High Sensitivity to Salt in Kininogen-Deficient Brown Norway Katholiek Rats

Masataka Majima, Osamu Yoshida, Harue Mihara, Takeshi Muto, Susumu Mizogami, Yoshikazu Kuribayashi, Makoto Katori, Sachiko Oh-ishi

Brown Norway Katholiek rats, which have very low levels of plasma kininogens, excreted a much smaller amount of kinin in the urine than normal rats of the same strain. The systolic blood pressure of 7-week-old kininogen-deficient rats fed low (0.3%) NaCl diets (131±4 mm Hg, n=12) was not different from that in normal rats. Two percent NaCl diets given from 7 weeks of age for 4 weeks caused rapid increases in blood pressure (167±4 mm Hg, n=12, 9 weeks old) in deficient rats, although the same diets induced no blood pressure increase in normal rats. Urinary excretion of active kallikrein and prokallikrein remained constant in both rat groups throughout NaCl loading. During this period, the deficient rats secreted less urine (9 weeks old, P<.05) and less urinary sodium (11 weeks old, P<.05). Serum levels of sodium in deficient rats were higher (P<.05) than in normal rats at 9 weeks of age. Intracellular concentrations of sodium in the erythrocytes of deficient rats were higher (P<.05) than in normal rats throughout NaCl loading. Subcutaneous infusion of bovine low molecular weight kininogen with an osmotic pump in NaCl-loaded deficient rats induced a reduction (P<.01) in blood pressure and increases (P<.05) in urine volume and urinary sodium and kinin levels. By contrast, subcutaneous infusion of the bradykinin antagonist Hoe 140 or of aprotinin in NaCl-loaded normal rats induced a hypertensive response. This antagonist treatment reduced urine volume and urinary sodium. These results indicate that the lack of kinin generation observed in the kininogen-deficient rats was related through sodium retention to the hypertensive response to NaCl loading. (Hypertension. 1993;22:705-714.)

KEY WORDS • hypertension, sodium-dependent • sodium, dietary • kininogens • bradykinin • aprotinin

The blood pressure-lowering effects of urinary kallikrein injected intravenously have been described for more than six decades, and bradykinin is well known to induce vasodilation and an increase in renal blood flow and excretion of water and sodium from the kidney. Urinary kallikrein therefore has been thought to be involved in hypertension, and its reduction has been reported in human and animal experiments. On the other hand, it is widely accepted that sodium retention may be related to the pathogenesis of hypertension, although the precise mechanisms of its contribution to the elevation of blood pressure are still unclear. The causal relation between these three factors—urinary kallikrein, sodium retention, and hypertension—has not been verified.

Recently, we reported that the kallikrein-kinin system may play a suppressive role in deoxycorticosterone acetate (DOCA)-salt hypertension. Using kininogen-deficient Brown Norway Katholiek (BN-Ka) rats and normal rats of the same strain (Brown Norway Kitasato [BN-Ki]), we were able to demonstrate that the urinary kallikrein-kinin system may contribute to lowering of systemic blood pressure in the initial phase of the development of DOCA-salt hypertension in uninephrectomized rats by acceleration of the excretion of sodium and water. However, it must be admitted that these experimental conditions, primarily DOCA-salt treatment of uninephrectomized rats, are very severe and biased and far removed from human hypertension. Therefore, in the present experiments, hypertension was induced by milder stimulation; it was found that mild NaCl loading in dietary chow, which did not increase systemic blood pressure in normal BN-Ki rats, induced hypertension through sodium retention in mutant BN-Ka rats, which excreted little kinin in the urine. We propose that the failure of excretion of excess sodium that is due to lack of renal kinin generation may result in the development of hypertension.

Methods

Animals

BN-Ka rats (Rattus norvegicus, BNf/Mai) were initially obtained from the Katholieke Universiteit of Leuven, Belgium. Normal rats of the same strain (BN-Ki) were transferred from the Microbiological Association, Frederick, Md, and kept at Kitasato University. Male rats 7 to 11 weeks of age were used. All animals were maintained at constant humidity (60±5%) and temperature (25±1°C) and kept continuously on a...
12-hour light/dark cycle throughout the duration of the experiment. The number of animals used for each experiment is shown as n below.

