A Nonpeptide Mimic of Bradykinin Blunts the Development of Hypertension in Young Spontaneously Hypertensive Rats

Masataka Majima, Izumi Hayashi, Naoya Inamura, Tomoe Fujita, Michiko Ogino

Abstract—We tested whether FR190997, a nonpeptide B 2 agonist, prevented the development of hypertension in young spontaneously hypertensive rats (SHR), which secrete less kallikrein into the urine than do Wistar-Kyoto rats. An intra-arterial (IA) injection of FR190997 (0.3 to 30 nmol/kg) caused dose-dependent hypotension in conscious Sprague-Dawley rats. Although the maximum hypotensive potency of FR190997 equaled that of bradykinin, its action lasted ~10 times as long. Hoe140 (100 nmol/kg IA) significantly blocked the hypotensive response induced by FR190997 (10 nmol/kg). Atropine (100 nmol/kg IA) did not affect this response. A selective infusion of FR190997 into the renal artery induced natriuresis and diuresis in anesthetized rabbits. A continuous infusion (2 nmol·10 mL⁻¹·h⁻¹ per rat) of FR190997 into the abdominal aorta of young SHR (6 weeks old, n=6) for 6 days significantly (P<0.05) reduced mean blood pressure to 114±6 (day 2) and 110±6 (day 5) mm Hg, from 149±7 and 162±6 mm Hg, respectively, in vehicle-infused rats (n=6). At 8 days after continuous infusion (day 14), mean blood pressure (148±5 mm Hg) in FR190997-infused rats remained significantly (P<0.05) lower than that in vehicle-infused rats (190±6 mm Hg), almost the peak value. The mesenteric artery isolated from FR190997-treated rats (day 14) had lower contractile sensitivity to norepinephrine than that from vehicle-treated rats. These results suggested that the continuous infusion of a nonpeptide B 2 agonist may prevent hypertension if performed in the critical phase. (Hypertension. 2000;35[part 2]:437-442.)

Key Words: rats, inbred SHR ■ bradykinin ■ natriuresis ■ hypertension, experimental ■ blood pressure

Bradykinin (BK) is known to increase renal blood flow and water and sodium excretion.1–3 Kinin is generated through the action of kallikrein secreted in the distal tubules, and its receptors are distributed on the tubular cells.1–3 Reportedly, less urinary kallikrein is secreted in patients with essential hypertension, and the renal kallikrein-kinin system (RKKS) may help to suppress hypertension in animal hypertensive models and in humans.1,4,5

Our study in kininogen-deficient Brown Norway-Katholiek (BN-Ka) rats and normal control animals of the same strain (BN-Kitasato and BN-Ki) suggested that RKKS may contribute to systemic blood pressure reduction early in the development of deoxycorticosterone acetate-salt hypertension through the acceleration of sodium and water excretion.6 Furthermore, we found that BN-Ka rats are sensitive to salt and a nonpressor dose of angiotensin II, showing an elevation in blood pressure, reduced urinary sodium excretion, and increased sodium retention.7,8 This suggested that a deficiency in RKKS function contributes to the development of hypertension when a high salt diet or nonpressor dose of angiotensin II is administered.

The facilitation of RKKS may be effective in the prevention of hypertension. In the kidney, kinin acts mainly on the BK receptors present on the collecting ducts1,9 and induces natriuresis through the inhibition of sodium reabsorption. Kinin is readily degraded by kininases in the kidney, so prevention of its degradation may facilitate its functions. Our previous reports on the blockade of renal-specific kinin degradation by inhibitors support this possibility.10–12 Alternatively, supplementation of kinin moieties to the renal tubules may facilitate RKKS function. A continuous infusion of low-molecular-weight kininogen, a preferential substrate for renal kallikrein, markedly inhibits hypertension induced by salt in the diet and by the prolonged administration of angiotensin II.7,8 Kinin analogs resistant to kinin-degrading protease may also be helpful in preventing hypertension, because kininases were abundant in both the circulation and the renal tubules.1

Recently, FR190997, the first nonpeptide mimic of BK, was developed.13,14 This compound acts only on B 2 , not inducible B 1 , receptors13,14 and should be useful for study of the pathophysiological role of BK. In the present study, we examined the inhibition by FR190997 of the development of hypertension in young spontaneously hypertensive rats (SHR), and the results provide the basis for the development of a novel treatment for hypertension.

