Hypertension Induced by a Nonpressor Dose of Angiotensin II in Kininogen-Deficient Rats

Masataka Majima, Susumu Mizogami, Yoshikazu Kuribayashi, Makoto Katori, Sachiko Oh-ishi

Abstract
Brown Norway Katholiek rats with 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 (132 ± 2 mm Hg, n=7) was not different from that of normal rats. Angiotensin II (Ang II) (20 μg/d SC) from 7 weeks of age for 2 weeks with a micro-osmotic pump caused significant increases in blood pressure (181 ± 5 mm Hg, n=7, 9 weeks old) in the deficient rats, although the same treatment induced no blood pressure increase in the normal rats. Also during this period, the deficient rats had significantly higher heart rates, tended to excrete less urinary sodium, and showed significantly higher sodium levels in serum, erythrocytes, and cerebrospinal fluid compared with the normal rats. Ang II increased urinary excretion of aldosterone in both deficient and normal rats (P<.05). Spironolactone treatment (50 mg/kg per day) for 7 days in deficient rats restored blood pressure and heart rate to normal levels and significantly reduced sodium levels in erythrocytes and cerebrospinal fluid. Subcutaneous infusion of bovine low-molecular-weight kininogen with an osmotic pump in Ang II-treated deficient rats induced significant reductions in blood pressure, heart rate, and erythrocyte sodium levels. By contrast, subcutaneous infusion of the bradykinin antagonist Hoe 140 in Ang II-treated normal rats induced a hypertensive response in parallel with significant increases in heart rate and erythrocyte sodium level. These results suggest that the lack of kinin generation observed in the kininogen-deficient rats may cause the hypertensive response during the administration of a nonpressor dose of Ang II mainly through sodium retention probably caused by aldosterone release.

Key Words
• hypertension, experimental
• angiotensin II
• sodium • spironolactone • kininogens • bradykinin

The biologically active peptide bradykinin is well known to induce increases in renal blood flow and water and sodium excretions. Kinin is generated by the action of kallikrein secreted in the distal tubules, and its receptors are distributed on the tubular cells of the distal tubules.1,2 It has been claimed that urinary kallikrein may be involved in hypertension in humans3-6 and animal models of hypertension.7-17 Using kininogen-deficient Brown Norway Katholiek (BN-Ka) rats that excreted little kinin in the urine18 and normal rats of the same strain (Brown Norway Kitasato [BN-Ki]), we previously reported that the urinary kallikrein-kinin system may contribute to lowering systemic blood pressure in the initial phase of the development of deoxycorticosterone acetate (DOCA)-salt hypertension in uninephrectomized rats by acceleration of the blood pressure in normal BN-Ki rats. These results indicated that urinary kinin generation is a critical factor in the development of this type of hypertension.

Angiotensin II (Ang II) is known to be a potent vasoconstrictor21 and involved in some types of hypertension. Moreover, a nonvasoconstrictive dose of Ang II caused aldosterone release from the adrenal glands.22,23 In connection with the regulation of body fluids, Ang II also induces drinking,24 causes sodium absorption from intestine,25 and has an antinatriuretic action on the renal tubules.26 The present article reports that a low dose of Ang II, which did not induce vasoconstriction in normal BN-Ki rats, induced hypertension in mutant kininogen-deficient BN-Ka rats, probably through aldosterone-mediated sodium retention in the body.

Methods

Animals
BN-Ka rats (Rattus norvegicus, BN/MAi) were initially 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 7 to 10 weeks of age were used. All animals were housed at constant humidity (60±5%) and temperature (25±1°C) and kept on a 12-hour light/dark cycle throughout the duration of the experiment. Numbers of animals used for each experiment are stated below. This study was performed in accordance with the guidelines for animal experiments of Kitasato University School of Medicine.

