

Increase in Vascular Sensitivity to Angiotensin II and Norepinephrine after Four-Day Infusion of 0.3 M Sodium Chloride in Conscious Kininogen-Deficient Brown Norway Katholiek Rats

Masataka Majima, Keiichi Adachi, Yoshikazu Kuribayashi, Susumu Mizogami and Makoto Katori

Department of Pharmacology, Kitasato University School of Medicine, Sagamihara, Kanagawa 228, Japan

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ABSTRACT—Kininogen-deficient Brown Norway Katholiek (deficient BN-Ka) rats excreted a small amount of kinin in their urine, compared with normal BN Kitasato (normal BN-Ki) rats from the same strain. Intra-arterial (i.a.) infusion (6 ml/kg/hr) of conscious deficient BN-Ka rats with 0.15 M NaCl did not increase mean arterial blood pressure (MBP) [from 103 ± 2 (pre) to 93 ± 6 mmHg (day 4)] and did not cause sodium accumulation in the serum, cerebrospinal fluid or erythrocytes, but 0.3 M NaCl infusion significantly increased MBP from 104 ± 3 (pre) to 130 ± 5 mmHg (day 4) with increased sodium levels in the serum, cerebrospinal fluid and erythrocytes. Infusion of 0.3 M NaCl in normal BN-Ki rats neither increased MBP nor accumulated sodium. The dose-response curve of the increase in MBP for angiotensin II injection (i.a., bolus, 1–1000 pmol/kg) in 0.3 M NaCl-infused deficient BN-Ka rats shifted to the left by a factor of 10 compared with that in 0.15 M NaCl-infused deficient BN-Ka rats, and that for norepinephrine injection shifted to the left by a factor of 30. Normal BN-Ki rats did not show any enhancement in MBP elevation with 0.3 M NaCl. These results suggest that the sodium accumulation attributable to a lack of kinin generation may be related to increased vascular reactivity to angiotensin II and norepinephrine.

Keywords: Sodium accumulation, Kininogen-deficient rat, Angiotensin II, Norepinephrine, Vasoconstriction

We have published a series of papers on the crucial role of the renal kallikrein-kinin system in the development of hypertension in animal models. In kininogen-deficient Brown Norway Katholiek (deficient BN-Ka) rats, which do not generate kinin in their urine, hypertension was induced easily in comparison with normal rats from the same strain, that is BN-Kitasato (normal BN-Ki) rats (1–6).

Deficient BN-Ka rats, which excreted less sodium and water after the loading of their diets with a mild concentration (2%) of sodium, readily accumulated sodium and showed increased systemic blood pressure (4, 6). Subcutaneous infusion of the deficient BN-Ka rats with a non-pressor dose (20 μ g/day/rat) of angiotensin II caused an increase in the systemic blood pressure and accumulation of sodium in the cerebrospinal fluid and the erythrocytes through aldosterone release (5). In deoxycorticosterone acetate (DOCA)-salt hypertension models, the systemic blood pressure was increased gradually up to 180 mmHg 10 weeks after the start of treatment in normal BN-Ki rats, whereas deficient BN-Ka rats,

with their lower sodium and water excretion, exhibited systemic blood pressure rises to the maximum values within two weeks after DOCA-salt treatment was initiated (1–3).

We therefore hypothesized that reduced excretion of sodium and water either after an increase in the dietary sodium concentration or after infusion of a non-pressor dose of angiotensin II may play a crucial role in the development of experimental hypertension. However, accumulation of sodium is generally accompanied with water retention, and cannot be separated from increase in blood volume, which may well induce a blood pressure increase.

In the present experiment, we infused conscious kininogen-deficient BN-Ka rats with either isotonic (0.15 M) NaCl solution or hypertonic (0.3 M) NaCl solution to clarify whether sodium accumulation or blood volume increase is the predominant factor in the development of experimental hypertension. Furthermore, we tested whether or not the sodium accumulation, which was seen in the kininogen-deficient BN-Ka rats, enhances the

arteriolar response to angiotensin II and norepinephrine.

MATERIALS AND METHODS

Animals

Deficient BN-Ka rats (*Rattus norvegicus*, BN/fMai) were initially obtained from the Katholiek Universiteit of Leuven, Belgium, and they were fed in our animal facilities (1–5). Normal rats from the same strain were already being kept in Kitasato University (BN-Ki rats). The animals used were males of 10 weeks of age. They were all housed at constant humidity ($60 \pm 5\%$) and temperature ($25 \pm 1^\circ\text{C}$), with a 12-hr light/12-hr dark cycle throughout the experiment. The number of animals used for each experiment is stated in the corresponding section. This study was performed in accordance with the animal experimentation guidelines of Kitasato University School of Medicine.

Prolonged infusion of sodium chloride solutions and blood pressure determination

From a few days after weaning throughout the experimental periods, the deficient BN-Ka and normal BN-Ki rats were fed ad libitum a low (0.3%) sodium chloride (NaCl) diet (NMF; Oriental Yeast Corp., Tokyo). The rats were given free access to distilled water for drinking. All rats were housed individually in the metabolic cages throughout the experimental period. At 10 weeks of age, a polyethylene cannula (PE-10; Clay-Adams, Parsippany, NJ, USA) was inserted into the abdominal aorta through the femoral artery under light ether anesthesia, and it was then connected to a PE-50 cannula (Clay-Adams) and exteriorized in the interscapular region. These cannulas were used to infuse the sterilized NaCl solution (0.15 M or 0.3 M, at an infusion rate of 6 ml/kg/hr) with a syringe-type infusion pump (Syringe Pump 11; Harvard Apparatus, Millis, MA, USA). The exteriorized cannula was fed through a plastic cannula cover (MK-77; Medical Agent Co., Ltd., Kyoto) to protect it from the rats. One end of the cover was attached to the subcutaneous tissue of the interscapular region with Aron Alpha adhesive (Toagosei Chemical Industry, Co., Ltd., Tokyo), and the other end was attached to a swivel (375/22; Instech Lab., Inc., Plymouth Meeting, PA, USA) mounted above the metabolic cage (7), so as to allow the rat free movement within the cage. When the systemic blood pressure was determined, the arterial infusion of NaCl solution was stopped, and a blood pressure transducer (TP-200T; Nihon Kohden, Tokyo) was attached to the external end of the intra-arterial (i.a.) catheter. The mean arterial blood pressure (MBP) was monitored on a polygraph (WS-641-G, Nihon Kohden) for 30 min daily, and immediately after the de-