**Measurement of Systemic Blood Pressure**

The systolic blood pressure (SBP) of unanesthetized rats was determined weekly with tail-cuff plethysmography (model UR-1000, Ueda Seisakusho, Tokyo, Japan), as reported previously. Each pressure value was obtained by averaging four to six individual readings.

Mean arterial blood pressure (MAP) was also determined in conscious rats. The rats were anesthetized with light ether anesthesia, and a polyethylene cannula (PE-10, Clay Adams, Parsippany, NJ) was inserted into the abdominal aorta by way of the femoral artery. The tip of the cannula was placed between the bifurcation of the femoral arteries and the branching of the renal arteries. The cannula was connected to a PE-50 cannula and exteriorized in the interscapular region, filled with heparinized saline (5 x 10^5 U/L), and plugged with stainless steel pins. The rats were allowed to recover in individual cages with free access to food and water. The day after cannula insertion, a blood pressure transducer (model TP-200T, Nihon Koden, Tokyo) was attached to the intra-arterial catheter, and MBP and heart rate were recorded on a thermal array recorder (model WS-641G, Nihon Koden). During measurement, rats remained in their own cages. Recordings were made over 1 hour after 30 minutes of stabilization. The average of these readings was used for estimation of MBP and heart rate.

**Induction of Hypertension**

BN-Ka and BN-Ki rats were fed a low-NaCl diet (NMF, Oriental Yeast Corp, Tokyo, Japan) ad libitum containing 0.3% NaCl from a few days after weaning until 7 weeks of age. From the day they became 7 weeks of age, the rats were fed a 2%, 3%, 4%, 6%, or 8% NaCl diet ad libitum over 4 weeks. Each diet was prepared by uniformly mixing different amounts of NaCl with a standard 0.3% NaCl diet in powder form (NMF) with a special mixer (Irie Shokai Corp, Tokyo, Japan) to give final concentrations and was fed to rats as chow pellets. For control rats, the 0.3% NaCl diet was given for 4 weeks from 7 weeks of age ad libitum. The rats drank distilled water ad libitum.

**Measurement of Left Ventricle Weight**

Rats were killed by bleeding, and the hearts were excised and fixed with a 10% formaldehyde solution. After removal of the atrium and right ventricle from the fixed hearts, left ventricles were weighed.

**Blood Collection**

With rats under light ether anesthesia, blood was collected from the carotid artery of each strain at 7, 8, 9, and 11 weeks of age through the PE-50 cannula into plastic tubes containing 1/10 vol of 3.8% sodium citrate or into siliconized tubes containing EDTA (final concentration, 1 mg/mL plasma) and was centrifuged at 1500g for 15 minutes at 25°C to separate the plasma. For the preparation of serum, blood from the carotid artery was collected into glass tubes without anticoagulant, left at room temperature for 2 hours, and then centrifuged at 1500g for 15 minutes at 25°C. For determination of the sodium concentration of erythrocytes, blood was collected directly into tubes containing ice-chilled isotonic lithium chloride solutions.

**Collection of Urine and Measurement of Urinary Levels of Creatinine, Sodium, and Potassium**

Twenty-four-hour urine samples from individual rats were collected with rats in metabolic cages. Urine volume and amounts of drinking water consumed were recorded at the end of the 24-hour period. Urinary creatinine levels were measured by a kinetic method using Jaffe's reaction. Urinary sodium and potassium levels were determined electrometrically using coated wire electrodes selective for sodium and potassium, respectively.