Induction of Hypertension

From a few days after weaning, BN-Ka and BN-Ki rats were fed a low NaCl (0.3% NaCl) diet ad libitum (NMF, Oriental Yeast Corp). Rats were given free access to distilled drinking...
water. From 7 weeks of age, Ang II (Peptide Institute; 20 μg/d, dissolved in sterilized physiological saline) was infused subcutaneously for 2 weeks by means of a micro-osmotic pump (Alzet model 2002, Alza Corp) implanted under the skin of the back. In vehicle control animals, sterilized physiological saline was weighed.20

Measurement of Systemic Blood Pressure

Systolic blood pressure (SBP) of unanesthetized rats was determined weekly by tail-cuff plethysmography (Ueda model UR-1000, Ueda Seisakusho), as reported previously.18-20 Mean arterial blood pressure (MBP) was also determined in conscious rats as described previously.20 Briefly, a polyethylene cannula (PE-10, Clay-Adams) was inserted into the abdominal aorta through the femoral artery with rats under light ether anesthesia, was connected to a PE-50 cannula (Clay-Adams), and was exteriorized in the interscapular region. A blood pressure transducer (TP-200T, Nihon Koden) was attached to the intra-arterial catheter, and MBP was monitored on a polygraph (WS-641-G, Nihon Koden). Starting 30 minutes after the recording of the transducer, recording was carried out for more than 1 hour on the rats, which were housed in separate cages.

Measurement of Systemic Blood Pressure by Bolus Intravenous Injection of Ang II in Anesthetized Rats

MBP of sodium pentobarbital–anesthetized rats (50 mg/kg IP) was also monitored through the femoral artery cannula (PE-50). A bolus of Ang II (10 to 100 pmol/kg, dissolved in physiological saline [1 mL/kg]) was injected into the femoral vein through the cannula. The maximal response in the elevation of MBP was noted.

Measurement of Left Ventricular Weight

After the rats were killed by bleeding, the hearts were excised and fixed with a 10% solution of formaldehyde. The atrium and right ventricle were removed, and the left ventricle was weighed.20

Blood Collection

With rats under light ether anesthesia, blood was collected from the carotid artery of each rat strain at 7, 8, 9, and 10 weeks of age through the cannula (PE-50) into plastic tubes containing a 1/10 vol of 3.8% sodium citrate18 or into siliconized tubes containing EDTA (final concentration, 1 mg/mL plasma). These samples were then 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, blood was also collected directly into tubes containing ice-chilled isosmotic lithium chloride solution for the determination of the sodium concentration of erythrocytes.

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

Twenty-four-hour urine samples from individual rats 7, 8, 9, and 10 weeks old were collected using metabolic cages. The 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.18,20 Urinary sodium and potassium levels were determined electrometrically using coated wire electrodes selective for sodium and potassium, respectively.18,20 Urinary aldosterone levels were determined by a specific radioimmunoassay27 after the extraction of the aldosterone fraction with dichloromethane.

Measurement of Urinary Active Kallikrein and Prokallikrein

The activities of active kallikrein and prokallikrein in the urine collected in metabolic cages over 24 hours were measured using Pro-Phe-Arg-MCA (Peptide Institute), a peptide fluorogenic substrate, as reported previously.18,20 One arbitrary unit was defined as the amount of urinary kallikrein that released 1 x 10^{-10} mol 7-amino-4-methylcoumarin from 1 μL of urine in 10 minutes at 37°C.

Measurement of Urinary Kinin

Free kinin was measured in the urine collected via catheters (PE-10, Clay-Adams) inserted into both ureters of rats of both strains under pentobarbital anesthesia (60 mg/kg SC). Kinin levels were determined with a bradykinin enzyme immunoassay kit (Markit A, Dainippon Pharmaceutical Corp) after separation with a Sep-Pak C18 column (Waters Associates) and high-performance liquid chromatography.18,20 The amounts of kinin secreted are expressed as nanograms per 24 hours.

Measurement of Kinogen Levels in Plasma

Plasma kinogen levels in citrated plasma were determined by the amount of kinin released from the plasma, as described previously.18,20 The levels are expressed as nanograms bradykinin per milligram plasma protein.

Measurement of Plasma Renin Activity

Plasma renin activity in EDTA-treated plasma was determined by the generation rate of Ang I, as reported previously.18,20 The activity is expressed as nanograms Ang I generated per millilitre plasma over a period of 1 hour.

Measurement of Levels of Creatinine, Sodium, and Potassium in Serum and Erythrocyte Sodium Concentrations

The serum levels of creatinine, sodium, and potassium were determined by the same methods as used for those in urine, as described above. Erythrocyte sodium concentration (RBC[Na⁺]) was determined using atomic absorption spectrophotometry,26 as reported previously.20 Erythrocyte sodium concentrations are expressed as millimoles per liter red blood cells.

Measurement of Sodium Levels in Cerebrospinal Fluid

Cerebrospinal fluid from rats under light ether anesthesia was obtained by aspiration of the cisterna magna with a 26-gauge needle. Sodium levels in the cerebrospinal fluid were determined with an atomic absorption spectrophotometer.