termination of blood pressure, the i.a. infusion of NaCl solution was started again.

On the fourth day of the experiment, some of the rats were taken from the metabolic cages, and the end of the arterial cannula was plugged with a stainless steel pin. The systolic blood pressure was determined by the tail cuff method (7).

Intra-arterial injection of angiotensin II and norepinephrine

On the fourth day of the infusion period, i.a. infusion was interrupted to determine the hypertensive responses for i.a. injections of angiotensin II. The solution of angiotensin II (2–2000 pmol/ml in physiological saline containing 0.1% (W/W) gelatin; Peptide Institute, Minoh) was administered as a single i.a. injection of 0.5 ml/kg through the indwelling cannula used for the NaCl infusion, and the peak levels of the increased MBP were used to evaluate the hypertensive response.

The solution of norepinephrine (0.06–6 nmol/ml in physiological saline, 0.5 ml/kg) was also injected to determine the hypertensive response in the same manner as that of angiotensin II.

Blood collection

Under light ether anesthesia, blood was collected from the carotid artery of rats through the cannula (PE-50) into glass tubes without anticoagulant 4 days after the start of NaCl infusion. The collected blood was left at room temperature for 2 hr and then centrifuged at $1500 \times g$ for 15 min at 25°C to prepare serum. To prepare the citrated plasma, blood was collected into plastic tubes containing 1/10 volume of 3.8% sodium citrate solution and then centrifuged at $1500 \times g$ for 15 min at 25°C . Blood was also collected directly into plastic tubes containing ice-chilled iso-osmotic lithium chloride solution for the determination of the sodium concentration of erythrocytes.

Collection of urine and measurement of urinary levels of creatinine, sodium and potassium

The volume of urine collected in each metabolic cage was recorded at the end of each 24-hr period. Urinary creatinine levels were measured by a kinetic method using Jaffe's reaction (1, 4, 5). Urinary sodium and potassium levels were determined electrometrically using coated wire electrodes selective for sodium and potassium, respectively (1, 4, 5).

Measurement of urinary active-kallikrein and pro-kallikrein

The activities of the active-kallikrein and pro-kallikrein in the urine collected in the metabolic cages over 24 hr

was measured using Pro-Phe-Arg-MCA (Peptide Institute), a peptidyl fluorogenic substrate, as reported previously (4, 5, 8). One arbitrary unit (AU) was defined as the amount of urinary kallikrein that released 0.1 nmole 7-amino-4-methyl-coumarin (AMC) for 10 min/ μ l urine at 37°C.

Measurement of urinary kinin

Free kinin was measured in the urine collected via catheters (PE-10, Clay Adams) inserted into bilateral ureters of rats of the deficient BN-Ka and normal BN-Ki strains under pentobarbital anesthesia (60 mg/kg, s.c.). The kinin concentrations were determined with a recently developed bradykinin enzyme immunoassay kit (Markit M; Dainippon Pharmaceutical Corp., Osaka) (9, 10) after extraction with ethanol. The amounts of kinin secreted were expressed as ng/24 hr.

Measurement of kininogen concentrations in plasma

Plasma kininogen concentrations in citrated plasma were determined by the amount of kinin released from the plasma, as described in previous reports (1, 4, 5). The concentrations were expressed in terms of nanograms of bradykinin released per milligram of plasma protein.

Measurement of concentrations of creatinine, sodium and potassium in serum and sodium concentrations in erythrocytes

The concentrations of creatinine, sodium and potassium in the serum were determined by the methods used for those in urine, as described above. The sodium concentration in the erythrocytes (RBC[Na]_i) was determined by atomic absorption spectrophotometry (11), as reported previously (4, 5). The sodium concentrations in the erythrocytes are expressed as mEq/l.

Measurement of concentrations of sodium in cerebrospinal fluid

Cerebrospinal fluid from rats under light ether anesthesia was obtained by aspiration from the cisterna magna with a 26G needle. The concentrations of sodium in the cerebrospinal fluid were determined with an atomic absorption spectrophotometer (5).

Statistical analyses

Values are expressed as means \pm S.E.M., 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 by Wilcoxon's rank sum test. A *P* value less than 0.05 was considered to be significant.