**Measurement of Urinary Active Kallikrein and Prokallikrein**

The activity of the active kallikrein in urine collected in metabolic cages over 24 hours was measured using a peptidyl fluorogenic substrate selective for glandular kallikrein, Pro-Phe-Arg-methyl-coumarinylamide (Pep-tide Institute, Minoh, Osaka, Japan). After 10-fold dilution of the original urine with 0.2 mol/L Tris-HCl buffer (pH 7.8), 10 μL of the diluted urine was incubated with 1 mL of 5 x 10^-3 mol/L substrate solution in 0.05 mol/L Tris-HCl buffer containing 0.1 mol/L NaCl and 0.01 mol/L CaCl₂ (pH 8.0). One arbitrary unit (AU) was defined as the amount of urinary kallikrein that released 1 x 10^-10 mol/L 7-amino-4-methylcoumarin for 10 minutes per microliter of urine at 37°C. Active kallikrein activity was calculated as the difference between amidase activity in the presence of soybean trypsin inhibitor (0.5 μg/mL urine) and that in the presence of aprotinin (0.5 μg/mL urine). This determination method can exclude the contamination by proteases other than kallikrein in urine. The activity of prokallikrein was determined by the increase in kallikrein activity after treatment with trypsin (0.1 μg/mL urine) at 37°C for 10 minutes using the same method as for urinary active kallikrein.

**Measurement of Urinary Kinin**

Free kinin was measured in the urine collected via PE-10 catheters inserted into both ureters of rats of both strains under pentobarbital anesthesia (60 mg/kg SC). During urine collection, physiological saline was infused (6 mL/kg per hour) from the femoral vein. Kinin levels were determined with a bradykinin enzyme immunoassay kit (Markit A, Dainippon Pharmaceutical Corp, Osaka, Japan) after separation with a Sep-Pak C18 column (Waters Associates, Milford, Mass) and high-performance liquid chromatography. Kinin was separated by high-performance liquid chromatography by the method reported previously. The amounts of kinin secreted are expressed in nanograms per 24 hours.

**Measurement of Plasma Kininogen and Plasma Protein**

Plasma levels of high molecular weight (HMW) and low molecular weight (LMW) kininogens were determined by the previously reported method of Uchida et al. with some modification. Briefly, kininogens were converted to bradykinin, and the amounts of kinin generated were measured by the bradykinin enzyme.
immunoassay kit in which the antibody is equally cross-reactive to kallidin. Kininogen levels were expressed as nanograms bradykinin equivalent per milligram of plasma protein. The total protein in citrated plasma was measured by the method of Lowry et al.28

**Measurement of Plasma Renin Activity**

EDTA-treated plasma was incubated at 37°C for 90 minutes, and the amounts of angiotensin I generated were measured by radioimmunoassay (Gamma Coat 125I plasma renin activity kits, Baxter Healthcare Corp, Cambridge, Mass).29 Plasma renin activity was expressed as nanograms of angiotensin I generated per milliliter plasma over a period of 1 hour.

**Creatinine, Sodium, and Potassium Levels in Serum**

The levels of creatinine, sodium, and potassium in serum were determined by the same methods described above for those in urine.

**Measurement of Erythrocyte Sodium Concentration**

Intracellular erythrocyte sodium concentration (RBC[Na]) was determined with atomic absorption spectrophotometry.30 Briefly, erythrocytes (RBC) from 1 mL of blood were washed three times with ice-chilled isosmotic lithium chloride. After the last wash, 250 μL of the packed RBC was resuspended in 250 μL lithium chloride. A sample was taken to determine the hematocrit of the RBC suspension. This washed RBC suspension (500 μL) was added to distilled water (500 μL), and the resulting sample was freeze-lysed at −20°C. The sodium level of the lysate was determined by atomic absorption spectrophotometry. 30 Briefly, erythrocytes (RBC) from (RBC[Na]) was determined with atomic absorption spectrophotometry.30 Briefly, erythrocytes (RBC) from 1 mL of blood were washed three times with ice-chilled isosmotic lithium chloride. After the last wash, 250 μL of the packed RBC was resuspended in 250 μL lithium chloride. A sample was taken to determine the hematocrit of the RBC suspension. This washed RBC suspension (500 μL) was added to distilled water (500 μL), and the resulting sample was freeze-lysed at −20°C. The sodium level of the lysate was determined by atomic absorption spectrophotometry. (Shimadzu Corp, Tokyo, Japan) after centrifugation to remove RBC membranes. Results are expressed as millimoles per liter (mEq) RBC.

