Effect of Chronic Blockade of the Kallikrein-Kinin System on the Development of Hypertension in Rats

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Abstract—The kallikrein-kininogen-kinin system is an important vasodilator and vasodepressor component of the cardiovascular system. Acting mainly through B2 receptors, kinins may counterbalance the pressor effect of angiotensin II, salt, and mineralocorticoids plus salt. Using rats lacking the bradykinin precursors low- and high-molecular-weight kininogen or a B2 kinin receptor antagonist (icatibant), we investigated whether absence or blockade of the kallikrein-kinin system alters blood pressure (BP) in rats given (1) chronic infusion of Ang II, (2) a normal or high salt diet, or (3) chronic administration of deoxycorticosterone acetate (DOCA) plus salt. We confirmed the genotype and phenotype of Brown Norway Katholiek rats (BNK) and found that they had a G-to-A point mutation on the kininogen gene compared with Brown Norway (BN) and Sprague-Dawley (SD) rats, very low levels of high-molecular-weight kininogen (17±3 ng/mL) compared with BN and SD (1814±253 and 2397±302 ng/mL, respectively; P<0.01), and plasma low-molecular-weight kininogen concentrations below detectable limits compared with 1773±4 and 1781±140 ng/mL for BN and SD, respectively. Basal BP was the same in BNK and BN. Chronic infusion of icatibant did not alter BP in BN or Wistar rats. At doses that blocked the acute effect of bradykinin, icatibant did not potentiate the pressor effect of a chronic suppressor or pressor dose of angiotensin II in male and female Wistar rats nor that of a high salt diet (2%) plus unilateral nephrectomy in male Wistar rats. Moreover, blockade of the kallikrein-kininogen-kinin system in either BN rats given a very high dose of icatibant or kinin-deficient rats (BNK) did not potentiate the pressor effect of angiotensin II (nonpressor dose) or a high salt (3% NaCl) diet given for 2 weeks. Established DOCA-salt hypertension was not exaggerated in rats treated with icatibant but was partially attenuated by ramipril (1.5 mg · kg−1 · d−1 for 7 days; P<0.002). This antihypertensive effect was abolished by icatibant (P<0.002, ramipril versus ramipril plus icatibant). These results suggest that endogenous kinins do not participate in the maintenance of normal blood pressure or antagonize the development of hypertension induced by chronic infusion of angiotensin II, a high salt diet, or DOCA-salt. However, kinins appear to play an important role in the antihypertensive effect of angiotensin-converting enzyme inhibitors in DOCA-salt hypertension. (Hypertension. 2001;37:121-128.)

Key Words: kinins ▪ receptors, B2 ▪ angiotensin II ▪ sodium ▪ deoxycorticosterone acetate ▪ hypertension, experimental ▪ rats, Brown Norway Katholiek

Three types of kininogen are found in rat plasma: a low-molecular-weight form (LMWK, 9% to 14% of total plasma kininogen), a high-molecular-weight form (HMWK, 12% to 21%), and T-kininogen (65% to 77%).1 HMWK and LMWK are natural substrates of kallikrein, whereas T-kininogen is resistant to kallikrein but releases T-kinin by interacting with trypsin. Kinins are endogenous paracrine-autocrine vasodilators; acting by means of B2 receptors, they mediate the synthesis and release of prostaglandins and nitric oxide from the endothelium.2 This vasodilator effect has been extensively documented and reportedly plays an important role in the acute effect of angiotensin-converting enzyme inhibitors (ACEi).3–5 although its role in the chronic effects of ACEi remains controversial.6–8

Majima et al9–11 published a series of reports that indicated that Brown Norway Katholiek rats (BNK), which are deficient in kininogen, became hypertensive earlier than normal Brown Norway rats (BN) when subjected to both suppressor and pressor doses of angiotensin II (Ang II), a high salt diet, or deoxycorticosterone acetate (DOCA) plus salt. These authors also reported that normal rats given a subpressor dose of Ang II or a high salt diet became hypertensive when treated with either a kallikrein inhibitor (aprotinin) or a potent B2 kinin receptor antagonist (icatibant, administered subcutaneously). Using a different approach, Madeddu et al12–14 reported that chronic blockade of B2 receptors with icatibant (administered intraperitoneally) made rats hypertensive when chronically infused with either a pressor or nonpressor dose of Ang II or DOCA-salt. Both Majima et al and Madeddu et al concluded that defective kinin generation could contribute to arterial hypertension by fostering vasoconstrictor tone and sodium retention. In addition, Madeddu et al14,15 claimed that...
female rats were better models in which to study the cardiovascular effects of endogenous kinins because their renal kallikrein-kinin system was more active.