RESULTS

Figure 1 summarizes the basal parameters of the kallikrein-kinin system of untreated deficient BN-Ka rats and normal BN-Ki rats (10-weeks-old). The plasma concentrations of high-molecular-weight (HMW) kininogen in deficient BN-Ka rats were very low (less than 0.2 ng bradykinin equivalent/mg plasma protein, *n*=6), as were those of low-molecular-weight (LMW) kininogen (less than 0.1 ng bradykinin equivalent/mg plasma protein, *n*=6), whereas the plasma concentrations of HMW and LMW kininogens in normal BN-Ki rats were 16.1 ± 0.9 (*n*=6) and 10.0 ± 0.9 (*n*=6) ng bradykinin equivalent/mg plasma protein, respectively. The urinary excretion of active-kallikrein and pro-kallikrein for 24 hr did not differ

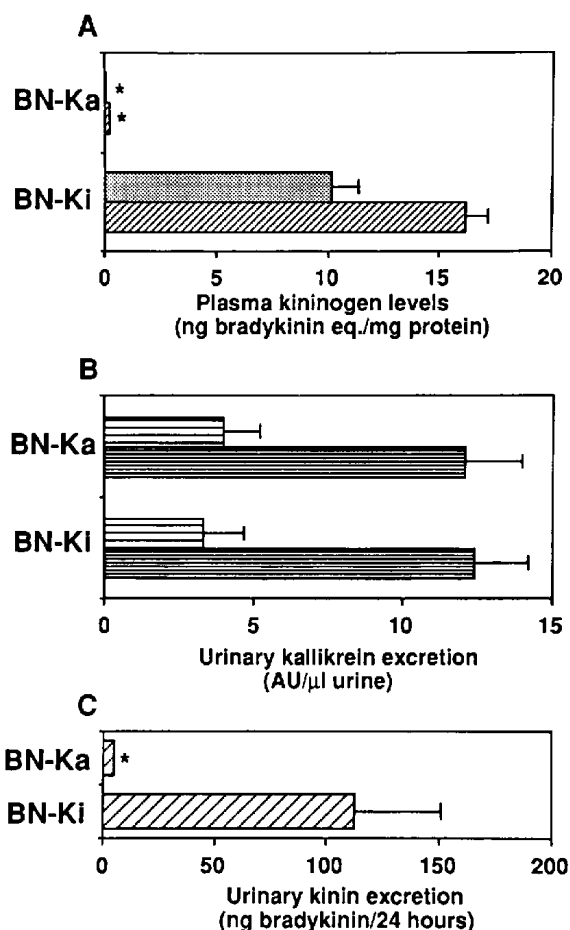


Fig. 1. Plasma kininogens (panel A), urinary active-kallikrein and pro-kallikrein (panel B) and urinary kinin (panel C) in untreated deficient Brown Norway Katholeik (BN-Ka) rats and normal Brown Norway Kitasato (BN-Ki) rats (10-weeks-old). Values show the means \pm S.E.M. from six animals for panels A and B and five animals for panel C. Values in deficient BN-Ka rats were compared with those in normal BN-Ki rats, **P* < 0.05. ▨, high-molecular-weight (HMW) kininogen; ▩, low-molecular-weight (LMW) kininogen; ▤, urinary pro-kallikrein; ▥, urinary active-kallikrein.

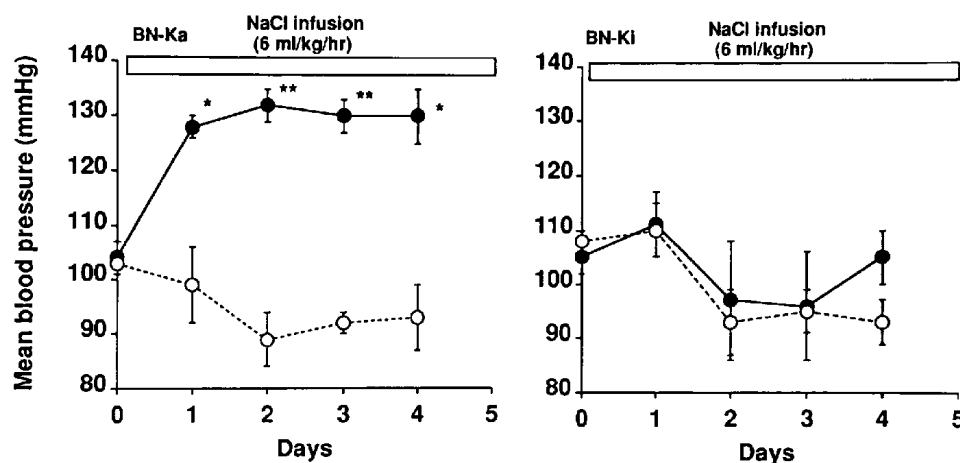


Fig. 2. Changes in the mean blood pressure during the intra-arterial infusion of NaCl solution in conscious deficient Brown Norway Katholeik (BN-Ka) rats and conscious normal Brown Norway Kitasato (BN-Ki) rats. Values show the means \pm S.E.M. from five rats. Sodium chloride solutions (0.3 M or 0.15 M) were infused (6 ml/kg/hr) into the abdominal aorta for 4 days from 10 weeks of age. Values from rats infused with 0.3 M sodium chloride solution (closed circles) were compared with those infused with 0.15 M sodium chloride solution (open circles) on the same day, * $P < 0.05$, ** $P < 0.01$.

between untreated deficient BN-Ka rats and normal BN-Ki rats. The amount of immuno-reactive free kinin excreted in the ureteral urine in the normal BN-Ki rats was 112.7 ± 39.8 ng bradykinin/24 hr ($n=5$), but was very low in the deficient BN-Ka rats (less than 4.8 ng bradykinin/24 hr, $n=5$). The results from deficient BN-Ka and normal BN-Ki rats in the present experiments were essentially the same as those in our previous reports (1, 4, 5).