**Continuous Administration of Kininogen, a Bradykinin Antagonist, and Aprotinin**

One week after the start of administration of 2% NaCl diets (when rats were 8 weeks old), purified bovine LMW kininogen31 (5 mg/kg per 20 μL per day, dissolved in physiological saline; Seikagaku Kogyo, Tokyo, Japan), the bradykinin antagonist Hoe 140 32 (5 mg/kg per 20 μL per day, dissolved in physiological saline; Seikagaku Kogyo, Tokyo, Japan) after centrifugation to remove RBC membranes. Results are expressed as millimoles per liter (mEq) RBC.

**Statistical Analysis**

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

**Results**

As shown in Fig 1, the plasma levels of HMW kininogen in mutant BN-Ka rats at 7 weeks of age were below the detection limit, and those of LMW kininogen were also very low in mutant BN-Ka rats. The plasma concentrations of HMW and LMW kininogens in normal BN-Ki rats were 15.8±0.8 (n=4) and 8.9±0.6 ng bradykinin equivalent per milligram plasma protein (n=4), respectively, results that were approximately the same as those in the Sprague-Dawley rat strain.35 The amount of free kinin excreted in the ureteral urine in normal 7-week-old BN-Ki rats was 114.3±48.9 ng bradykinin per 24 hours (n=4) but was very low in mutant BN-Ka rats (6.1±0.6 ng bradykinin per 24 hours, n=4).

SBP of 7-week-old mutant BN-Ka rats fed a low-NaCl diet (0.3%) after weaning was 131±4 mm Hg (n=12), which was not significantly different from that of normal BN-Ki rats (128±4 mm Hg, n=7) (Fig 2). Two weeks after the start of the 2% NaCl diet, the SBP of mutant BN-Ka rats was markedly increased to 167±4 mm Hg and fell slightly thereafter (Fig 2), although the SBP of normal BN-Ki rats loading 2% NaCl did not change throughout the 4-week study period (Fig 2) and did not differ from that of BN-Ki rats fed a low (0.3%) NaCl diet (Table).

Chronic direct blood pressure measurement showed MBP of 7-week-old mutant BN-Ka rats and normal
Fig 2. Line graph shows changes in systolic blood pressure in normal Brown Norway Kitasato (BN-Ki) rats and mutant Brown Norway Katholiek (BN-Ka) rats fed 2% NaCl diet. Values are mean±SEM of n rats. After blood pressure measurement at 7 weeks of age, diets were changed from low NaCl (0.3%) to 2% NaCl. Values in BN-Ka rats were compared with those in BN-Ki rats at the same age; **P<.01, ***P<.001.

BN-Ki rats fed a low-NaCl diet (0.3%) to be 103±4 (n=5) and 105±4 mm Hg (n=6), respectively. One week and 2 weeks after the start of 2% NaCl diets, MBP values of mutant BN-Ka rats were significantly elevated to 127±4 (n=5) (P<.05) and 131±5 mm Hg (n=5) (P<.05), respectively. However, MBP values of BN-Ki rats were constant even 2 weeks after the start of the 2% NaCl diet (106±3 mm Hg, n=6). Heart rate of 7-week-old mutant BN-Ka rats (314±5 beats per minute, n=5) was markedly increased to 455±11 beats per minute (n=5) (P<.05) after loading of 2% NaCl for 2 weeks, but that in normal BN-Ki rats (356±26 beats per minute, n=6) was not changed (332±14 beats per minute, n=6).

Left ventricular weight was larger in mutant BN-Ka rats (745±55 mg, n=5) after 2% NaCl loading for 2 weeks than in normal BN-Ki rats (662±47 mg, n=5), although the difference was not statistically significant.