ACEi act mainly by (1) abolishing the conversion of Ang I to Ang II, a potent vasopressor agent, and (2) inhibiting the breakdown of bradykinin into inactive fragments.\textsuperscript{16,17} Locally produced kinins appear to play an important role in the cardioprotective effect of ACEi in heart failure induced by myocardial infarction\textsuperscript{18} and preconditioning in a rat model of ischemia-reperfusion.\textsuperscript{19} These protective effects were absent in the presence of a B\textsubscript{2} kinin receptor antagonist (icatibant).

Given the relevance of the findings of Majima et al\textsuperscript{9–11} and Maddeddu et al\textsuperscript{12–14} we attempted to confirm or refute them by testing the hypothesis that kinins play a key role in the balance between vasodilators and vasoconstrictors needed to maintain normal blood pressure (BP). For this, we compared BP in BNK versus BN rats and in BN or Wistar rats given a B\textsubscript{2} kinin receptor antagonist versus controls. We also carefully examined changes in BP in BNK rats given either a high salt diet or chronic subpressor doses of Ang II using conventional tail-cuff plethysmography or telemetry and studied whether blockade of B\textsubscript{2} kinin receptors with icatibant increases the pressor effect of chronic treatment with (1) a subpressor or pressor dose of Ang II, (2) a high salt diet, or (3) DOCA-salt. Finally, we tested whether the antihypertensive effect of ACEi in rats given DOCA-salt is mediated by kinins.

**Methods**

**Animals**

Five-week-old male BN and BNK (obtained from Kitasato University, Kanagawa, Japan, and maintained and bred in Henry Ford Hospital animal care facilities) and 8- to 9-week-old female and male Wistar and male Sprague-Dawley rats (SD; Charles River, Wilmington, Mass) were used for these experiments. Rats were housed in an air-conditioned room with a 12-hour light/dark cycle and received standard rat chow (0.4% sodium) and tap water ad libitum. BN and BNK were given standard 0.3% NaCl rat chow obtained from NMF Oriental Yeast Co (Tokyo, Japan) and tap water. This study was approved by the Henry Ford Hospital Care of Experimental Animals Committee.

**Surgical Procedures**

All surgical procedures were performed under aseptic conditions in rats anesthetized with either pentobarbital (50 mg/kg IP) or urethane (1.25 g/kg IP) and blood withdrawn through the abdominal aorta with a heparin-soaked syringe. Plasma HMWK and LMWK were measured by a modification of the method of Majima et al.\textsuperscript{10} For HMWK, plasma prekallikrein was primed through activation of coagulation factor XII by incubating plasma with glass powder at 37°C for 30 minutes; kininase inhibitors o-phenanthroline (6 mmol/L) and Na\textsubscript{2}EDTA (60 mmol/L) were also added to the reaction. HMWK was converted to kinins by plasma kallikrein, and released bradykinin was measured by radioimmunoassay.\textsuperscript{20} For LMWK, an additional plasma sample was incubated with glass powder in the absence of kininase inhibitors. Thus, kinins converted from HMWK by plasma kallikrein were destroyed by kininases in plasma.\textsuperscript{10} Peptidases, kallikrein inhibitors, and plasma prekallikrein were destroyed by acidification (pH 2): for this, HCl (1 mol/L) was added for 30 minutes at 37°C. Plasma was incubated with purified rat urinary kallikrein after pH was adjusted to 7.8 with 0.1 N NaOH to release kinins from LMWK. Kinins released from kininogen were assayed by radioimmunoassay.\textsuperscript{20} Kininogen levels were expressed as nanograms of kinin generated per milliliter of plasma.