MBP of conscious and untreated deficient BN-Ka rats (10-weeks-old) was 106 ± 3 mmHg ($n=10$), which was not

significantly different from that of conscious normal BN-Ki rats of the same age (112 ± 3 mmHg, $n=10$). Although there was no increase in MBP after i.a. infusion of 0.15 M NaCl solution, the i.a. infusion of hypertonic (0.3 M) NaCl solution in conscious deficient BN-Ka rats caused a marked increase in MBP (127 ± 3 mmHg, $n=5$) even one day after the start of infusion, and the increased concentrations remained fairly constant throughout the experimental period (Fig. 2). The difference between the MBPs of 0.3 M NaCl-infused and 0.15 M NaCl-infused

Table 1. Urinary excretions of potassium and creatinine during the infusion of NaCl solution

Rats Measurements	NaCl	Days				
		0	1	2	3	4
Deficient BN-Ka						
Potassium	0.3 M	15.9±1.2	29.3±4.3*	31.0±5.5*	36.3±11.9	23.7±8.6
(mEq/day)	0.15 M	15.0±1.2	14.6±1.2	15.9±2.1	13.7±1.8	13.1±1.3
Normal BN-Ki						
Potassium	0.3 M	15.0±0.4	25.7±1.7*	25.7±2.9*	31.0±4.2*	25.0±2.6*
(mEq/day)	0.15 M	15.6±0.4	12.3±1.8	12.8±1.8	16.9±1.0	13.0±1.5
Deficient BN-Ka						
Creatinine	0.3 M	7.9±0.4	6.8±0.5	9.3±1.2	12.9±0.9	15.9±1.3
(mg/day)	0.15 M	8.5±0.8	8.7±1.4	10.1±0.6	11.6±0.8	14.8±0.5
Normal BN-Ki						
Creatinine	0.3 M	7.9±0.4	8.3±1.6	9.2±1.1	11.4±1.2	15.3±0.9
(mg/day)	0.15 M	8.5±0.8	8.3±1.4	9.6±0.9	12.8±0.5	16.6±0.5

BN-Ka, Brown Norway Katholeik rats; BN-Ki, Brown Norway Kitasato rats. Each value represents the mean \pm S.E.M. from six animals. Values from rats infused with 0.3 M NaCl were compared with those infused with 0.15 M NaCl on the same day, * $P < 0.05$.

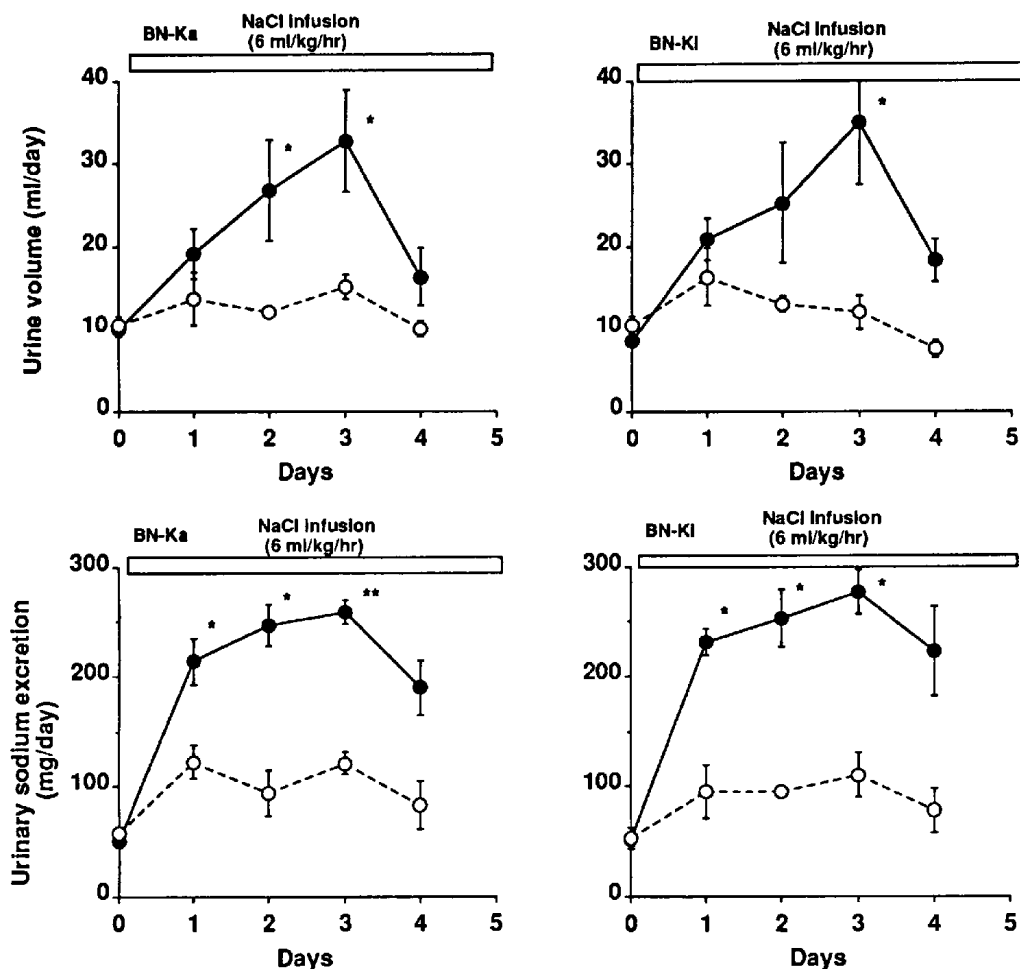


Fig. 3. Changes in urine volume and urinary sodium excretion during the intra-arterial infusion of NaCl solution in deficient Brown Norway Katholick (BN-Ka) rats and normal Brown Norway Kitasato (BN-Ki) rats. Values show the means \pm S.E.M. from five rats. Sodium chloride solutions (0.3 M or 0.15 M) were infused (6 ml/kg/hr) into the abdominal aorta for 4 days from 10 weeks of age. Values from rats infused with 0.3 M sodium chloride solution (closed circles) were compared with those infused with 0.15 M sodium chloride solution (open circles) on the same day, * $P < 0.05$, ** $P < 0.01$.

deficient BN-Ka rats was statistically significant ($P < 0.05$ or $P < 0.01$). In contrast, the infusion of neither 0.3 M nor 0.15 M NaCl solution to normal BN-Ki rats at the same infusion rate resulted in any significant increase in MBP (Fig. 2). The systolic blood pressure in 0.3 M NaCl-infused deficient BN-Ka rats, determined by the tail cuff method at the end of experimental period, was 166 ± 3 mmHg ($n=3$). The systolic blood pressure in untreated deficient BN-Ka rats of the same age was 133 ± 2 mmHg ($n=7$).