Methods

Animals
We used male SHR (specific pathogen free, 6 weeks old; Hoshino-Sankyo Laboratory Service) and male Sprague-Dawley (SD) strain...
rats (specific pathogen free, 6 weeks old; SLC, Shiznoka Laboratory Animal Center). All rats received normal rat chow (NMF; Oriental Yeast Corp) and tap water ad libitum immediately after weaning, were housed at a constant humidity (60±5%) and temperature (25±1°C), and were maintained on a continuous 12-hour light/dark cycle.6,8

We also used male Japanese White rabbits (specific pathogen free, weigh 2 to 2.5 kg; SLC) in the experiments for selective renal artery infusion. This study conformed to the guidelines for animal experiments of the Kitasato University School of Medicine.

Compound
FR190997 (8-[2,6-dichloro-3-[N{(E)-(4-N-methylcarbamoyl)-
cinnamidoacetyl]N-methyl-amino}[benzoyloxy]-2-methyl-4-(2-
pyridylmethoxy)quinoline) was developed as the first nonpeptide
MgCl2 1.2, NaHCO3 15, NaH2PO4 1.2, and glucose 6, through which
the addition of phenylephrine at a final concentration of 10
specific gravity of 1. Urinary sodium and potassium levels were
measured with an ion-selective electrode (Fuji Dri-Chem Slide
Na-K-Cl, 800 V; Fuji Film Co, Ltd), as reported previously.11

Continuous Infusion of FR190997 Into Abdominal Aorta in Young SHR
As a preliminary experiment, 5-week-old male SHR and Wistar-Kyoto (WKY) rats were put into a metabolic cage, and the 24-hour secretion of renal active kallikrein was determined as reported previously.16 We confirmed the report16 that WKY rats secreted about half the amount of kallikrein secreted by SHR. These rats were used in the following infusion experiments.

A continuous intra-arterial infusion (2 nmol·10 mL−1·h−1 per rat)
of FR190997 into young SHR (male, 6 weeks old) was performed for 6 days with miniosmotic pumps (2 ML1; Alzat). Briefly, a polyethylene cannula (PE-10) was inserted into the abdominal aorta through the femoral artery with the animal under light ether anesthesia and connected to a PE-50 cannula. This was connected to a Y-shaped tube, 1 arm of which was blocked with a stainless steel pin for later connection to a miniosmotic pump, and the other arm was exteriorized in the interscapular region for blood pressure determination. One day after intra-arterial cannula insertion, a blood pressure transducer (TP-200T; Nihon Koden) was attached to the free end of the cannula, and MAP was monitored on a polygraph. On the day of implantation of the pumps, an osmotic pump containing either FR190997 or vehicle was implanted subcutaneously into the backs of rats and connected to the Y-tube of the indwelling cannula with the animals under light ether anesthesia. The site of the intra-arterial infusion, which was determined with the use of SHR of the same age, was proximal to the branches of renal arteries in 1 group and distal to them in the other. One group of rats received FR190997 proximal to the renal arteries, and the other group of rats received FR190997 distal to the renal arteries. After a 6-day intra-arterial infusion, the pumps were removed with the animals under light ether anesthesia.

Twenty-four-hour urine samples were collected during the experiment from individual rats, with the use of metabolic cages, and the volume of each was recorded. Urinary sodium excretion was also determined.

Eight days after withdrawal of the intra-arterial infusion (day 14 of the experiment), the rats were exsanguinated, and the mesenteric arteries were isolated. The contractions induced by norepinephrine were tested with the use of an isometric myograph. The ED50 values of the FR190997-treated rats for the contractions and those of the vehicle-treated rats were compared.

Statistical Analysis
Values are expressed as mean±SEM. Factorial ANOVA, followed by a post hoc test, or repeated ANOVA was used for the results from multiple groups. A value of P<0.05 was considered statistically significant.

Results
Effect of FR190997 on Isolated Small Arteries Contracted by Phenylephrine
To test the effect of FR190997 on arterial constriction by phenylephrine, we determined the isometric contraction of the mesenteric arteries from normotensive SD rats. Phenylephrine (10−6 mol/L) caused the contractions in the presence or absence of indomethacin (10−5 mol/L). Phenylephrine was added to the bath, as was FR190997 (10−7 mol/L) 8 to 10 minutes later (Figures 1A and 1B). Without indomethacin (Figure 1A), the artery became dilated after a transient contraction occurred on the addition of FR190997. This contraction disappeared with indomethacin treatment, although the same magnitude of dilation was seen (Figure 1B).