As the Table shows, the diets containing more than 3% NaCl also induced a sudden and significant increase in the SBP of mutant BN-Ka rats within 2 weeks. The 8% NaCl diet induced a smaller increase in SBP in mutant BN-Ka rats. However, even in the normal BN-Ki rats, the diets containing more than 3% NaCl also induced gradual increases in SBP, which reached levels significantly higher than those seen with the 0.3% NaCl diet (Table). The increased SBP levels in mutant BN-Ka rats fed 6% and 8% NaCl diets were not significantly higher than those in normal BN-Ki rats.

In the further precise analysis of subsequent experiments, the 2% NaCl diet was selected because no increase in SBP was observed in normal BN-Ki rats after NaCl loading.

Analyses of water and sodium metabolism were studied in BN-Ka and BN-Ki rats fed 2% NaCl diets in the metabolic cages. As Fig 3A indicates, both mutant BN-Ka and normal BN-Ki rats tended to show an increased water intake over 24 hours after starting to eat the 2% NaCl diet, although the water intake of mutant BN-Ka rats was slightly higher than that of normal BN-Ki rats, with a statistically significant difference emerging at 11 weeks of age. Urine volume over 24 hours of both BN-Ka and BN-Ki rats tended to increase after NaCl loading (Fig 3B). Mutant BN-Ka rats generally excreted slightly less urine, and at 9 weeks of age the difference was significantly less in these rats (12.4±1.2

### Changes in Systolic Blood Pressure of Mutant Brown Norway Katholiek and Normal Brown Norway Kitasato Rats Fed High NaCl Diet

<table>
<thead>
<tr>
<th>Strain</th>
<th>NaCl, %</th>
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<th>8</th>
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<tr>
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<td>155±5*</td>
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<td></td>
<td>4</td>
<td>127±4</td>
<td>166±5§</td>
<td>182±10‡</td>
<td>180±20‡</td>
<td>179±1‡</td>
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<td>6</td>
<td>125±5</td>
<td>145±12§</td>
<td>196±9§</td>
<td>175±10*</td>
<td>170±10§</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>126±3</td>
<td>150±5*</td>
<td>168±8*</td>
<td>162±6*</td>
<td>181±4</td>
</tr>
<tr>
<td>BN-Ki</td>
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<td>127±2</td>
<td>130±3</td>
<td>132±3</td>
<td>133±5</td>
<td>134±4</td>
</tr>
<tr>
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<td>3</td>
<td>128±4</td>
<td>141±1*</td>
<td>138±4</td>
<td>138±5</td>
<td>134±3</td>
</tr>
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<td>4</td>
<td>126±4</td>
<td>136±4</td>
<td>142±4</td>
<td>150±35</td>
<td>148±4*</td>
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<td>146±1§</td>
<td>163±35</td>
<td>160±3</td>
<td>167±5*</td>
</tr>
</tbody>
</table>

BN-Ka indicates Brown Norway Katholiek rats; and BN-Ki, Brown Norway Kitasato rats. After blood pressure measurements at 7 weeks of age, diets were changed to high NaCl (3% to 8%). For control rats, low-NaCl diets (0.3%) were given. Values are mean±SEM from five to seven rats in millimeters of mercury.

*P<.05, **P<.01, ***P<.001, high-NaCl (3% to 8%) compared with low-NaCl (0.3%) diet.

†P<.01, ‡P<.05, §P<.001, BN-Ka compared with BN-Ki rats fed the same diet.
Urinary sodium excretion was markedly increased a week after 2% NaCl loading in both BN-Ka and BN-Ki rats. However, mutant BN-Ka rats secreted significantly less sodium than normal BN-Ki rats at 8 and 9 weeks of age (Fig 3C). Urinary potassium and creatinine excretions were slightly increased after the start of NaCl loading in both BN-Ka and BN-Ki rats, but no significant differences between these two strains were observed (Fig 3D and 3E).