Figure 3 shows the changes in urine volume and urinary sodium excretions during the 4-day infusion of NaCl solutions. Infusion of 0.15 M NaCl solution did not markedly change the urine volume in either conscious deficient BN-Ka rats or normal BN-Ki rats. However, 0.3 M NaCl-infusion increased the volume of urine from the first day of the experiment in the deficient BN-Ka rats,

showing the peak level on the third day. The time-dependent increase of urine volume in normal BN-Ki rats receiving 0.3 M NaCl solution was essentially the same as that in deficient BN-Ka rats. The urinary sodium excretion of the deficient BN-Ka rats receiving 0.15 M NaCl solution were on a par with those of the normal BN-Ki rats having the same treatment, and marked increases in the excretion of urinary sodium were seen in both strains of rats infused with 0.3 M NaCl.

Urinary excretion of potassium did not change during the infusion of 0.15 M NaCl in either deficient BN-Ka rats or normal BN-Ki rats (Table 1). There was significantly greater urinary excretion of potassium in rats of both strains infused with 0.3 M NaCl solution compared with rats receiving 0.15 M NaCl solution (Table 1). However, no differences were observed in potassium excretion between deficient BN-Ka rats and normal BN-Ki rats (Table

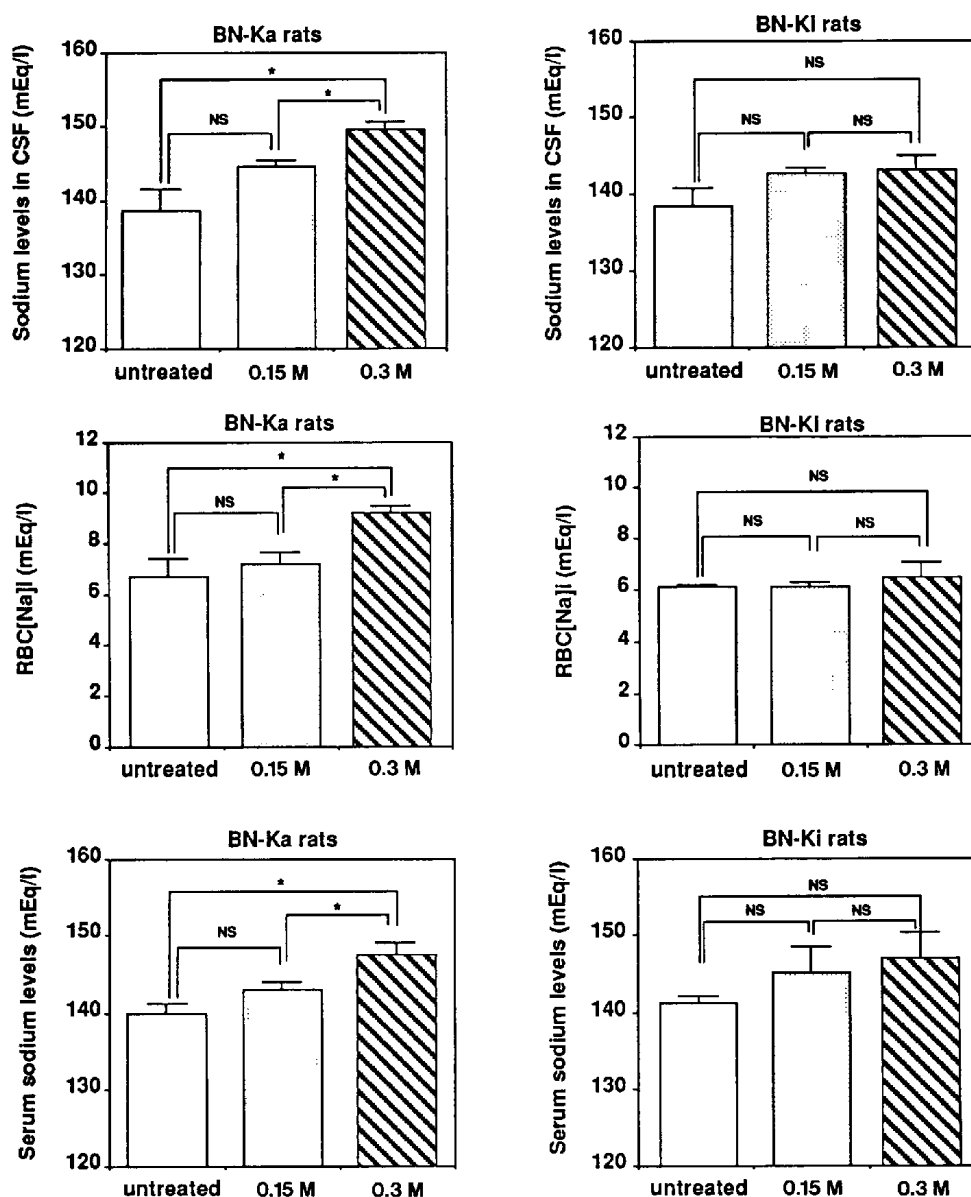


Fig. 4. Concentration is sodium in cerebrospinal fluid (CSF), erythrocytes and serum after 4-day infusion of NaCl solution in deficient Brown Norway Katholeik (BN-Ka) rats and normal Brown Norway Kitasato (BN-Ki) rats. Values show the means \pm S.E.M. from seven rats. Sodium chloride solutions (0.3 M or 0.15 M) were infused (6 ml/kg/hr) into the abdominal aorta for 4 days from 10 weeks of age. Values from rats infused with 0.3 M sodium chloride solution were compared with those infused with 0.15 M sodium chloride solution or those of untreated rats, * $P < 0.05$. RBC[Na]_i, sodium concentration in the erythrocytes.

