Blunted Renal Responses to Angiotensin II Infusion in Lifetime Captopril-Treated Spontaneously Hypertensive Rats

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Abstract

Previously, we had found that inhibition of the renin-angiotensin system in the early lifespan of spontaneously hypertensive rat could prevent the development of hypertension in this animal model. In the present study we evaluated the responses of blood pressure and renal function to intracerebroventricular administration of angiotensin II in long-term captopril-treated spontaneously hypertensive rats. Spontaneously hypertensive rats had been mated and their pups were treated with captopril through drinking water after birth. Age-matched Wistar-Kyoto and spontaneously hypertensive rats drinking tap water were used as control groups. At 4 months of age, the basal mean arterial blood pressure of captopril-treated hypertensive rats was the lowest among those of controlled hypertensive and normotensive rats (98±5 vs. 160±4 and 126±4 mmHg, respectively). Intravenous administration of angiotensin II caused similar increments of blood pressure in all rat groups. However,intracerebroventricular administration of angiotensin II to captopril-treated hypertensive rats induced a significantly less increase of arterial blood pressure in comparison with other groups. The sensitivity of baroreflex in captopril-treated hypertensive rats was also the lowest among all rat groups. The basal urine flow, sodium and potassium excretion rates, and osmolar clearance of captopril-treated hypertensive rats were significantly higher than those of controlled hypertensive rats. Intracerebroventricular infusion of angiotensin II caused significant increases in urine flow, electrolytes excretion, osmolar clearance, and free water reabsorption rate of both normotensive and controlled hypertensive rats. However, the same angiotensin II treatment did not change any of the renal excretion indices in captopril-treated hypertensive rats. Our results suggest that lifetime captopril treatment can decrease the activity of the renin-angiotensin system in the brain of hypertensive animals, which caused increases in basal urine flow and excretion of electrolytes and enhanced the sensitivity of baroreflex. It is likely that changes in the renal and baroreflex functions underlie the prevention of hypertension elicited by long-term captopril treatment.

Key Words: angiotensin II, captopril, spontaneously hypertensive rats

Introduction

There is abundant evidence for the existence of a localized renin-angiotensin system (RAS) in the brain. Anatomical and functional studies have provided evidence that an enhanced activity of tissue RAS plays a significant role in the pathogenesis and maintenance of hypertension in spontaneously hypertensive rats (SHRs). In comparison to Wistar-Kyoto rats (WKYs), SHRs have been shown increases in sympathetic tone, vasopressin secretion, densities of neuronal angiotensin II receptors, and pressor

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responsiveness to vasopressors (5, 8, 26, 29, 31, 32). In our previous studies, we had found that administration of the angiotensin converting enzyme (ACE) inhibitor, captopril, to SHR breeders could permanently prevent the development of hypertension in their offspring, lifetime captopril-treated SHRs (38). Although the exact mechanism of the prolonged antihypertensive effect of ACE inhibitors remains unclear, there is evidence that the permanent antihypertensive effect of ACE inhibitors administered to fetal and neonatal animals may attribute to a reduction in the RAS activity, a remodeling of cardiovascular structures, and/or changes in sodium metabolism (13,15, 17, 34, 38).

The maintenance of sodium and water homeostasis by the kidney is widely believed to be the primary long-term determinant of systemic arterial blood pressure. An abnormal renal function is critical for the initiation, development, and maintenance of hypertension (7, 12). The inhibition of tissue RAS in the brain can also regulate the blood pressure and sodium and water balance through the influence to renal function (9, 14, 20). Therefore, studies on pathophysiological effects of central angiotensin II in blood pressure and renal function are important for better understanding the underlying mechanisms of antihypertensive effect of ACE inhibitors.

In the present study we thoroughly examined the influence of lifetime captopril treatment on the responses of drinking, pressor, and renal function induced by intracerebroventricular infusion of angiotensin II in WKYs and SHRs.