Levels of active kallikrein and prokallikrein were measured in urine collected for 24 hours in the experiment using 2% NaCl diets. Fig 4A and 4B indicate the levels of active kallikrein and prokallikrein, respectively, over 24 hours. The urinary active kallikrein levels of mutant BN-Ka and normal BN-Ki rats at 7 weeks of age were 12.1±1.1 (n=5) and 12.2±1.1 AU per 24 hours (n=5), respectively, and remained fairly constant even after the start of the 2% NaCl diet. There was no significant difference between mutant BN-Ka and normal BN-Ki rats throughout the experiments. No change in urinary prokallikrein levels was observed.

A further increase in NaCl concentration to higher than 2% in the diet resulted in reduction of the activity of urinary active kallikrein not only in normal BN-Ki rats but also in mutant BN-Ka rats. Two weeks after the start of NaCl loading (at 9 weeks of age), the reductions for 3%, 4%, 6%, and 8% NaCl in chow in normal BN-Ki rats were 86%, 58%, 45%, and 61%, respectively, of the activity in the 0.3% NaCl diet (Fig 4C). A similar reduction in the secretion of urinary active kallikrein was observed in mutant BN-Ka rats after the increase in NaCl concentration in rat chow. A slight reduction in urinary prokallikrein secretion was also observed in both BN-Ka and BN-Ki rats given higher concentrations of dietary NaCl (Fig 4D).

Serum levels of sodium, potassium, and creatinine are shown in Fig 5A, 5B, and 5C, respectively. Sodium levels in mutant BN-Ka rats rose slightly after 2% NaCl loading of the diets, whereas in normal BN-Ki rats, levels remained constant over the experimental period. The serum level of sodium at 9 weeks of age in mutant BN-Ka rats was significantly higher than in normal BN-Ki rats (Fig 5A). The levels of serum potassium and creatinine were not changed during the period of 2% NaCl loading in either rat strain (Fig 5B and 5C).

Fig 5D indicates changes in RBC[Na+], during 2% NaCl loading of the diet. RBC[Na+], in mutant BN-Ka
rats was significantly increased from 1 week after 2% NaCl loading, whereas that in normal BN-Ki rats remained constant throughout the experimental period.

As Fig 5E shows, plasma renin activity in mutant BN-Ka rats was not different from that in normal BN-Ki rats throughout the experiments, although the activity was transiently reduced at 8 weeks of age and recovered thereafter in both rat strains.

Subcutaneous infusion of LMW kininogen was given with a microosmotic pump for 7 days to mutant BN-Ka rats fed 2% NaCl diets. Fig 6A indicates that this infusion lowered SBP significantly from 157±5 (n=7) to 133±3 mm Hg (n=5) (P<.01). This treatment induced generation of 58±14 ng per 24 hours of urinary kinin measured in urine collected from the ureter of another comparable set of mutant BN-Ka rats (n=3) under pentobarbital anesthesia, whereas mutant BN-Ka rats that received physiological saline vehicle by the same pump secreted only marginal amounts of urinary kinin (<4.8 ng per 24 hours) (Fig 7A). The significant decrease in SBP in mutant BN-Ka rats was accompanied by significant increases in urine volume (from 12.4±1.0 mL per 24 hours [n=7] in the physiological saline-infused group to 19.5±0.6 mL per 24 hours [n=5], P<.01, Fig 7C) and in urinary sodium excretion of 1.8 times (from 79±9 mg per 24 hours [n=7] to 142±12 mg per 24 hours [n=5], P<.001, Fig 7B).

In contrast, subcutaneous infusion of the bradykinin antagonist Hoe 140 in normal BN-Ki rats fed 2% NaCl diets resulted in an increase in SBP to 166±3 mm Hg (n=5), which was significantly higher (P<.001) than the SBP of 131±5 mm Hg (n=6) of BN-Ki rats receiving physiological saline vehicle (Fig 6B). This treatment with the bradykinin antagonist significantly (~35%, P<.05) reduced urine volume (from 19.6±0.9 mL per 24 hours [n=6, vehicle saline] to 12.2±0.3 mL per 24 hours [n=5], Fig 7E) and excretion of urinary sodium (~24%, P<.01) (Fig 7D).