**Infusion of Ang II, Icatibant, or Vehicle**

Infusions were performed by implanting intraperitoneal or subcutaneous osmotic minipumps (Alzet 2001 for 7 days or Alzet 2002 for 14 days) as indicated below. Pumps were replaced when necessary.

**Telemetry of BP**

The telemetric device (Data Sciences International) was implanted surgically in anesthetized rats in accord with the manufacturer’s specifications and institutional procedures. Rats were allowed to recover for 1 week in a quiet room. Baseline BPs were measured for 1 week with a Data Science acquisition program, which allows us to collect BP readings every 10 seconds for 24 hours. After implanting osmotic minipumps filled with Ang II or vehicle, BP was recorded for 2 more weeks. BP readings were averaged and plotted against 4 different time frames: 6 AM to 12 PM, 12 to 6 PM, 6 PM to 12 AM, and 12 to 6 AM.

**Phenotype and Genotype of BNK**

We investigated whether the genotype and phenotype that characterize BNK rats are conserved in these rats as described below.

**Measurement of Plasma Kininogen**

To confirm that BNK are deficient in plasma kininogen, we measured plasma kininogen from BN (n = 10), SD (n = 10), and BNK rats (n = 17). Rats given a normal diet were anesthetized with pentobarbital sodium (50 mg/kg IP) and blood withdrawn through the abdominal aorta with a heparin-soaked syringe. Plasma HMWK and LMWK were measured by a modification of the method of Majima et al.\textsuperscript{10} For HMWK, plasma prekallikrein was primed through activation of coagulation factor XII by incubating plasma with glass powder at 37°C for 30 minutes; kininase inhibitors o-phenanthroline (6 mmol/L) and Na\textsubscript{2}EDTA (60 mmol/L) were also added to the reaction. HMWK was converted to kinins by plasma kallikrein, and released bradykinin was measured by radioimmunoassay.\textsuperscript{20} For LMWK, an additional plasma sample was incubated with glass powder in the absence of kininase inhibitors. Thus, kinins converted from HMWK by plasma kallikrein were destroyed by kininases in plasma.\textsuperscript{10} Peptidases, kallikrein inhibitors, and plasma prekallikrein were destroyed by acidification (pH 2): for this, HCl (1 mol/L) was added for 30 minutes at 37°C. Plasma was incubated with purified rat urinary kallikrein after pH was adjusted to 7.8 with 0.1 N NaOH to release kinins from LMWK. Kinins released from kininogen were assayed by radioimmunoassay.\textsuperscript{20} Kininogen levels were expressed as nanograms of kinin generated per milliliter of plasma.

**Determination of a G-to-A Point Mutation on the Kininogen Gene**

The kinin deficiency in BNK rats is reportedly the result of a G-to-A point mutation at kininogen nucleotide position 487.\textsuperscript{21} To confirm that our BNK rat colony had this point mutation, we amplified 164-bp fragments containing kininogen nucleotide position 487 that our BNK rat colony had this point mutation, we amplified 164-bp fragments containing kininogen nucleotide position 487. To confirm that our BNK rat colony had this point mutation, we amplified 164-bp fragments containing kininogen nucleotide position 487. To confirm that our BNK rat colony had this point mutation, we amplified 164-bp fragments containing kininogen nucleotide position 487. To confirm that our BNK rat colony had this point mutation, we amplified 164-bp fragments containing kininogen nucleotide position 487. To confirm that our BNK rat colony had this point mutation, we amplified 164-bp fragments containing kininogen nucleotide position 487.

**Protocol 1**

In protocol 1, BP response to chronically infused Ang II in BNK rats (kinin deficient) or normal rats chronically treated with a B\textsubscript{2} kinin receptor antagonist (icatibant) was tested.

**Protocol 1a**

To determine whether BNK rats (kinin deficient) are sensitive to a subpressor dose of Ang II and examine diurnal changes in BP, we used telemetry, which is less stressful and is well suited for long-term drug studies. Rats weighing 100 to 135 g were subdivided into 2 groups that received either vehicle (n = 4) or Ang II (30 µg/d SC; n = 5). After measuring basal MBP, osmotic minipumps filled...
with either vehicle or Ang II were implanted and MBP was recorded for 2 more weeks.