Materials and Methods

Subjects

Male WKYs and SHRs at 20 to 21 weeks of age were used in this study. The lifetime captopril-treated SHRs were produced as previous study (38). Briefly, three mating cages of the breeders from SHRs were given captopril (Research Biochemicals Inc., Natick, MA, USA) in their drinking water at a dose of 1.84 mmol/L. Their pups (CAPSHRs) continuously drank captopril water throughout the whole life. Age-matched WKYs and SHRs were used as control groups.

Animal Preparation

In surgical preparation, rats were first anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/Kg). The animal was placed on a stereotaxic apparatus and its skull was exposed for cerebroventricular cannulation. A sterile stainless steel cannula (24-gauge spinal needle with 30-gauge inner cannula) was implanted into the right lateral ventricle through a small hole drilled in the skull. The cannula was placed to the stereotaxic position of 1.0 mm posterior to bregma, 1.5 mm lateral from midline, and 4 mm inferior to brain surface. The cannula was anchored to the skull and 2 small screws with dental cement. The left femoral artery and vein were catheterized with cannulae tunneled under the skin. The free ends of the cannulae were externalized at the back of the neck. The cannula of femoral artery was used for measurement of mean arterial blood pressure (MABP) and heart rate (HR) via a Statham P23XL pressure transducer and RS 3800 polygraph (Gould Inc., Cleveland, OH, USA). Before further experiments, the rat received at least 24 hour's recovery period from surgery.

Angiotensin II Injection and Blood Pressure Measurement

First part of experiments was carried out in conscious, freely moving rats in their cages after a stabilization period of 30 minutes. Each rat received different dosages of intravenous and intracerebroventricular injections of angiotensin II (Sigma Chemical Co., St. Louis, MO, USA). The doses of intravenous injection of angiotensin II were 10, 30, and 100 ng in 100 μL normal saline. The dosages of drug administration were randomized. The doses of intracerebroventricular injection of angiotensin II were 100 ng in 10 μL artificial cerebrospinal fluid [133.3 mM NaCl, 3.4 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.6 mM Na_2HPO_4, 32 mM $NaHCO_3$, 3.4 mM D-Glucose, pH 7.4]. The arterial blood pressure, heart rate, and accumulative water intake in 20 and 40 minutes were measured. Drug treatments were separated by a 24-hour interval.

Angiotensin II Infusion and Renal Function Test

After the conscious experiments had been completed for three days, rats were further anesthetized with Somnotol (sodium pentobarbital 65mg with benzyl alcohol 2% as preservative in an aqueous propylene glycol base per mL, MTC Pharmaceuticals, Cambridge, Ontario, Canada) to perform renal function test. The procedures of renal function experiment had been previously described (39). Mainly, the animal received tracheotomy and cannulation of urinary bladder. The rat was placed under servo-controlled heated table to keep its core temperature at 37°C. A prime dose of 1 mL 7.5% Inutest (polyfructosan, Laeavosan-Gesellschaft, Linz, Austria) in normal saline was administered to the animal. An infusion of the same solution at a rate of 0.02 mL/min was followed throughout the experiment. The animal was allowed to rest for an hour to achieve

a steady state of urine flow. Throughout the experiment, each collection period of urine samples was 20 minutes. Urine samples of the control period were collected for 40 minutes. After the control period, the rat received intracerebroventricular infusion with angiotensin II in artificial cerebrospinal fluid in the concentration of 10 μM at infusion rate of 100 μL/hr for one hour. Blood samples were collected during control and experiment periods for measurements of sodium, potassium, osmolality, and inutest concentrations in plasma. The concentrations of inutest in plasma and urine samples were measured with a semimicroanthrone colorimetric technique as previous study (18). The electrolytes were measured with a flame photometer (Model 343, Instrumentation Lab., Lexington, MA, USA). The osmolality was measured with an osmometer (Model 3MO, Advanced Instrument, Inc., Needham Heights, MA, USA).

After the experiment, the animal was sacrificed with overdosed sodium pentobarbital and the wet weights of both kidneys were measured. Placement of the lateral cerebroventricular cannula was checked by injection of 10 μL gentian violet into the cannula. Correct placement was verified by the presence of dye in the cerebroventricular system.