Even when BK was used without indomethacin, transient contraction occurred, followed by dilatation (Figure 1C). Indomethacin treatment inhibited this contraction but did not

Effects of FR190997 on Contracted Isolated Small Arteries
Mesenteric arteries were isolated from 6-week-old SD rats while the animals were under pentobarbital anesthesia. Segments (5 mm long) of mesenteric artery were dissected free as described previously15 and mounted as rings on an isometric myograph (Dual Myograph, model 410A; J.P. Trading), which allows the direct measurement of vessel wall force while the internal circumference is controlled.15 Vessels were bathed in normal Na+Krebs solution maintained at 37°C and containing (in mmol/L) NaCl 119, KCl 4.6, CaCl2 1.5, MgCl2 1.2, NaHCO3 15, NaH2PO4 1.2, and glucose 6, through which carbogen (95% O2/5% CO2) was bubbled. From 8 to 10 minutes after

Effects of FR190997 on Systemic Blood Pressure in Conscious, Unrestrained Rats
Mean arterial blood pressure (MAP) was determined in conscious, unrestrained rats (male SD, 6 weeks old), as in our previous report.3,4 Briefly, a polyethylene cannula (PE-10; Clay-Adams) was inserted into the abdominal aorta through the femoral artery with the animals under light ether anesthesia, connected to a PE-50 cannula (Clay-Adams), and exteriorized in the interscapular region. One day after cannula insertion, a blood pressure transducer (TP-200T; Nihon Koden) was attached to the free end of the intra-arterial cannula, and the MAP was monitored on a polygraph.

Eight days after withdrawal of the intra-arterial infusion (day 14 of the experiment), the rats were exsanguinated, and the mesenteric arteries were isolated. The contractions induced by norepinephrine were tested with the use of an isometric myograph. The ED50 values of the FR190997-treated rats for the contractions and those of the vehicle-treated rats were compared.

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Even when BK was used without indomethacin, transient contraction occurred, followed by dilatation (Figure 1C). Indomethacin treatment inhibited this contraction but did not

Determination of Urine Volume and Urinary Excretions of Sodium and Potassium During Selective Infusion of FR190997 Into Renal Artery of Anesthetized Rats
Male rabbits were anesthetized with sodium pentobarbital (30 mg/kg IV), and an arterial branch to the left adrenal gland was cannulated with a PE-10 cannula. The left renal artery was infused continuously and retrogradely with FR190997 (0.1 nmol/min). Urine was collected from the left kidney through a cannula inserted into the ureter and weighed, and its volume was determined with the use of a specific gravity of 1. Urinary sodium and potassium levels were
affect the dilatation by BK-induced dilatation (as with FR190997) (Figure 1D).

The reproducibility of the results was confirmed in 3 sets of arteries from different rats.

**Effect of Single Intra-Arterial FR190997 Injections on Systemic Blood Pressure**

To test the effect of FR190997 on systemic blood pressure, single intra-arterial injections were administered to conscious, unrestrained normotensive rats. BK induced short-lived dose-dependent hypotension (Figure 2). FR190997 induced more prolonged hypotension (over 20 minutes) than the same dose of BK (Figure 2). Thus, the area under the curve (AUC) of the hypotensive response to FR190997 significantly exceeded that to BK (Figure 2). The hypotensive potency of FR190997 was not apparently different from that of BK, because the 2 ED$_{50}$ values did not differ significantly. Preinjection of Hoe140 (100 nmol/kg IA), but not of atropine (100 nmol/kg IA), markedly inhibited FR190997-induced hypotension (Figure 2).

**Effects of Selective Infusion of FR190997 Into Renal Artery on Urine Volume and Excretions in Sodium and Potassium in Anesthetized Rabbits**

A selective retrograde intra-arterial infusion of FR190997 into the renal artery caused diuresis in anesthetized rabbits and significantly increased sodium excretion but not potassium excretion (Figure 3).

**Figure 1.** Effect of FR190997 on isolated small arteries constricted by phenylephrine in vitro. Small mesenteric arteries were isolated from SD rats, and dilatation by FR190997 or BK was tested with an isometric myograph. Arteries were preconstricted by addition of phenylephrine (Ph.). Indomethacin (IDM; 10$^{-5}$ mol/L) was added 20 minutes before application of FR190997 or BK. W indicates washing with normal Na$^+$-Krebs solution.