**Protocol 1b**

BN rats weighing 135 to 155 g were studied, because this strain was used in the study of Majima et al. Rats were subdivided into 2 groups that received either Ang II plus vehicle (n=6) or Ang II plus icatibant (n=3). SBP was measured twice during the first week (baseline), and then an infusion of Ang II (20 μg/d SC) was started with osmotic minipumps and continued until the end of the study. On day 7, a very high dose of icatibant (5 mg · kg⁻¹ · d⁻¹) IP was infused and continued until the end of the study. SBP was measured twice a week until day 14. MBP was measured in awake rats on days 16 and 17.

**Protocol 1c**

Because Madeddu et al reported that the renal kallikrein-kinin system is more active in female than male Wistar rats, we compared the effect of a subpressor dose of Ang II (20 μg/d) on SBP and MBP in groups that received either (1) icatibant 500 μg · kg⁻¹ · d⁻¹ or (2) Ang II plus icatibant (500 μg · kg⁻¹ · d⁻¹), n=7 females and n=5 males, respectively. After basal SBP was measured, osmotic minipumps containing Ang II combined with either vehicle or icatibant were implanted intraperitoneally and SBP was measured twice a week for 2 weeks following the protocol of Madeddu et al. On day 14, MBP was measured directly in awake rats after which a single dose of bradykinin (100 ng IA) was injected into anesthetized rats to confirm B₁ receptor blockade.

**Protocol 1d**

Male Wistar rats weighing 175 to 215 g were used in this study as in the study of Madeddu et al. SBP was measured twice during the first week (baseline), and then a subpressor to pressor dose of Ang II (20, 40, 80, or 160 μg/d IP) combined with vehicle or icatibant (500 μg · kg⁻¹ · d⁻¹) IP was infused by minipump and continued until the end of the study (n=4 for 5 each). SBP was measured twice a week until day 14. MBP was measured in awake rats on days 16 and 17.

**Protocol 2**

This protocol measured the effect of a high salt diet on BP in BNK (kinin deficient) and normal rats chronically treated with a B₂ kinin receptor antagonist (icatibant).

**Protocol 2a**

Male BNK rats (120 to 180 g) were subdivided into 2 groups: (1) those that received a 3% NaCl diet (n=7) and (2) those that received standard 0.3% NaCl rat chow (n=8) for 3 weeks. SBP, 24-hour urinary volume (UV) and sodium excretion (UNaV, V) and body weight were measured in each rat before the diet was started and twice a week thereafter. At the end of the experiment, MBP was measured in conscious rats.

**Protocol 2b**

Male BN rats (120 to 175 g) were divided into 2 groups that received either (1) icatibant 5 mg · kg⁻¹ · d⁻¹ (n=11) or (2) vehicle (n=12). Powdered rat chow containing 3% NaCl was given for 3 weeks until the end of the protocol. One week after the high salt diet was begun, osmotic minipumps containing icatibant or vehicle were implanted subcutaneously. SBP, 24-hour UV and UNaV, and body weight were measured in each rat before the diet was begun and twice a week thereafter. At the end of the experiment, MBP was measured in conscious rats, after which the rats were anesthetized and bradykinin injected through the femoral vein.

**Protocol 2c**

Male Wistar rats (180 to 210 g) were divided into 2 groups that received either (1) icatibant 500 μg · kg⁻¹ · d⁻¹ (n=6) or (2) vehicle (n=6). Rats were anesthetized and uninephrectomy performed. Powdered rat chow containing 2% NaCl was given for 3 weeks until the end of the protocol. One week after the high salt diet was begun, osmotic minipumps containing icatibant or vehicle were implanted subcutaneously. SBP was measured before the diet was begun and twice a week thereafter. At the end of the experiment, MBP was measured in conscious rats.

**Protocol 3**

Protocol 3 measured the effect of chronic blockade of B₂ kinin receptors on maintenance of hypertension and the antihypertensive effect of ACE inhibition in DOCA-salt hypertensive rats.