Subcutaneous infusion of the kallikrein inhibitor aprotinin with a microosmotic pump to normal BN-Ki rats loaded with 2% NaCl in the diet significantly increased SBP from the 122±2 mm Hg of normal BN-Ki rats that received physiological saline vehicle alone (n=7) to 154±4 mm Hg (n=5, P<.05) (Fig 6C). The validity of the inhibition of kallikrein activity in the urine was shown by marked inhibition of urinary active kallikrein from 12.2±1.1 (n=7, saline vehicle-infused) to 1.2±0.2 AU per 24 hours (n=5).

**Discussion**

BN-Ka rats were congenitally lacking in both HMW and LMW kininogens in the plasma and also in urinary kinin excretions (Fig 1), confirming the results of a previous experiment.18
As the Table shows, increases in the salt content of chow from 3% to 8% caused rapid and significant increases in SBP in mutant BN-Ka rats, although the increase in SBP in normal BN-Ki rats was mild and gradual. Interestingly, feeding rats with higher salt concentrations (3% or more) reduced the excretion of urinary active kallikrein and prokallikrein in normal BN-Ki rats as well as in mutant BN-Ka rats, indicating that the loading of higher salt concentrations in rats might induce renal damage. In fact, the urine volume of normal BN-Ki rats decreased as the NaCl concentrations in the diet increased (data not shown).

Salt loading has been discussed in relation to the development of hypertension in some species. Sapirstein et al reported that drinking water, not chow, containing 1.5% to 2.5% NaCl caused a significant increase in SBP in rats. Meneely et al reported that Sprague-Dawley rats receiving more than 2.8% NaCl in the diet over several months showed significantly higher SBP than rats given 0.01% NaCl diets. However, rats on diets containing more than 7% NaCl showed significant histological damage in the kidneys with systemic edema. Recently, chow containing 5% NaCl has generally been used to induce hypertension in rats. Dahl salt-sensitive rats were reported to secrete less activity of urinary kallikrein than Dahl salt-resistant rats, but the causal relation between the renal kallikrein-kinin system and salt-induced hypertension has never been fully understood.

In the present experiments, the SBP of normal BN-Ki rats whose diets contained 2% NaCl (Fig 2) was not different from that of normal BN-Ki rats fed 0.3% NaCl in the diet (Table), whereas 2% NaCl in the diet induced a rapid elevation of SBP in mutant BN-Ka rats (Fig 2). The elevation of systemic blood pressure was also confirmed in the experiments in the chronic direct determination of MBP. This MBP increase was accompanied by an increase in heart rate. Left ventricular weight tended to be increased in mutant BN-Ka, suggesting that MBP was sustained at higher levels in these mutant rats.

When both rat strains were fed a diet containing 2% NaCl, they excreted kallikrein at fairly constant levels (Fig 4A and 4B), in contrast to the reduced excretion of urinary active kallikrein and prokallikrein in rats fed a diet containing more than 3% NaCl. The lack of increase in serum creatinine level (Fig 5C) and that in secretion of urinary creatinine (Fig 3E) in both rat strains fed a 2% NaCl diet probably exclude the signifi-
FIG 6. Line graphs show effects of continuous subcutaneous administration of low molecular weight kininogen (A), bradykinin antagonist (Hoe 140, B), and aprotinin (C) on systolic blood pressure in normal Brown Norway Kitasato rats (B and C) and mutant Brown Norway Katholie rats (A) fed 2% NaCl diet. After blood pressure measurement at 7 weeks of age, diets were changed from low NaCl (0.3%) to 2% NaCl. After blood pressure determination at 8 weeks of age, subcutaneous infusion was started. Values are mean±SEM of n rats and were compared between a drug-infused group (•) and vehicle control group (○); **P<.01, ***P<.001.

The increase in SBP was independent of the increase in plasma renin activity, which showed a transient reduction and no difference in activity between BN-Ka and BN-Ki rats (Fig 5E). Thus, the contribution of angiotensin II to the increase in SBP can be excluded in the present experiment.