Statistical Analysis

The glomerular filtration rate (GFR), absolute sodium excretion rate ($U_{Na}V$), absolute potassium excretion rate (U_KV), fractional sodium excretion rate (FE_{Na}), fractional potassium excretion rate (FE_K), osmolar clearance (Cosm), and free water reabsorption rate ($T^C_{H_2O}$) were computed according to the standard clearance formula. Data are expressed as mean±SEM. Analysis of variance and Student-Newman-Keuls' post-test were used to evaluate whether there were differences in blood pressures, heart rates, and renal function among three animal groups. Paired Student's *t* test was used to evaluate renal function before and after angiotensin II infusion. Statistical significance is assumed when $p<0.05$.

Results

Effects of Captopril Treatment on Blood Pressure

The averaged body weights of WKYs, SHRs, and CAPSHRs were 326±6, 308±4, and 289±7 g, respectively. CAPSHRs showed the lowest mean body weight among all rat groups. The baseline MABP of conscious WKYs, SHRs, and CAPSHRs were 126±4, 160±4, and 98±5 mmHg, respectively (Figure 1). SHRs possessed the highest MABP and CAPSHRs had the lowest MABP among all rat groups. The baseline HR of conscious WKYs, SHRs, and CAPSHRs were 345±18, 327±11, and 311±23 beats per minute, respectively. There were no significant differences in HR among all rat groups.

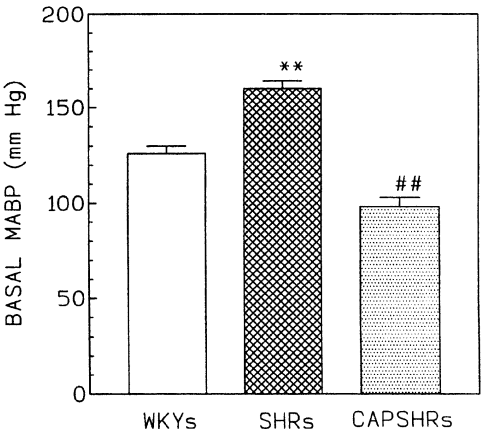

Fig. 1. The basal mean arterial blood pressure (MABP) in conscious Wistar-Kyoto rats (WKYs, n=11), spontaneously hypertensive rats (SHRs, n=12), and captopril-treated SHRs (CAPSHRs, n=12). The data represented means±SEM. **, $p< 0.01$ compared with WKY group. ##, $p< 0.01$ compared with SHR group.

Effects of Peripheral Angiotensin II Injection on Blood Pressure

Intravenous injection of angiotensin II significantly increased MABP in all rat groups. The changes of MABP with 10, 30, and 100 ng angiotensin II injection were 22±4, 36±5, and 43±3 mmHg in WKYs, 15±3, 28±4, and 39±2 mmHg in SHRs, and 19±3, 29±4, and 39±2 mmHg in CAPSHRs, respectively. There were no significant differences in changes of MABP among all rat groups at all three doses. However, SHRs showed significantly fewer changes in HR at all three doses of angiotensin II administrations in comparison to WKYs and CAPSHRs. No obvious drinking behavior after these intravenous injections of angiotensin II was observed in all rat groups. Figure 2 depicts the relationships between changes in MABP and changes in HR during intravenous administrations of angiotensin II. We found that the slope of SHRs (-0.80±0.17) was significantly higher than that of WKYs (-1.85±0.36). The slope of CAPSHRs (-2.09±0.32) was significantly lower than that of SHRs but similar to that of WKYs.