Male SD weighing 225 to 250 g were given standard rat chow (0.28% NaCl) with free access to tap water and given 1 week to acclimate to the housing conditions. Basal SBP was measured by tail cuff, and then a silicone rubber sheet (No, 3110 RTV) containing DOCA (150 mg/kg) was implanted subcutaneously in uninephrectomized rats. Rats that received DOCA were given a solution of 1% NaCl and 0.2% KCl to drink. SBP was measured once a week for 4 weeks, the time needed to develop sustained hypertension. Osmotic minipumps filled with (1) vehicle (saline; n=7); (2) ramipril (1.5 mg · kg⁻¹ · d⁻¹; n=8); (3) icatibant (35 μg/d; n=7); or (4) ramipril plus icatibant (n=8) were implanted IP and the femoral artery catheterized as above. MBP was recorded every day for 1 week in conscious animals housed in a plastic restrainer. After MBP was measured, 100 ng bradykinin dissolved in 100 μL saline was injected intra-arterially into anesthetized rats and MBP recorded to determine whether blockade of B₂ receptors was successful.

**Materials**

Porcine pancreatic kallikrein, o-phenanthroline, NaCl, NaEDTA, and DOCA were purchased from Sigma. Ang II was obtained from Peptides International. Osmotic minipumps were obtained from Alza, polyethylene catheters from Clay-Adams, and silicone rubber from Dow Corning. Ramipril and icatibant were generously donated by Hoechst (Cincinnati, Ohio).

**Data Analysis**

Results are expressed as mean±SEM. Values were compared by ANOVA for repeated measures, except for the UV, UNaV, and BP responses to bradykinin (in BN and Wistar rats), which were analyzed by paired t test. BP response to bradykinin in BNK rats given different levels of salt in the diet was compared using a 2-sample t test. Pairwise comparisons were made using Holm’s method to adjust for multiple testing to compare BP of the 4 groups listed in protocol 3. P<0.05 was considered significant.

**Results**

**Kininogen Deficiency and Presence of a G-to-A Point Mutation on the Kininogen Gene in BNK Rats**

Plasma HMWK in BNK was 17±3 mg/mL, 107-fold lower than BN (1814±253) and 140-fold lower than SD (2397±302; P<0.01). Plasma LMWK was below detectable limits in BNK (<5 ng/mL) compared with BN (1773±74) and SD (1781±140; P<0.01). We also confirmed by polymerase chain reaction and sequence analysis that BNK rats (but not BN or SD) have a G-to-A point mutation of nucleotide 487 on the amplified cDNA fragment, in the heavy chain of the kininogen gene.

**Effect of Kininogen Deficiency or Chronic Blockade of B₂ Kinin Receptors on Basal BP**

Comparison of SBP among all rats showed that BNK (kinin deficient) had SBP almost identical to BN (124±5 versus 123±3 mm Hg; n=15 and 23, respectively). Icatibant (500 μg · kg⁻¹ · d⁻¹ for 14 days) tended to increase SBP in Wistar
rats (123±3 mm Hg; n=5) compared with controls (115±4 mm Hg; n=5), but did not reach significance.

Protocol 1

Protocol 1a
Telemetric BP in BNK rats given a subpressor dose of Ang II (30 μg/d) was not significantly different from rats given saline (Figure 1); BP was higher at night than during the day in rats given Ang II or vehicle, which is to be expected since rats are nocturnal animals. Although it did not reach significance, BP was lower at week 2 compared with baseline or week 1 during the day in both Ang II and vehicle groups.

Protocol 1b
In BN, chronic infusion of a subpressor dose of Ang II (20 μg/d) did not alter SBP or MBP either in the absence or presence of a very high dose of icatibant (5 mg·kg⁻¹·d⁻¹) (Figure 2).

Protocol 1c
SBP and MBP were measured during chronic infusion of a subpressor dose of Ang II (20 μg/d) alone or combined with icatibant in Wistar rats. Icatibant did not increase SBP or MBP in either females or males (Figure 3); however, in the

Figure 2. Effect of a subpressor dose of Ang II (20 μg/d IP) alone (○) or combined with a very high dose of icatibant (5 mg·kg⁻¹·d⁻¹; ●) on SBP and MBP in BN. The effect of Ang II was unaltered by chronic blockade of B₂ receptors. Veh indicates vehicle; B₂-ant, B₂ antagonist. Values are mean±SEM.

same rats bradykinin (100 ng) given intra-arterially decreased BP by 15±2 mm Hg in females and 14±2 mm Hg in males. This depressor effect was significantly lowered to 0±1 (P=0.0004) and 2±2 mm Hg (P=0.0001) by chronic icatibant treatment.