1), and there were no difference in urinary excretion of creatinine between the two strains of rats (Table 1).

As shown in Fig. 4, after the 4-day infusion of 0.3 M NaCl solution, the sodium concentration in the cerebrospinal fluid in deficient BN-Ka rats was significantly higher than in those in deficient BN-Ka rats receiving 0.15 M NaCl solution and in untreated deficient BN-Ka rats. The sodium concentration in the erythrocytes of the deficient BN-Ka rats during the 4-day infusion

of 0.3 M NaCl solution was significantly higher than in those receiving 0.15 M NaCl solution and in untreated deficient BN-Ka rats. The same results were observed for the serum sodium concentration. In normal BN-Ki rats, the 4-day infusion of 0.3 M NaCl solution did not result in a significantly higher sodium concentration in the cerebrospinal fluid, erythrocytes or serum than in 0.15 M NaCl-infused normal BN-Ki rats or untreated BN-Ki rats.

The serum potassium level in deficient BN-Ka rats after

Table 2. Serum levels of potassium and creatinine in rats after 4 days of NaCl infusion and those in untreated rats

Rats	NaCl	Potassium (mEq/l)	Creatinine (μ g/ml)
Deficient BN-Ka	0.3 M	4.8 ± 0.3	7.9 ± 0.6
	0.15 M	4.7 ± 0.5	7.3 ± 0.3
	Untreated	4.6 ± 0.2	7.9 ± 1.1
Normal BN-Ki	0.3 M	5.0 ± 0.2	6.6 ± 0.4
	0.15 M	4.9 ± 0.2	6.6 ± 0.6
	Untreated	4.7 ± 0.2	6.9 ± 0.5

BN-Ka, Brown Norway Katholiek rats; BN-Ki, Brown Norway Kitasato rats. Each value represents the mean \pm S.E.M. from five animals.

the 4-day infusion of 0.3 M NaCl solution was not statistically different from that of untreated deficient BN-Ka rats, which in turn was not different from that after the infusion of 0.15 M NaCl solution (Table 2). The same was true in normal BN-Ki rats. The serum creatinine concentration of deficient BN-Ka rats receiving 0.3 M NaCl for 4 days was not different from that of untreated deficient BN-Ka rats (Table 2). No significant increase was observed after 0.15 M NaCl infusion (Table 2). In the case of normal BN-Ki rats, there were no significant changes after infusion of 0.3 M or 0.15 M NaCl solutions (Table 2).

As shown in Table 3, hematocrit values were not changed significantly after the 4-day infusion of 0.3 M

Table 3. Hematocrit values in rats after 4 days of NaCl infusion and those in untreated rats

Rats	NaCl	Hematocrit (%)
Deficient BN-Ka	0.3 M	42.3 ± 1.0
	0.15 M	44.3 ± 0.5
	Untreated	44.8 ± 0.9
Normal BN-Ki	0.3 M	42.0 ± 1.6
	0.15 M	43.2 ± 0.5
	Untreated	44.2 ± 0.6

BN-Ka, Brown Norway Katholiek rats; BN-Ki, Brown Norway Kitasato rats. Each value represents the mean \pm S.E.M. from nine animals.

NaCl solution or of 0.15 M NaCl solution in rats of either strain.

Figure 5 depicts the dose-response curves for angiotensin II-induced elevation of MBP after the 4-day infusion of NaCl solution. Single injections of angiotensin II (1–1000 pmol/kg) in conscious deficient BN-Ka rats increased the MBP by 2 to 52 mmHg after 4 days of 0.15 M NaCl infusion. The dose-response curve was not different from that in untreated deficient BN-Ka rats. After 4 days of 0.3 M NaCl infusion, however, the increases of MBP ranged from 21 to 57 mmHg when the same dose range of angiotensin II was administered. The dose-response curve for deficient BN-Ka rats after 0.3 M NaCl infusion was shifted to the left by a factor of 10 compared with that

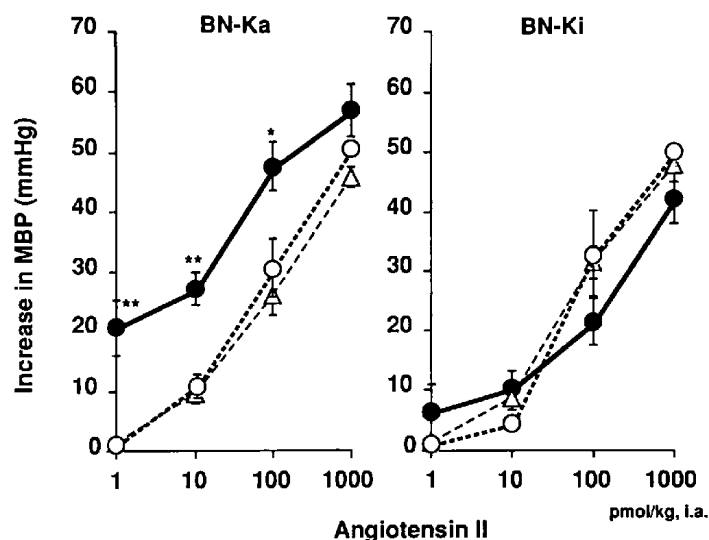


Fig. 5. Changes in the elevation of the mean blood pressure (MBP) after a bolus intra-arterial injection of angiotensin II to NaCl-infused conscious deficient Brown Norway Katholiek (BN-Ka) rats and conscious normal Brown Norway Kitasato (BN-Ki) rats. Values show the means \pm S.E.M. from six rats. Sodium chloride solutions (0.3 M or 0.15 M) were infused (6 ml/kg/hr) into the abdominal aorta for 4 days from 10 weeks of age. Values from rats infused with 0.3 M sodium chloride solution (closed circles) were compared with those infused with 0.15 M sodium chloride solution (open circles), * $P < 0.05$, ** $P < 0.01$. Values represented by open triangles are those from untreated rats.