Administration of Spironolactone, Low-Molecular-Weight Kininogen, and Bradykinin Antagonist to Rats

One week after the start of subcutaneous infusion of Ang II (when rats were 8 weeks old), purified low-molecular-weight (LMW) bovine kininogen26 (5 mg/kg per 20 μL per day, dissolved in physiological saline; Seikagaku Kogyo) or a bradykinin antagonist, Hoe 1409 (5 mg/kg per 20 μL per day, dissolved in physiological saline; a generous gift from Hoechst AG), was administered for 7 days by continuous subcutaneous infusion via a micro-osmotic pump (Alzet model 2001, Alza Corp) implanted under the skin of the back. Control animals received physiological saline (20 μL per day) via the same type of pump. Spironolactone (50 mg/kg per day SC, dissolved in sesame oil) was injected daily to the back for 7 days. Control rats received only vehicle solution (sesame oil, 1 mL/kg per day). SBP was determined by the tail-cuff method before and after the start of drug administration mentioned above. After the last blood pressure measurement, the blood and cerebro-
spinal fluid of the animals were collected for determination of sodium levels in erythrocytes and cerebrospinal fluid.

**Statistical Analysis**

Values 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 method or Wilcoxon's rank sum test.

**Results**

Plasma levels of high-molecular-weight (HMW) kininogen in 7-week-old mutant BN-Ka rats were very low (0.12±0.12 ng bradykinin equivalent/mg plasma protein, *n=6*) and those of LMW kininogen were also very low (0.05±0.01 ng bradykinin equivalent/mg plasma protein, *n=6*) in mutant BN-Ka rats, whereas the plasma concentrations of HMW and LMW kininogens in normal BN-Ki rats were 15.4±0.5 and 9.5±0.3 ng bradykinin equivalent/mg plasma protein (*n=6* in each case), respectively. The amount of immunoreactive free kinin excreted in the urethral urine in normal 7-week-old BN-Ki rats was 101.5±20.8 ng bradykinin/24 h (*n=4*) but was very low in mutant BN-Ka rats (<4.8 ng bradykinin/24 h, *n=4*). The results from BN-Ka and BN-Ki rats in the present experiments were the same as those in our previous reports.\(^ {18-20}\)

SBP of 7-week-old mutant BN-Ka rats was 132±2 mm Hg (*n=7*), which was not significantly different from that of normal BN-Ki rats (129±3 mm Hg, *n=7*) (Fig 1A). From the third day after the start of subcutaneous infusion of Ang II (20 μg/d), SBP of mutant BN-Ka rats was significantly increased to 166±4 mm Hg (*n=7*) and rose continuously thereafter until the end of the infusion period, whereas SBP of normal BN-Ki rats given the same treatment did not change throughout the 3-week study period and did not differ from that of BN-Ki rats receiving saline vehicle (Table 1).

When a high dose of Ang II (100 μg/d) was infused subcutaneously, SBP of normal BN-Ki rats was significantly increased, but the elevated SBP of mutant BN-Ka rats was markedly higher throughout the experiments (Table 1). For the subsequent experiments, the lower dose of Ang II (20 μg/d) was used, as no increase in SBP was observed in normal BN-Ki rats during subcutaneous infusion.

Heart rates of 7-week-old mutant BN-Ka and normal BN-Ki rats were 374±18 (n=7) and 389±9 beats per minute (n=7), respectively. Heart rates of mutant BN-Ka rats were higher than those of normal BN-Ki rats during the Ang II infusion period (*P<.05 to .01*) (Fig 1B).

Chronic direct blood pressure measurement (Table 2) showed the MBP of 7-week-old mutant BN-Ka and normal BN-Ki rats to be 103±4 (n=5) and 105±2 mm Hg (n=6), respectively. One and 2 weeks after the start of Ang II infusion, the MBP values of mutant BN-Ka rats were significantly elevated to 147±10 mm Hg (n=5) (*P<.01*) and 150±3 mm Hg (n=5) (*P<.001*), respectively. However, those of BN-Ki rats remained constant even 2 weeks after the start of Ang II treatment.

Fig 1C indicates the changes in RBC[Na], during Ang II infusion. RBC[Na] in mutant BN-Ka rats was significantly increased from a week after the start of Ang II infusion, whereas that in normal BN-Ki remained constant throughout the experimental period.