Effects of Central Angiotensin II Injection on Blood Pressure

The increases of the MABP in WKYs, SHRs, and CAPSHRs induced by intracerebroventricular injections of angiotensin II were 40±3, 40±5, and 23±3 mmHg, respectively. There were no significant

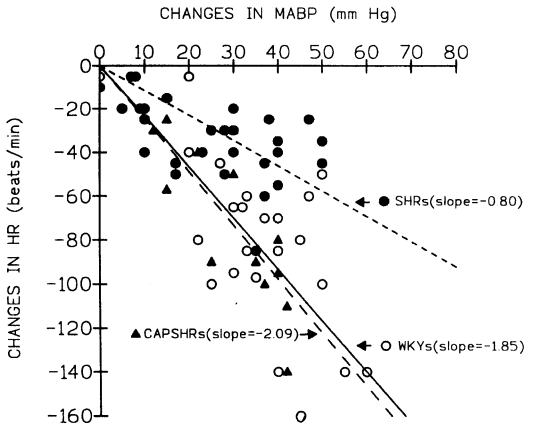

Fig. 2. The relationships between changes in mean arterial blood pressure (MABP) and changes in heart rate (HR) of conscious Wistar-Kyoto rats (WKYs, n=11), spontaneously hypertensive rats (SHRs, n=12), and captopril-treated SHRs (CAPSHRs, n=12). The changes in blood pressure and heart rate were induced by intravenous injections of angiotensin II (10, 30, and 100 ng).

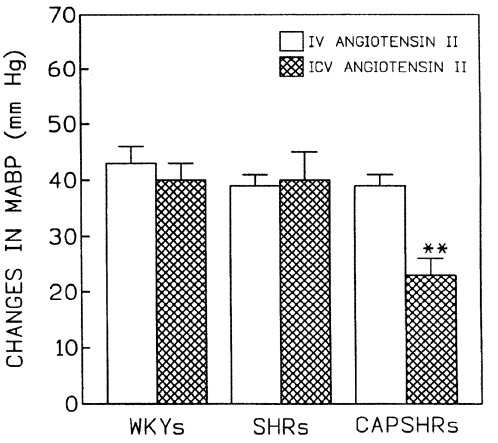

Fig. 3. The changes in mean arterial blood pressure (MABP) to intravenous (IV) or intracerebroventricular (ICV) administration of angiotensin II (100 ng) in conscious Wistar-Kyoto rats (WKYs, n=11), spontaneously hypertensive rats (SHRs, n=12), and captopril-treated SHRs (CAPSHRs, n=12). The data represented means(SEM. **, $p < 0.01$ compared with the changes in MABP treated by IV angiotensin II injection.

differences in changes of HR by the intracerebroventricular injection of angiotensin II among all rat groups. The water intake of WKYs, SHRs, and CAPSHRs induced by intracerebroventricular injections of angiotensin II were 14±1, 16±1, and 9±1 mL, respectively. CAPSHRs showed significant less water intake than WKYs and SHRs. Figure 3 illustrates the changes in MABP of WKYs, SHRs, and CAPSHRs with intravenous and intracerebroventricular injections of 100 ng angiotensin II.

Effects of Central Angiotensin II Infusion on Renal Function

Table 1 shows MABP, HR, and renal function indices of WKYs, SHRs, and CAPSHRs before and after intracerebroventricular infusion of angiotensin II. The basal GFR of WKYs, SHRs, and CAPSHRs under anesthetization were 1.96±0.20, 1.77±0.22, and 2.13±0.17 mL/min, respectively. SHRs showed lower basal urine flow, absolute excretion of potassium, free water reabsorption rate, and osmolar clearance than those of WKYs. CAPSHRs possessed significantly higher basal urine flow, and absolute excretions of potassium and sodium rates, and osmolar clearance than those of SHRs. Intracerebroventricular infusion of angiotensin II did not significantly increase MABP in all rat groups. However, the infusion of angiotensin II significantly increased urine flow, absolute and fractional excretions of sodium and potassium, and free water reabsorption rate in WKYs. SHRs possessed similar responses to the same treatment. However, in CAPSHRs, angiotensin II did not significantly change any renal function indices measured.

The averaged wet weights of left and right kidneys in WKYs were 1.39±0.20 and 1.42±0.20 g; in SHRs were 1.39±0.19 and 1.40±0.18 g; in CAPSHRs were 1.29±0.20 and 1.30±0.19 g, respectively. There were no significant differences in kidney weights of either side among all rat groups.