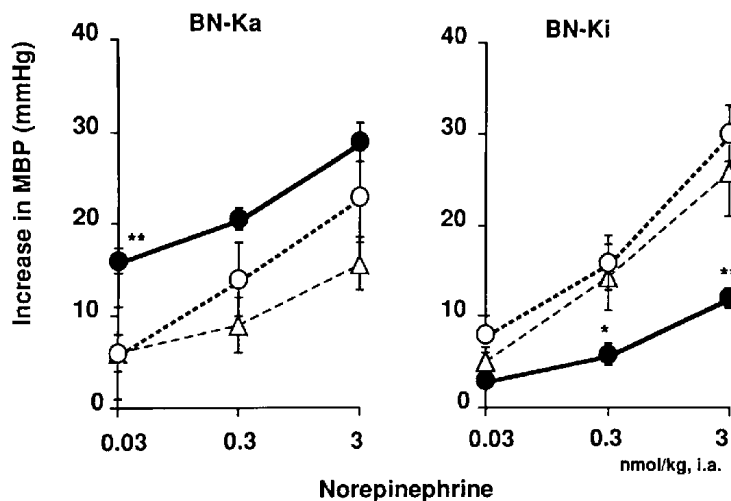


Fig. 6. Changes in the elevation of the mean blood pressure (MBP) after a bolus intra-arterial injection of norepinephrine to NaCl-infused conscious deficient Brown Norway Katholiek (BN-Ka) rats and conscious normal Brown Norway Kitasato (BN-Ki) rats. Values show the means \pm S.E.M. from six rats. Sodium chloride solutions (0.3 M or 0.15 M) were infused (6 ml/kg/hr) into the abdominal aorta for 4 days from 10 weeks of age. Values from rats infused with 0.3 M sodium chloride solution (closed circles) were compared with those infused with 0.15 M sodium chloride solution (open circles), * $P < 0.05$, ** $P < 0.01$. Values represented by open triangles are those from untreated rats.

after 0.15 M NaCl infusion. In contrast, even after the 4-day infusion of 0.3 M NaCl, the dose-response curve for normal BN-Ki rats was not shifted significantly compared with that after the infusion of 0.15 M NaCl, which was the same as that for the untreated normal BN-Ki rats.

Figure 6 presents the dose-response curves for norepinephrine-induced elevation of MBP after 4 days of infusion of NaCl solution. Infusion of 0.15 M NaCl solution in deficient BN-Ka rats did not shift the dose-response curve. However, that of 0.3 M NaCl solution caused a marked leftward shift of the dose-response curve by a factor of 30 in the deficient BN-Ka rats compared with that of 0.15 M NaCl solution. In contrast, 0.3 M NaCl infusion in normal BN-Ki rats did not shift the dose-response curve to the left.

DISCUSSION

The low kininogen concentrations in the plasma and the marginal generation of urinary kinin in mutant deficient BN-Ka rats reported in our previous papers (1, 4, 5) were also observed in the present experiments. The very low plasma concentrations of HMW and LMW kininogens were attributable to the inability of the liver of this rat strain to secrete kininogens (12), because of one point mutation of alanine¹⁶³ to threonine in the kininogen moiety, although the hepatic cells of deficient BN-Ka rats produced kininogens that are not different in molecular weight (about 110 kDa) from that in normal BN-Ki rats (13). Deficient BN-Ka rats that cannot generate kinin in

urine excreted less sodium and water in urine under a mild dietary NaCl loading or subcutaneous infusion of a non-pressor dose of angiotensin II. Hence, sodium was accumulated in the cerebrospinal fluid and erythrocytes, causing an elevation in blood pressure (1–6). During the treatment of rats with sodium loading or angiotensin II infusion, supplementation of LMW kininogen to deficient BN-Ka rats reduced the systemic blood pressure with increased excretion of urinary kinin, urinary sodium and urine volume, whereas subcutaneous infusion of a bradykinin B₂ receptor antagonist, Hoe 140, increased the blood pressure with reduced excretion of urinary sodium and urine volume, indicating direct linkage between kinin release, sodium excretion and high blood pressure.

In the present experiment, 4-day infusion of 0.3 M NaCl solution in conscious deficient BN-Ka rats resulted in the significant increase in MBP, whereas 0.15 M NaCl solution did not elevate it. Normal BN-Ki rats, receiving either 0.15 M or 0.3 M NaCl solution, did not show any hypertensive response during the experimental period. It is easy to imagine that sodium accumulation may be accompanied with water retention in this hypertension model. Thus, we first tested which of these two factors (sodium accumulation and water retention) is mainly involved in the development of this hypertension.