Exact intake and excretion of NaCl were not determined in this study; however, excretion of urinary sodium was reduced in mutant BN-Ka on the 2% NaCl diet (Fig 3C), suggesting sodium retention. Sodium retention in the body was certainly verified by the increase in sodium concentration in serum (Fig 5A), although the value was statistically significant only at 9 weeks of age. The increase seen in the sodium content of RBCs firmly indicated this retention (Fig 5D).

It is plausible to conclude that there is an interrelation between the lack of renal kinin generation, the reduced excretion of sodium and water, the retention of sodium in the body, and hypertension when sodium was in excess in the body. The causal relations between these parameters are not clear, but the following three experiments may help clarify this interrelation.

The subcutaneous infusion of LMW kininogen by a microosmotic pump for 7 days decreased blood pressure (Fig 6A). This hypotensive effect of LMW kininogen was interpreted as inducing kinin generation, because in short-term experiments with rats under pentobarbital anesthesia, infusion of the LMW kininogen into the femoral vein of mutant BN-Ka rats resulted in immediate detection of kinin in urine collected from the ureter (data not shown), indicating that kininogen infused intravenously was easily transferred to the renal tubules and was subject to cleavage by renal kallikrein. Kinin formation in urine collected from the ureter of mutant BN-Ka rats was also observed during subcutaneous infusion of LMW kininogen, as shown in Fig 7A. The hypotensive effect of LMW kininogen was accompanied by increased urinary excretion of sodium (Fig 7B) and increased urine volume (Fig 7C), indicating that once kinin is generated, it accelerates sodium and water excretion.

Subcutaneous infusion of the potent bradykinin antagonist Hoe 140 at a sufficient dose to normal BN-Ki rats loading with 2% NaCl increased SBP elevation (Fig 6B), which was a mirror image of the SBP decrease in mutant BN-Ka rats receiving kininogen treatment (Fig 6A). Again, this elevation in SBP was accompanied by a decrease in urinary sodium excretion (Fig 7D) and urine volume (Fig 7E). Continuous infusion of aprotinin by a microosmotic pump was successfully used in DOCA-salt treatment and in spontaneously hypertensive rats to demonstrate the suppressive role of the kallikrein-kinin system on SBP. This treatment also induced a significant increase in SBP in the salt-loaded normal BN-Ki rats in the present experiment (Fig 6C).

Bradykinin is known to exert a potent action on the renal excretion of sodium and water. It was reported that bradykinin receptors are distributed only in the collecting tubules of the kidney, and this distribution is known to be quite different from the sites of action of loop diuretics and thiazide-type diuretics, which are thick ascending limb of Henle's loop and distal tubules, respectively. Thus, the role of kinin in sodium excretion may be different from the ordinary regulatory mechanism of sodium excretion. In fact, changes with age in SBP and sodium excretion rate in mutant BN-Ka rats under no treatment are not different from those in normal BN-Ki rats. Furthermore, subcutaneousifu-
sion of aprotinin to Wistar-Kyoto rats did not increase SBP, whereas the same treatment in spontaneously hypertensive rats accelerated the elevation of SBP. These results indicate that the renal kallikrein-kinin system may play a part in increased excretion of sodium and water only when excess amounts of sodium are taken.

According to Guyton, a mere 1% increase in body water can, in the absence of reflexes, increase the mean arterial pressure of an experimental dog from 100 to 175 mm Hg. On the other hand, excretion of urinary kallikrein was also reported to be lower in patients affected by essential hypertension than in normotensive control subjects. In spontaneously hypertensive rats, the excreted activity of urinary kallikrein is also lower than in Wistar-Kyoto rats.

Despite the many proposals that have been put forward, no linkage between these parameters in the development of hypertension has been verified. The present experiment showed that the lack of generation of urinary kallikrein caused reduced excretion of sodium and water in urine, retention of sodium and water in the body, and hypertension. These results may advance the understanding of the pathogenesis of essential hypertension.

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