The sodium concentration of cerebrospinal fluid in 7-week-old mutant BN-Ka rats was not different from that of normal BN-Ki rats, but the 2-week Ang II infusion resulted in significant increases in the sodium levels in cerebrospinal fluid of mutant BN-Ka rats (Table 3).
Subcutaneous Ang II infusion tripled or quadrupled urinary aldosterone secretion in both mutant BN-Ka and normal BN-Ki rats, giving significant rises over basal levels \((P<.05\) to .01). However, not only the basal secretions but also the increased secretions in mutant BN-Ka rats were not different from those observed in normal BN-Ki rats (Fig 2).

Left ventricular weight of mutant BN-Ka rats (760±35 mg, \(n=5\)) after 2 weeks of Ang II infusion (at 9 weeks of age) was significantly \((P<.05)\) higher than that of normal BN-Ki rats (637±37 mg, \(n=5\)). When 50 mg/kg per day spironolactone, sufficient to cause antagonism of aldosterone, was given from 8 weeks of age for 7 days to the mutant BN-Ka rats receiving Ang II, the elevated SBP was significantly \((P<.01)\) reduced to 141±4 mm Hg (\(n=5\)), a level similar to that in normal BN-Ki rats given Ang II (Fig 1A). Vehicle-treated BN-Ka rats maintained a high SBP even after 7 days (185±10 mm Hg, \(n=6\)). The reduction in SBP in BN-Ka rats was accompanied by significant decreases in RBC\[Na\], levels (Fig 1C, \(P<.05\)) and heart rate (Fig 1B, \(P<.01\)). Spironolactone treatment also resulted in a significant reduction in the sodium levels in the cerebrospinal fluid in mutant BN-Ka rats, which had been elevated by Ang II (Table 3).

During Ang II infusion, the body weight of mutant BN-Ka rats increased \((P<.05\) to .001, Table 4), becoming larger than that of normal BN-Ki rats. The difference was statistically significant at 8 weeks of age \((P<.05)\) (Table 4).

Table 2. Changes in Mean Blood Pressure Determined by Chronic Methods During Subcutaneous Infusion of Low Doses (20 \(\mu g/d\)) of Ang II

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN-Ka</td>
<td>103±4</td>
<td>147±10*†</td>
<td>150±3†</td>
<td>153±3†</td>
</tr>
<tr>
<td>BN-Ki</td>
<td>105±4</td>
<td>102±5</td>
<td>99±8</td>
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</tbody>
</table>

Definitions are as in Table 1. After blood pressure measurement at 7 weeks of age, Ang II infusion was not significantly different except for the urine volume of normal BN-Ki rats at 9 weeks of age (Table 4).

Although there were no apparent changes in the urinary excretions of potassium and creatinine in either rat strain during Ang II treatment, sodium excretion in normal BN-Ki rats was slightly (but not significantly) increased, whereas in mutant BN-Ka rats tended to be suppressed, and the value was reversed after the infusion was stopped (Table 4).

Urinary active kallikrein levels of 7-week-old mutant BN-Ka rats were not different from those of normal BN-Ki rats. Ang II infusion resulted in significant increases \((P<.05\) to .01) in active kallikrein levels in both rat strains from the 7-week-old values. However, the elevated levels of kallikrein in BN-Ki rats were not different from those in BN-Ka rats at the same age (Table 4). Ang II also raised urinary prokallikrein levels, but there was no significant difference between BN-Ka and BN-Ki rats of the same age (Table 4).

As shown in Table 5, serum sodium level in mutant BN-Ka rats rose slightly after Ang II infusion, whereas that in normal BN-Ki rats remained constant over the experimental period. Serum sodium level at 8 weeks of age in mutant BN-Ka rats was significantly higher than that in normal BN-Ki rats. Hematocrit in mutant BN-Ka rats during the infusion period was significantly lower than that of normal BN-Ki rats (Table 5). Serum potassium and creatinine levels did not change during the infusion period in either rat strain (Table 5). 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 markedly reduced during Ang II infusion and recovered thereafter in both rat strains.