There have been many reports that described the effects of acute volume expansion on the systemic blood pressure and natriuresis in rats. Rapid infusion of physiological saline or Ringer's solution, which infusion rates were

ranged 30 ml/kg/hr to 600 ml/kg/hr, for 2 min to 40 min (the total infused volume; 10 ml/kg to 100 ml/kg) did not result in the significant increase in systemic blood pressure (14–17). Under these experimental conditions, diuresis and natriuresis were markedly increased to over 10-folds of the basal concentrations after volume expansion, but no increase in blood pressure was observed (14–16). In the present experiment, continuous infusion of isotonic NaCl solution at 6 ml/kg/hr also did not increase the blood pressure even after a 4-day infusion (Fig. 2). In terms of the amount of sodium, the present infusion rate of 0.15 M NaCl was approximately 5 mEq/day/rat.

Although some authors reported the elevation of systemic blood pressure in rats by chronic infusion of NaCl (7, 18), they used solutions containing high concentrations of NaCl, over 0.25 M. Kanagy and Fink reported the development of hypertension in renal mass-reduced rats, not in normal rats, during infusion with 6 mEq/day/rat (the total infused volume: 5 ml/day/rat) of sodium from the first day of the infusion experiment (7). Greene et al. reported the hypertensive response from 121 mmHg to 146 mmHg in MBP in Dahl-salt sensitive rats after a 4-day infusion of 20 mEq/day/rat of sodium (the total infused volume: 80 ml/day/rat) (18). When compared with our experimental condition, their rates of sodium infusion were 2-fold higher than ours using 0.3 M NaCl (10 mEq/day/rat). In Greene's experiment, the hypertensive response was accompanied with a marked reduction in hematocrit, from 37.7% to 27.5%, and an increase in body weight, suggesting that sodium infusion caused marked water retention in the body. However, in the present experiment, hematocrit values did not fall significantly after the 4-day infusion of 0.3 M NaCl or 0.15 M NaCl in either deficient BN-Ka rats or normal BN-Ki rats in spite of the difference in kinin generation. These results exclude the possibility that volume expansion is the cause of the hypertension in the present model.

The selective elevation of MBP in deficient BN-Ka rats during infusion of 0.3 M NaCl solution was accompanied with the accumulation of sodium in the serum, erythrocytes and cerebrospinal fluid. In contrast, the treatment of deficient BN-Ka rats with 0.15 M NaCl solution did not show any significant increase in sodium concentrations in these body fluids and cells. No increase in MBP and sodium concentrations in the cerebrospinal fluid and erythrocytes in normal BN-Ki rats receiving 0.3 M NaCl solution indicated that the MBP increase in deficient BN-Ka rats was due to the lack of kinin generation in the body. These results also strongly suggested that sodium accumulation in the body may be a cause of hypertension development in the present experiments.

It has been reported that the elevation of sodium concentration in the cerebrospinal fluid by intraventricular

injection of hypertonic NaCl solutions increased the systemic blood pressure in rats in a concentration-dependent manner (19, 20), and that reduction of cerebrospinal fluid sodium concentrations by intracerebroventricular infusion of NaCl-free isoosmotic mannitol solution resulted in significant reduction of MBP (21), indicating that sodium plays a crucial role in the induction of hypertension.

The increase in the sodium concentrations in the cerebrospinal fluid may increase the sodium retention in the nerve cells including the sympathetic nerve cells and the sympathetic discharge. The increased sympathetic tone increases vasoconstriction and heart rate or the cardiac output. In our previous experiments, the treatment of angiotensin II-induced hypertension with spironolactone immediately reduced the increased heart rate and the increased systemic blood pressure to the normal levels, together with a reduction of the sodium concentrations in the erythrocytes and cerebrospinal fluid (5). The increased activity in the sympathetic system in the development of hypertension has been repeatedly reported (22). Thus, it is plausible that the increased activity of the sympathetic tone due to sodium retention in the cerebrospinal fluid induces the hypertensive response.

The present experiment added further evidence that the sensitivity of the resistant vessels to vasoconstrictive substances, such as angiotensin II and norepinephrine, was increased after sodium accumulation. The sensitivity of arterioles to angiotensin II or norepinephrine was not different between deficient BN-Ka rats and normal BN-Ki rats without NaCl infusion or even after 0.15 M NaCl infusion. However, as shown in the present experiment, once sodium accumulation occurred in the deficient BN-Ka rats after 0.3 M NaCl infusion, the dose-response curve for angiotensin II or for norepinephrine was shifted to the left by a factor of 10 to 30. The reason why the dose-response curve of MBP increase by norepinephrine shifted to the right in 0.3 M NaCl infused normal BN-Ki rats is unknown.

Feeding a high sodium (3.4% NaCl) diet to rats for 3 weeks reduced the plasma renin activity by 73% and increased the expression of AT_{1A} receptors by 155% compared with low sodium intake (23). In humans, high sodium intake (100 mEq/day) caused the increase in AT receptor density by 80%, compared with low sodium intake (10 mEq/day) (24). However, in our previous hypertension models induced by DOCA-salt treatment (1) and sodium loading (4), although the reduction in plasma renin activity was observed, the reduced concentrations were not different between deficient BN-Ka rats and normal BN-Ki rats, suggesting that even if the up-regulation of the vascular AT receptors (25) occurred, it may occur to a minimum level in the present experiment. The intracellular signal transduction pathway may be influenced

by sodium accumulation in the present experiments. Furthermore, it has been reported that sodium loading (8.5% NaCl diet) to rats for 3 weeks increased the density of α_2 -adrenoreceptors by 24% (26). This rate of increase in receptor density (26) may not account for the leftward-shift of the dose-response curve for norepinephrine by a factor of 30 in the present experiment.

In conclusion, the sodium accumulation, rather than volume expansion, which is attributable to the lack of kinin generation, may be relevant to increased sensitivity to vasoconstrictors such as angiotensin II and norepinephrine.

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