**Figure 2.** Hypotension induced by intra-arterial injections of FR190997. MAP was determined in conscious and unrestrained SD rats. Top, Actual traces of MAP before and after single intra-arterial injections of FR190997 or BK. Bottom, Maximum fall in MAP and AUC of hypotension. A B$_2$ antagonist, Hoe140 (100 nmol/kg; B2 Ant) or atropine (100 nmol/kg IA) was injected intra-arterially 10 minutes before injections of FR190997 or BK. Values are mean with SEM obtained from various numbers (n) of rats.

**Figure 3.** Effects of FR190997 infused into renal artery on urinary excretions of water (top), sodium, and potassium (bottom). Shaded area (top) indicates urine volume at steady state. FR190997 was infused (0.1 nmol/min) selectively and retrogradely into renal artery of anesthetized rabbits. Five-minute urine samples were collected. Values are mean with SEM obtained from 6 rabbits. ANOVA was used to evaluate significance of differences (*P<0.05). Comparison was made between basal values and values during infusion.
Effect of Continuous Infusion of FR190997 Into Abdominal Aorta on Development of Hypertension in Young SHR

We tested the effect of a continuous intra-arterial infusion of FR190997 into the abdominal aorta of 6-week-old SHR (MAP 142±2 mm Hg, n = 18). In rats infused proximal to the renal arteries with vehicle solution, MAP increased gradually (Figure 4) and continued to increase even after removal of the pump that delivered the vehicle solution. Although FR190997 infusion into the aorta distal to the renal arteries did not reduce MAP, FR190997 infusion into the aorta proximal to the renal arteries did. After removal of the FR190997 pumps, MAP increased gradually. However, recovery did not reach the level in vehicle-infused rats, and the difference in MAP between FR190997-treated and vehicle-treated rats was statistically significant when the experiment ended (day 14).

In vehicle-treated rats, urine volume and urinary sodium excretion remained fairly constant during and after pump implantation, whereas in FR190997-treated rats, sodium excretions and urine volume increased significantly (Figure 5). After the final blood pressure determination (day 14), the mesenteric artery was excised, and its sensitivity to norepinephrine-induced contraction was determined with an isometric myograph (Figure 6). Norepinephrine at 100 nmol/L induced contraction in the control artery but little contraction in the FR190997-treated rat artery. The difference in contraction with 300 nmol/L norepinephrine appeared significant. The reproducibility of the results was confirmed in 4 sets of arteries from different rats. The ED50 value for norepinephrine-induced contraction of mesenteric artery was 690±60 nmol/L (n = 4), which is significantly greater than that of the control artery (290±20 nmol/L, n = 4), suggesting that the FR190997-treated rat artery was less sensitive to norepinephrine even after treatment withdrawal.

Discussion

The SHR is the most widely studied animal model of genetic hypertension. ACE inhibitors such as captopril and cilazapril were reported early to prevent the development of hypertension in SHR treated from a young age,17,18 although hypertension developed as usual if the treatment was discontinued after 4 to 12 weeks. Later reports, however, suggested that the early initiation of captopril treatment in SHR produces profound and lasting effects on blood pressure after discontinuation. Freslon and Giudicelli19 prevented hypertension in SHR through captopril administration from 6 to 20 weeks of age, and a lower blood pressure persisted for weeks after drug withdrawal. Harrap et al20 reported that systolic blood pressure in SHR increased after brief ACE inhibition but then remained at 25 to 30 mm Hg below control values. The withdrawal of ACE inhibition in older SHR (20 weeks old) with established hypertension has been shown to produce no long-term reduction in blood pressure.20 A more recent
report\textsuperscript{21} that involved the use of radiotelemetric blood pressure monitoring in SHR confirmed the report of Harrap et al. Such reports suggest the existence in hypertension of a critical phase that may be amenable to pharmacological intervention.\textsuperscript{22}

We previously reported that in BN-Ka rats, with BN-Ki rats as controls, RKKS may contribute to the lowering of systemic blood pressure in the initial phase of deoxycorticosterone acetate-salt hypertension through the acceleration of sodium and water excretion.\textsuperscript{9} Furthermore, we found that BN-Ka rats were sensitive to salt and to a nonpressor dose of angiotensin II, exhibiting elevated blood pressure and sodium retention and reduced urinary sodium excretion.\textsuperscript{7,8} These results suggested that RKKS prevented hypertension mediated through activation of the renin-angiotensin-aldosterone system (RAAS) and that deficient function of RKKS led to hypertension when salt-related bias or activation of the RAAS was introduced. We previously reported that a newly weaned SHR, in the phase characterized by Unger and Retting\textsuperscript{22} as critical, secreted less kallikrein than age-matched WKY rats.\textsuperscript{16} Thus, not only blockade of the RAAS, but also facilitation of the RKKS, may be useful in the prevention of critical-phase hypertension.