Protocol 1d
The pressor effect of Ang II at doses of 20, 40, 80, or 160 μg/d for 14 days was measured in male Wistar rats infused with vehicle or icatibant (500 μg·kg⁻¹·d⁻¹). Icatibant did not increase SBP in rats chronically infused with subpressor or pressor doses of Ang II (Figure 4).

Protocol 2

Protocol 2a
A high salt diet did not increase SBP or MBP in BNK (kinin deficient) (Figure 5A). In BNK given a high salt diet (3% NaCl), UV and UNa,V increased to 46.9±5.5 mL per 100 g body weight per 24 hours and 7.1±0.4 mmol per 100 g body weight per 24 hours, respectively, compared with rats on a normal diet (UV, 10.9±0.7; UNa,V, 0.8±0.06).

Protocol 2b
BN given a high salt diet did not exhibit any significant changes in SBP, either in the absence or presence of a very high dose of icatibant (5.0 mg·kg⁻¹·d⁻¹; Figures 5B and 5D). MBP was very similar in BN given high salt alone or combined with icatibant (114±2 versus 113±3 mm Hg; n=10 in each group). With sodium loading, BN excreted higher amounts of sodium (UNa,V, 8.1±1.6 mmol per 100 g body weight per 24 hours) and urine (UV, 50.5±3.9 mL per 100 g body weight per 24 hours) compared with rats given normal sodium (UNa,V, 0.58±0.1; UV, 8.4±0.5). Icatibant did not affect UNa,V or UV in rats given a high salt diet (UNa,V, 8.2±0.7; UV, 43.5±3.3). Exogenous bradykinin (10 μg IV) lowered MBP in anesthetized BN given high salt alone by 13±1 mm Hg (n=5), and its effect was almost completely blocked in rats given high salt plus icatibant (3±1 mm Hg; n=9; P<0.01).

Protocol 2c
SBP was significantly increased in Wistar rats fed a high salt diet (P<0.05). Icatibant did not increase the pressor effect of
MBP was also similar in rats given a high salt diet alone or combined with icatibant.

**Protocol 3**

In DOCA-salt hypertensive rats treated with an ACE inhibitor (ramipril 1.5 mg \( \cdot \) kg\(^{-1} \cdot \) d\(^{-1} \) for 7 days), MBP decreased from 176±9 (n=7) to 158±7 mm Hg (n=8; \( P=0.066 \)). This effect was blocked by icatibant, which resulted in an MBP of 184±7 mm Hg (n=5; \( P<0.05 \)). Blockade of B\( _2 \) kinin receptors with icatibant (35 \( \mu \)g/d IP) did not aggravate the hypertension induced by DOCA-salt in conscious rats (Figure 6) but significantly attenuated the hypotensive effect of exogenous bradykinin (100 ng IA) from 56±8 to 13±3 mm Hg (n=3 for each group; \( P<0.01 \)).

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**Figure 4.** Changes in SBP caused by Ang II (0 to 160 \( \mu \)g/d SC) either alone (C) or combined with icatibant (500 \( \mu \)g \( \cdot \) kg\(^{-1} \cdot \) d\(^{-1} \)) in male Wistar rats. SBP was determined by tail cuff before treatment (time 0) and every 3 days for 2 weeks after Ang II infusion was begun. Icatibant did not alter the pressor effect of Ang II. Values are mean±SEM.

