the SBP 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 hyperten-

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

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.

These results are summarized in Table 3. Normal BN-Ki rats, which had kininogens in the plasma and excreted kinin in the urine, showed increased excretion of urinary kallikreins and sodium during DOCA-salt treatment and consumed LMW kininogen, and so the early rise of the SBP was mild. In contrast, mutant BN-Ka rats, which are deficient in kininogen in the plasma and devoid of kinin release in the urine, excreted less urinary kallikrein and exhibited a rapid early increase in the SBP during DOCA-salt treatment.
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 \( ^{3}H \) bradykinin in rabbits. Kinin may inhibit the reabsorption of sodium and chloride under the stimulus of mineral corticoids or the antidiuretic hormone through the formation of prostaglandins.

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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