Discussion

Spontaneously hypertensive rats (SHRs) demonstrate numerous abnormalities in the regulation of blood pressure and are widely used to mimic the essential hypertension in human (1, 2, 11). In our results, SHRs did show high blood pressure and the captopril treatment significantly prevented the development of hypertension (Figure 1). Intravenous injection of angiotensin II increased MABP to similar amplitude in all rat groups. Captopril treatment did not change the pressor response to intravenous administration of angiotensin II in SHRs. Intracerebroventricular injections of angiotensin II significantly increased MABP in both WKYs and SHRs, but lifetime captopril-treated SHRs showed diminished pressor and drinking responses to the same manipulation. These results are in agreement with our previous study (38). It suggested that long-term captopril treatment did not considerably affect the peripheral vascular system but mainly decreased the responsibility of the RAS in the brain. We highly suspected that the central nervous system was the main target by long-term treatment with the pharmacological antihypertensive drug captopril.

Table 1. The Responses of the Mean Arterial Blood Pressure, Heart Rate, and Renal Function to Intracerebroventricular Administrations of Angiotensin II in Wistar-Kyoto Rats (WKYs), Spontaneously Hypertensive Rats (SHRs), and Captopril-Treated SHRs (CAPSHRs)

		WKYs	SHRs	CAPSHRs
Parameter		(n=11)	(n=12)	(n=12)
MABP (mm Hg)	basal	128±3	157±5	$93\pm3^{aa,bb}$
	A II	137±4	166±5	$94\pm4^{aa,bb}$
HR (beat/min)	basal	293±17	304±9	334±8
	A II	319±16	320±9	349±9
GFR (mL/min)	basal	1.96±0.20	1.77±0.22	2.13±0.17
	A II	1.99±0.22	1.98±0.27	2.17±0.18
V (μL/min)	basal	6.42±0.40	5.36 ± 0.48^{a}	7.53 ± 0.57^{b}
	A II	$8.59\pm0.61^{\#}$	$8.06\pm1.26^{\#\#}$	7.43±0.56
$U_{Na}V$ (μEq/min)	basal	0.20±0.03	0.17±0.02	$0.37\pm0.08^{a,bb}$
	A II	$0.38\pm0.08^{\#\#}$	$0.35\pm0.15^{\#}$	0.42±0.15
FE_{Na} (%)	basal	0.10±0.03	0.11±0.04	0.14±0.04
	A II	$0.18\pm0.02^{\#\#}$	$0.27\pm0.16^{aa,\#\#}$	0.17±0.05
U_KV (μEq/min)	basal	1.11±0.09	0.73 ± 0.12^{a}	1.44 ± 0.15^{bb}
	A II	$1.79\pm0.16^{\#\#}$	$1.22\pm0.14^{aa,\#\#}$	1.56±0.21
FE_K (%)	basal	15.2±2.4	17.2±3.9	14.0±2.1
	A II	$27.3\pm4.8^{\#\#}$	$32.5\pm9.2^{\#\#}$	$17.0\pm3.2^{a,bb}$
Cosm (μL/min)	basal	37.7±1.9	21.7 ± 3.2^{aa}	34.5 ± 2.8^{bb}
	A II	$56.3\pm3.8^{\#\#}$	$47.2\pm4.8^{\#\#}$	$39.1\pm4.4^{a,bb}$
$T^C_{H_2O}$ (μL/min)	basal	31.3±1.8	24.2 ± 3.2^{a}	26.9 ± 2.7^{a}
	A II	$47.8\pm3.1^{\#\#}$	$39.2\pm3.5^{a,\#\#}$	31.9 ± 3.8^{a}

MABP, mean arterial blood pressure; HR, heart rate; GFR, glomerular filtration rate; V, urine flow; $U_{Na}V$, absolute sodium excretion rate; U_KV, absolute potassium excretion rate; FE_{Na} , fractional excretion of sodium; FE_K, fractional excretion of potassium; Cosm, osmolar clearance; $T^C_{H_2O}$, free water reabsorption rate. Values are mean±SEM; n is the number of animals. Basal and AII values represent the averages of periods before and after angiotensin II infusion. $^{a}P<0.05$, $^{aa}P<0.01$ compared with WKYs and $^{b}P<0.05$, $^{bb}P<0.01$ compared with SHRs by ANOVA with Newman-Keuls posttest procedure. $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with basal level by paired Student's t test.