**Figure 5.** A, BP changes in BNK given a normal (E) or high salt diet (F). B and C, BP changes in BN and Wistar rats due to high salt in the absence (C) or presence of icatibant (F). High salt increased BP in Wistar but not BN rats independent of B\( _2 \) kinin receptor blockade. D, BP was not affected by B\( _2 \) receptor blockade with icatibant or lack of kinins in rats given a high salt diet. Wistar rats had lower BP than BN or BNK. Values are mean±SEM. *\( P<0.05 \) vs baseline.
Discussion

This study was designed to test whether chronic blockade or absence of kinins (1) increases BP in animals fed a normal diet; (2) potentiates the hypertensionogenic effect of Ang II, high salt intake, or DOCA-salt (a model of low renin and high kinins), or (3) prevents the antihypertensive effect of ACE inhibitors in established DOCA-salt hypertension. Two different approaches were followed: (1) use of kininogen-deficient BNK rats and (2) chronic blockade of kinin B₂ receptors with a kinin antagonist (icatibant). First, we confirmed the presence of a G-to-A point mutation at nucleotide 487, which causes a substitution of Thr for Ala₁₆₃ in the heavy chain of HMWK and LMWK in BNK but not normal BN or SD rats. Our results only partially confirm the findings by Yang et al.,¹⁹ since we found that BNK rats merely had traces of HMWK and LMWK, whereas Yang et al observed very low HMWK and normal LMWK concentrations in plasma. This could be due to the source of glandular kallikrein used in the present study and the studies of Majima et al.⁹–¹¹ (purified urinary kallikrein) compared with the study of Yang et al, in which glandular kallikrein (porcine pancreas) was obtained from Sigma. Indeed, we confirmed that porcine pancreatic kallikrein from Sigma was still capable of releasing kinins from LMWK. It is not clear how commercial kallikreins release kinins in BNK, but they could act like trypsin, releasing kinin from T-kininogen.

We found that chronic blockade of the kallikrein-kinin system did not increase SBP or MBP (1) under normal conditions, (2) in Wistar rats (male or female) given a chronic subpressor or pressor dose of Ang II, or (3) in BN or BNK given a chronic subpressor dose of Ang II. These data are at odds with those of Majima et al.,¹¹ who found a difference of >40 mm Hg in SBP between BN and BNK (kinin deficient) after infusion of a subpressor dose of Ang II for 2 weeks, and Maddedu et al.,¹⁴ who found that chronic blockade of the B₂ kinin receptor increased the effect of both pressor and subpressor doses of Ang II in female Wistar rats. At present, we cannot explain these discrepancies. Similarly, chronic blockade of the kallikrein-kinin system did not increase the pressor effect of a high salt diet in Wistar, BN, or BNK rats. Interestingly, we found that under normal conditions, Wistar rats had lower BP than either normal BN or BNK (Figure 5); while the reason for this is unknown, differences in strain and age could account for the variations in BP. Although we did not study sodium balance (nor did Majima et al.¹⁰), we confirmed that BNK had increased UV (9-fold) and UNa⁺ (5-fold) when given a high salt versus a normal diet; similarly, BN treated with icatibant exhibited increased UV (14-fold) and UNa⁺ (5-fold) on a high salt versus a normal diet, which confirms that the rats received a high salt diet. These data contrast with those previously reported by Majima et al.,¹⁰ for reasons that remain unclear but agree with those of Maddedu et al.¹² Majima and colleagues¹⁰ found that SBP was increased by ~40 mm Hg in BNK but not BN given a high salt diet, whereas Maddedu et al.¹² reported that icatibant did not increase the pressor effect of high salt in Wistar rats. Dr Majima was invited to our laboratory to discuss the reasons for these discrepancies. He kindly performed 2 different sets of experiments with us to try to confirm his previous studies⁹,¹⁰: (1) normal BN rats were given a subcutaneous infusion of Ang II or high salt diet alone or combined with icatibant (given intraperitoneally) and (2) BNK rats were given a subcutaneous infusion of a nonpressor dose of Ang II or a high salt diet. Although the experimental conditions (BNK rats, powdered rat chow, acidified water, and Ang II) were identical to those used in Japan, we were unable to reproduce any of their findings or those of Maddedu et al.¹⁴ The subpressor effect of Ang II was not increased by icatibant either intraperitoneally or subcutaneously (N.-E.R. and O.A.C., unpublished observations, 1999). Maddedu et al.¹⁴ showed that the BP response to chronic infusion of a subpressor dose of Ang II (subcutaneous) was increased in the presence of icatibant given intraperitoneally. We could not explain these dramatic differences between our findings and those of Majima et al or Maddedu et al. One could also question whether changes in BP occur only at night, when the rats are active; however, this possibility was ruled out because telemetry showed no difference in BP between BNK infused with vehicle or a subpressor dose of Ang II either during day or night.