Subcutaneous infusion of LMW kininogen was performed for 7 days with a micro-osmotic pump in mutant BN-Ka rats under Ang II treatment (Fig 3). In another comparable set of mutant BN-Ka rats \((n=3)\) under pentobarbital anesthesia, this treatment induced the excretion of 49±8 ng/24 h of urinary kinin in urine collected from the ureter, whereas mutant BN-Ka rats that received only physiological saline vehicle by the same procedure secreted only marginal amounts of urinary kinin \(<4.8\, ng/24\, h\). Fig 3A indicates that this infusion lowered SBP significantly from 166±8 (\(n=7\)) to 141±3 mm Hg (\(n=5\), \(P<.05\)). This significant decrease in SBP in mutant BN-Ka rats was accompanied by a
significant decrease in heart rate (Fig 3B, P<.05) and RBC[Na], (Fig 3C, P<.05).

By contrast, as shown in Fig 4, subcutaneous infusion of Hoe 140 in normal BN-Ki rats under Ang II treatment resulted in an increase in SBP to 159±5 mm Hg (n=5), which was significantly higher (P<.05) than the 130±5 mm Hg (n=6) seen in BN-Ki rats receiving physiological saline vehicle (Fig 4A). This treatment with the bradykinin antagonist significantly increased both heart rate (Fig 4B, P<.05) and RBC[Na], (Fig 4C, P<.01).

Fig 5 indicates the sensitivity of the arterioles of the mutant BN-Ka rats to Ang II under anesthesia. The hypertensive effect of single bolus intravenous injections of Ang II (10 to 100 pmol/kg) to anesthetized 7-week-old mutant BN-Ka rats was not different from that in normal BN-Ki rats of the same age.

**Discussion**

The present experiments confirmed the low kininogen levels in plasma and the marginal generation of urinary kinin in mutant BN-Ka rats reported in our previous articles.18-20 Very low plasma levels of HMW and LMW kininogen were attributable to lack of the ability to secrete kininogens by the liver of this rat strain, probably because of one-point mutation of amino acid, although the hepatic cells of the mutant BN-Ka rats produced kininogens, which are not different in molecular weight (approximately 110 kD).22 Subcutaneous infusion of a low dose (20 μg/d) of Ang II with a miniosmotic pump for 2 weeks did not change systemic blood pressure in normal BN-Ki rats, whereas the same treatment caused a marked increase in systemic blood pressure in kininogen-deficient mutant BN-Ka rats. This suggests that the hypertension in mutant BN-Ka rats was not induced simply by arteriolar constriction due to the potent vasoconstrictive effect of Ang II21 but by other factors, such as the sodium retention that was induced by Ang II doses below those necessary to exert a vasoconstrictive effect. It is known that a nonvasoconstrictive dose of Ang II increases the release of aldosterone from the adrenal glands22,23 and increases sodium retention in the body through the stimulation of aldosterone release,22,23 and it increases sodium retention by a direct action on renal function.26 Many authors of earlier reports of Ang II-induced hypertension in rats infused an Ang II dose between 6 and 15 times higher than that given in the present study using the same pump.33,34 However, we used rather a low dose of Ang II, which did not induce a pressor effect in normal BN-Ki rats.

Urinary aldosterone secretion was significantly increased by three to four times during Ang II infusion in both rat strains, but there were no differences in the secreted aldosterone concentrations between normal and mutant rats. The increase in urinary aldosterone excretions lasted more than 2 weeks. Bean et al15 reported that Ang II administration causes plasma aldosterone levels to rise only transiently. This difference may be due to the difference in the method of administration. The present results were consistent with those of Luft et al,35 who used the same pump. Intravenous administration of aldosterone was reported to increase sodium concentrations in plasma and cerebrospinal fluid and to raise MBP.36 The hypertension in mutant BN-Ka rats was not caused by their arterioles being more sensitive, because the blood pressure response to bolus intravenous injection of Ang II under pentobarbital anesthesia in these rats was not different from that in normal BN-Ki rats. Some genetic bias in mutant BN-Ka rats, related to arteriolar hypersensitivity to Ang II, may therefore be ruled out.

SBP was measured mainly with a tail cuff in the present experiments because of long-term safety determination, but the hypertension was not artificially induced by animal manipulation because even blood pressure measurement by direct cannulation in con-

**TABLE 3. Sodium Levels in Cerebrospinal Fluid Before and After Subcutaneous Infusion of Low Doses (20 μg/d) of Ang II**

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>Age, wk</th>
<th>Infusion of Low Doses (20 μg/d) of Ang II</th>
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<td>Infusion of Low Doses of Ang II (20 μg/d)</td>
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<td>RBC[Na], (Fig 3C, P&lt;.05)</td>
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<td>SBP (mm Hg) (Fig 3B, P&lt;.05)</td>
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<td>Heart rate (Fig 4B, P&lt;.05)</td>
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<td></td>
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<td>RBC[Na], (Fig 4C, P&lt;.01)</td>
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*Definitons are as in Table 1. From 8 weeks of age, spironolactone (50 mg/kg SC) was administered daily for a week. Each value represents mean±SEM from four to eight animals and is expressed in millimoles per liter.