In the kidney, kinin acts mainly on the BK receptors present on the collecting ducts\textsuperscript{9} and induces natriuresis. Kinin is readily degraded by kininases in the kidney, so the blockade of kinin degradation may facilitate kinin function. Our reports on the blockade of renal-specific kinin degradation by inhibitors support this possibility.\textsuperscript{10–12} The supplementation of kinin moieties to the renal tubules may also facilitate the RKKS. It was reported that the infusion in kininogen-deficient rats of low-molecular-weight kininogen, a preferential substrate for renal kallikrein, could increase kinin secretion from the kidney.\textsuperscript{7,8} The kinin secretion thus induced certainly inhibits salt- and angiotensin II–induced hypertension.\textsuperscript{7,8} Kinin analogs that are stable in vivo may help to prevent hypertension. The finding of FR190997, a nonpeptide mimic of BK that acts solely on B\textsubscript{2} receptors, prompted us to study its inhibition of hypertension.\textsuperscript{9,10} In the present study, we first tested the effect of FR190997 on the constricted small rat arteries in vitro. FR190997 induced the dilatation, as did BK (Figure 1). However, the hypotensive effect of FR190997 in vivo was considerably prolonged, and FR190997 markedly increased the AUC of hypotension (Figure 2). These may be results of the resistance of FR190997 to proteases that can degrade native BK. This resistance supports the in vivo use of this compound to test the inhibition of hypertension in SHR.

Furthermore, to test the diuretic and natriuretic effects of FR190997, we selectively infused it into the renal artery. BK infused into the renal artery at the same rate induced little diuresis or natriuresis (data not shown). However, FR190997 effectively induced the natriuresis and diuresis. The fact that FR190997 did not significantly increase urinary potassium excretion (Figure 3) was consistent with reports that kinin acts solely on the sodium channels in the renal tubules.\textsuperscript{1}

Last, we examined the inhibition by FR190997 of hypertension in young SHR. If the RAAS has a crucial role in the development of hypertension in young SHR, the facilitation of the RKKS in counteraction of the RAAS may also be important in the prevention of hypertension. The present results certainly provide evidence for the role of RKKS in the development of hypertension. The continuous infusion of a nondepressor dose of FR190997 that did not reduce the blood pressure when infused distal to the renal arteries induced hypotension on infusion proximal to the renal arteries. During this antihypertensive response, a significant increase in urinary sodium excretion occurred. The withdrawal of FR190997 resulted in a prolonged reduction in blood pressure; the level was not restored to that in vehicle-treated rats. This result resembled that of the short-term administration of an ACE inhibitor.\textsuperscript{20–22} The facilitation of the RKKS as a counterpart of RAAS produced profound and lasting effects on blood pressure even after the drug was discontinued.

Furthermore, we tested arterial sensitivity to norepinephrine to account for the lasting effect of FR190997. We previously reported that arterial sensitivity to norepinephrine and angiotensin II increased significantly after treatment inducing sodium retention in BN-Ka rats.\textsuperscript{23} This phenomenon may result from sodium retention, which finally increases intracellular calcium mobilization via the sodium-calcium exchanger present on the cell membrane of a wide variety of cells. It is plausible that the kinin mimic FR190997 can maintain low sodium levels in the smooth muscle cells through increased urinary sodium excretion. At least in part, this reduced sensitivity may help to explain the prolonged hypotension after FR190997 withdrawal.

A wide variety of antihypertensive agents can reduce established high blood pressure.\textsuperscript{24} Regarding the development of antihypertensive agents, it should be emphasized that a great many drugs exist that reduce high blood pressure manifested genetically or secondarily. Clinicians endeavor to select the optimum agent for the patient’s quality of life. However, little consideration has been given to the development of agents that inhibit or prevent hypertension. The present results suggest that a nonpeptide mimic of BK, such as FR190997, may be useful for this purpose.

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