However, the precise brain areas affected by this treatment need further study.

The averaged slope of changes in MABP to changes in HR of WKYs was stiffer than that of SHRs (Figure 2). It demonstrated that the response of baroreflex to increased blood pressure in SHRs were less sensitive than that in WKYs. Interestingly, long-term captopril treatment significantly enhanced the sensitivity of baroreflex in SHRs. It had been shown that angiotensin II has direct effects on neurons involved in sympathetic outflow and baroreflex function and indirect action on vasopressinergic and catecholaminergic systems in the brain (25, 36). Angiotensin II also modulates baroreceptor reflex control of heart rate via brain mechanisms (10, 16, 22, 27). Therefore, the enhancement of baroreflex sensitivity may contribute to the antihypertensive effects of captopril and this increment may be due to the inhibition of the local RAS in the brain.

In Table 1, there were no significant differences in the basal renal function between WKYs and SHRs except higher excretion rate of potassium and lower osmolar clearance in SHRs. Long-term captopril treatment enhanced the basal urine flow, sodium and potassium excretion rates, and osmolar clearance in SHRs. We had found previously that long-term captopril treatment decreased the density of angiotensin II receptor in kidneys of the young but not the adult SHRs (40). In addition, one previous study had indicated that the excretory function of captopril-treated SHRs in response to peripheral saline loading was similar to that of untreated SHRs. In the same study, they also found that histological examinations revealed similar renal structures of both rat groups (28). Therefore, a macro-change in renal tubular function *per se* induced by long-term captopril treatment was not likely. However, our results indicated that captopril treatment did change the basal renal excretory function. It was possible that the long-term inhibition of ACE enhanced the

physiological function in the kidney of SHRs through the influence of the RAS and/or the kallikrein-kinin system (21) and the resulting elevation of fluid and electrolyte excretions may contribute to the antihypertensive effects of captopril.

It is well known that the intracerebroventricular administration of angiotensin II induces elevation of blood pressure and natriuresis (6, 19). Using a dosage that would not significantly affect the systemic arterial blood pressure, we observed that intracerebroventricular infusion of angiotensin II, which enhanced the central renin-angiotensin system activity, induced diuresis and natriuresis in both WKYs and SHRs. Our findings agree with other studies, which suggested that diuresis might be due to the redistribution of renal plasma flow (24) and the natriuresis may be due to the inhibitions of aldosterone secretion and renal nerve activity (25, 37). Additionally, our results also showed that the free water reabsorption rate was increased by the intracerebroventricular infusion of angiotensin II. This increment is referred to the stimulation of the secretion of vasopressin (3, 33).

In long-term captopril-treated SHR, intracerebroventricular infusion of angiotensin II did not change renal functional indices. These results support our previous findings that in utero administration of ACE inhibitor would permanently inhibit the activity of RAS in the brain (38). Since angiotensin II generates its physiological function mainly through one of its specific receptors (AT1), which has been cloned and characterized (4, 23, 30, 35), our results suggest that a decrement in the density or affinity of AT1 receptors in the brain is induced by long-term captopril treatment in SHR.

In conclusion, our results imply that lifetime captopril treatment could inhibit the function of endogenous angiotensin II in CNS, which caused an enhancement of baroreflex function and an elevation of basal renal excretory indices in SHR. These functional enhancements may contribute to the prevention of the development of hypertension in SHR. In addition, we believe that an enhanced activity in central RAS, at least in part, plays important roles in the etiology of spontaneous hypertension.

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