*P<.05, BN-Ka vs BN-Ki rats at same age.

†P<.05 vs rats of the same strain at 7 weeks of age.

‡P<.01, spironolactone-treated vs nontreated at 9 weeks of age.

![Graph](http://hyper.ahajournals.org/DownloadedFrom://hyper.ahajournals.org)
TABLE 4. Changes in Body Weight; Water Intake; Urine Volume; and Urinary Excretions of Sodium, Potassium, Creatinine, and Kallikrein During Subcutaneous Infusion of Low Doses (20 \mu g/d) of Ang II

<table>
<thead>
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<th>Measurement</th>
<th>Rat Strain</th>
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<td>ΔBody weight, g/wk</td>
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<tr>
<td>BN-Ki</td>
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<tr>
<td>Water intake, mL/24 h</td>
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<td>Urine volume, mL/24 h</td>
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<td>Urinary sodium, mg/24 h</td>
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<td>Urinary potassium, mg/24 h</td>
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<td>Urinary creatinine, mg/24 h</td>
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<td>Urinary kallikrein</td>
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<tr>
<td>Active kallikrein, AU/24 h</td>
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<td>Active kallikrein, AU/24 h</td>
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Definitions are as in Table 1; AU, arbitrary units. After blood pressure measurement at 7 weeks of age, Ang II was subcutaneously infused for 2 weeks. Each value represents mean±SEM from five to eight animals.

*P<.05, BN-Ka vs BN-Ki rats at same age.
†P<.01, †P<.001 vs rats of the same strain at 7 weeks of age.

scious rats showed the same hypertension during the subcutaneous infusion of Ang II.

Ang II-induced hypertension was reduced to the level of normal BN-Ki rats by subcutaneous infusion of spironolactone, an aldosterone antagonist, during the second half of the Ang II infusion period. This indicates that the factor responsible for raising blood pressure was mainly aldosterone. The increase in sodium concentrations in serum, erythrocytes, and cerebrospinal fluid together with the increase in body weight and reduced hematocrit may indicate sodium retention in the body. Spironolactone treatment lowered markedly the sodium concentrations of erythrocytes and cerebrospinal fluid. It has been reported that elevation of the sodium concentration of cerebrospinal fluid by intraventricular injection of hypertonic NaCl solutions caused hyperten-

TABLE 5. Changes in Plasma Renin Activity; Serum Levels of Sodium, Potassium, and Creatinine; and Hematocrit During Subcutaneous Infusion of Low Dose (20 \mu g/d) of Ang II

<table>
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<tr>
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<tr>
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<td>BN-Ki</td>
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<td>Serum sodium, mmol/L</td>
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<td>BN-Ki</td>
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<td>Serum potassium, mmol/L</td>
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<td>BN-Ki</td>
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<td>Serum creatinine, mg/L</td>
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<td>BN-Ki</td>
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*P<.05, †P<.01, BN-Ka vs BN-Ki rats at same age.
‡P<.05, §P<.001 vs rats of the same strain at 7 weeks of age.
in rats\textsuperscript{37,38} and that reduction of cerebrospinal fluid sodium levels by intracerebroventricular infusion of isosmotic NaCl-free mannitol solution resulted in significant reductions of MBP.\textsuperscript{36} This observation supported our hypothesis that the Ang II-induced hypertension in mutant BN-Ka rats may be attributable to sodium retention caused by aldosterone release. The absence of any change either in the serum concentrations of potassium and creatinine or in their urinary excretion eliminated the possibility that sodium retention was caused by impaired kidney function, which was observed after infusion of the higher dose of Ang II.\textsuperscript{34}