Alfie et al.²⁵ found that mice genetically lacking the B₂ kinin receptor gene had higher SBP and MBP only when given a diet very high in salt. This study was recently confirmed by Cervenka et al.,²⁶ who started in a mouse model a high salt diet before birth and continuing it in the pups for 4 months after birth. Both groups found that permanent inactivation of B₂ receptors does not cause adult hypertension, which refutes the findings of Maddedu et al.²⁷ B₂ receptors are well established as the predominant if not the...
only means by which kinins may induce the release of endogenous vasodilators such as nitric oxide, hyperpolarizing factors, and prostaglandins. Therefore, eliminating B₂ receptors from the body might contribute to the imbalance between vasoconstrictor agents and kinins, and this may have occurred in mice in which the B₂ receptor gene was disrupted and a high salt diet was given longer than in BNK (6 to 8 versus 2 to 3 weeks). In contrast to these transgenic mice, BNK were still able to produce active kinins through release of T-kininogen following the action of trypsin-like enzymes on T-kininogen,21,28 which, in turn, may act on the B₂ receptor. Findings of Alfie et al21 and Cervenka et al26 contrast with those of the present study, since that we found that even a very high dose of icatibant, which completely blocked B₂ kinin receptors, did not increase the pressor effect of a high salt diet or chronic infusion of Ang II. This discrepancy may be due to 3 fundamental differences: (1) our protocol was designed for just 2 to 3 weeks follow-up after a subpressor dose of Ang II or high salt diet was begun, whereas Alfie and colleagues did not observe increased BP until 6 to 8 weeks after a high salt diet was started and did not notice any changes in BP after 3 to 4 weeks (M.E. Alfie, O.A. Carretero, unpublished observations, 1996); (2) B₂ receptors were absent in the transgenic mice since the fetal stage, whereas BN rats were treated with the B₂ kinin receptor antagonist for only a short time; and (3) icatibant may not be as efficient as animals that lack B₂ receptors, because icatibant may be degraded before it reaches the lumen of the distal nephron. We infused radiolabeled icatibant in rats and found that it was completely degraded in the urine (O.A.C, unpublished observations, 1999). Thus, whether the kallikrein-kinin system is physiologically linked to regulation of sodium balance (and, hence, BP regulation) remains unclear.

We found that ACE inhibitors have a mild antihypertensive effect on DOCA-salt hypertension, which was blocked by a B₂ kinin receptor antagonist. Icatibant alone did not increase BP further in rats with established DOCA-salt hypertension, in contrast to the results of Maddeddu et al.12,13 They found that chronic blockade of B₂ kinin receptors not only facilitated development of that chronic blockade of B₂ kinin receptors was due to 3 fundamental differences: (1) our protocol was designed for just 2 to 3 weeks follow-up after a subpressor dose of Ang II or high salt diet was begun, whereas Alfie and colleagues did not observe increased BP until 6 to 8 weeks after a high salt diet was started and did not notice any changes in BP after 3 to 4 weeks (M.E. Alfie, O.A. Carretero, unpublished observations, 1996); (2) B₂ receptors were absent in the transgenic mice since the fetal stage, whereas BN rats were treated with the B₂ kinin receptor antagonist for only a short time; and (3) icatibant may not be as efficient as animals that lack B₂ receptors, because icatibant may be degraded before it reaches the lumen of the distal nephron. We infused radiolabeled icatibant in rats and found that it was completely degraded in the urine (O.A.C, unpublished observations, 1999). Thus, whether the kallikrein-kinin system is physiologically linked to regulation of sodium balance (and, hence, BP regulation) remains unclear.

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We conclude that kinins do not oppose the pressor effect of a chronic infusion of Ang II or a high salt diet; thus, results of the present study fail to confirm the data of Majima et al.9–11 or Maddeddu et al.14,15 for reasons that remain unclear.

Although kinins did not regulate BP, they did mediate the beneficial effect of ACEi in DOCA-salt hypertensive rats.


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Effect of Chronic Blockade of the Kallikrein-Kinin System on the Development of Hypertension in Rats
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