During Ang II infusion, plasma renin activity was reduced in both BN-Ka and BN-Ki rats. Salt loading and DOCA-salt treatment reduced the renin activity in plasma.\textsuperscript{18,20} An increase in aldosterone secretion may be the cause of this decrease in plasma renin activity through negative feedback mechanisms. Thus, the possibility of the involvement of endogenous renin and Ang II can be excluded.
Despite the evidence of sodium retention in erythrocytes and cerebrospinal fluid in mutant BN-Ka rats, no clear reduction of urinary sodium excretion was observed, although the urinary sodium excretion in mutant BN-Ka rats appeared to be less than that of normal BN-Ki rats. In our previous experiments on induction of hypertension with 2% sodium in the diet, a significant decrease in urinary sodium excretion in mutant BN-Ka rats was seen together with sodium retention in serum and erythrocytes. The low sodium diet (0.3% [wt/wt] NaCl) in the present experiment may have been responsible for the lack of any significant difference in sodium excretion in either rat strain. It is also possible that pressure natriuresis prevented the appearance of a significantly reduced sodium excretion.

The lack of urinary kinin excretion may be related to hypertension resulting from sodium retention, because subcutaneous infusion of LMW kininogen to mutant kininogen-deficient rats by a miniosmotic pump in the second half of the Ang II infusion reduced the high blood pressure, the erythrocyte sodium concentration, and the heart rate almost to the levels of normal BN-Ki rats. In contrast, infusion of Hoe 140, a bradykinin antagonist, to normal BN-Ki rats increased blood pressure almost to the level of mutant BN-Ka rats and brought about increases in heart rate and erythrocyte sodium concentration. These results strongly suggest that excretion of urinary kinin is a factor in the blockade of both the elevation of SBP and retention of sodium in the erythrocytes during Ang II treatment.

Urinary kallikrein (either active kallikrein or prokallikrein) was slightly increased during Ang II infusion, but again there was no difference between the two rat strains. As the exogenous aldosterone caused the increase in urinary excretion of kallikrein, the rise in aldosterone level by a low dose of Ang II may have induced the kallikrein increase seen in the present experiment.

It is clear that loading of a low concentration (2%) of NaCl in the diet or aldosterone release caused by infusion of a low dose (20 μg/1) of Ang II (in the present article) induced hypertension when there was little kinin generation in the urine, probably through the reduction of sodium excretion by urinary kinin in the renal tubules. The increase in sodium concentration in the cerebrospinal fluid may reflect sodium retention in the nerve cells, including the sympathetic nerve cells. Stimulation of the sympathetic nerve cells may increase heart rate or cardiac output and cause vasoconstriction.

In the present experiments, treatment of mutant BN-Ka rats with spironolactone during Ang II infusion caused sharp decreases of heart rate and systemic blood pressure to normal levels, together with the reduction of sodium levels in erythrocytes (Fig 1) and in cerebrospinal fluid (Table 3). Increased activity in the sympathetic system in the development of hypertension has been repeatedly reported. Thus, the present results suggest that the hypertensive response arises from the greater activity of the sympathetic system caused by sodium retention in the cerebrospinal fluid. It has been reported that long-term Ang II treatment of rats resulted in greater basal sympathetic nerve activity than in control rats receiving vehicle solution, as shown by an increase in heart rate.

Once acceleration of sodium reabsorption or of sodium accumulation occurs as a result of aldosterone release, the action of aldosterone may be prevented by the natriuretic action of tubular kinin, if the renal kallikrein activity and the amount of kinin released are kept at normal levels. The hypertension that results from sodium retention in mutant BN-Ka rats may be explained by the lack of this counteraction.

Two sets of apparently conflicting observations were made in the present experiments. First, Ang II increased blood pressure in mutant BN-Ka rats but not in normal BN-Ki rats with the same dose, although there was no difference in arteriolar sensitivity to intravenous Ang II in the two rat strains. Second, despite the increased amounts of aldosterone excreted at similar levels by BN-Ka and BN-Ki rats on administration of Ang II, sodium retention, elevated heart rate, and high blood pressure were induced only in the mutant BN-Ka rats and were reduced to normal levels by spironolactone. Thus, we cannot exclude the possibility that a nonpressor dose of Ang II may cause sodium retention and hypertension through aldosterone release in kininogen-deficient BN-Ka rats, but a vasoconstrictive action, a direct renal effect of Ang II, or unknown direct or indirect effects of Ang II cannot be ruled out in seeking to explain the present results.

In conclusion, a nonpressor dose of Ang II induced sodium retention and hypertension in kininogen-deficient BN-Ka rats, which could not generate kinin in the kidney, and this was probably at least partly due to aldosterone release.

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