Prevention of Genetic Hypertension by Early Treatment of Spontaneously Hypertensive Rats With the Angiotensin Converting Enzyme Inhibitor Captopril

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Our purpose was to evaluate whether early treatment of spontaneously hypertensive rats (SHR) with the angiotensin converting enzyme inhibitor captopril could permanently alter the course of hypertension. Mating pairs of SHR were treated with captopril, and their pups were maintained on captopril until experimentation. Some captopril-treated rats were taken off treatment at 2 months of age, and then some of these rats were mated at 3 months of age. The mean arterial pressures of conscious captopril-treated rats, the rats removed from therapy, and the offspring of the rats removed from therapy were significantly smaller than control rats at 4 and 9 months of age. Central administration of angiotensin I or II induced significantly smaller increases in blood pressure and drinking in captopril-treated rats and the rats removed from therapy compared with control rats. The increase in blood pressure in response to intravenous injection of angiotensin I or II was similar among all groups, with the exception that captopril-treated rats showed lesser pressor responses to angiotensin I. Early administration of captopril, even after administration was stopped, prevented the subsequent development of hypertension in SHR and altered the course of development of hypertension in their progeny. This effect was associated with decreased central responses to angiotensin I and II. Our data suggest that captopril may permanently alter the development of hypertension in SHR through an alteration in the central renin-angiotensin system. (Hypertension 1993;22:139-146)

KEY WORDS • kininase II • angiotensin converting enzyme inhibitors • captopril • rats, inbred SHR

The brain renin-angiotensin system (RAS) has been recognized as one of various tissue RASs.1,2 All the components of the RAS have been identified in the brain.2-10 Anatomic and functional studies have provided evidence that enhanced RAS activity in brain may play a role in the pathogenesis of hypertension in spontaneously hypertensive rats (SHR). The strongest evidence for a potential role for the brain RAS in hypertension comes from studies showing that both short-term and long-term intracerebroventricular administration of either angiotensin (Ang) II receptor antagonists or angiotensin converting enzyme (ACE) inhibitors lower blood pressure in SHR at doses that are not effective peripherally.11-15 Furthermore, early treatment of young SHR with ACE inhibitors has been found to prevent the full expression of hypertension later in life even after the treatment was stopped.16,17 A possible critical phase sensitive to pharmacological interference has been proposed to exist during the development of hypertension.18 Interference with blood pressure regulation during this critical phase has been proposed to alter the long-term course of the disease. The prolonged effect of pharmacological intervention on the development and course of hypertension appears, however, to depend on the particular class of antihypertensive therapy used. ACE inhibitors have been found to produce a prolonged antihypertensive effect even after therapy is stopped. In contrast, no similar effect could be found with administration of β-blockers, direct vasodilators, or calcium antagonists.16,19,20 The exact mechanism or mechanisms resulting in the long-term antihypertensive effects after cessation of ACE inhibitor treatment remain unclear.

The purpose of the present study was to define the critical phase that may exist in the development of hypertension in SHR and determine the roles of central and peripheral RASs in the long-term antihypertensive effects of ACE inhibitors. The experimental SHR were divided into four groups: SHRCAP were given the ACE inhibitor captopril in their drinking water from in utero to 4 to 6 months of age; OFFCAP were treated like SHRCAP but taken off captopril at 2 months of age and then maintained on tap water; the first offspring of OFFCAP (2ndG) were given only tap water. Control rats (CON) were age-matched SHR given tap water until experimentation. Intracerebroventricular and in-
travenous injections of Ang I and Ang II were used to evaluate the status of central and peripheral ACE activity and Ang II receptors. Basal mean arterial blood pressure (MAP), change in blood pressure, and drinking response to exogenous Ang I and Ang II were measured. Early treatment with captopril prevented the subsequent development of hypertension. This antihypertensive response was associated with an alteration in the responsiveness of the rats to peripheral and central administration of Ang peptides. Alterations in the responsiveness of the brain RAS in SHR persisted even after captopril therapy was discontinued; hence, a long-term decrease in brain RAS activity may contribute to the prolonged antihypertensive effect of ACE inhibitors. Additionally, the ACE inhibitors may interfere with the remodeling of the cardiovascular system in SHR.

Methods

Animals

Four-month-old male SHR were used for this study. All experimental rats were offspring of breeders purchased from Harlan Sprague Dawley Inc, Indianapolis, Ind. At 3 months of age, 10 mating cages of the breeders were given captopril (Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ) in their drinking water at a dose of 1.84 mmol/L (100 mg/kg per day). They were maintained on this dosage throughout pregnancy and lactation. The pups were weaned at 4 weeks of age, and the captopril treatment was continued until 4 months of age (SHRCAP, n=19). Some SHRCAP were taken off captopril therapy at 2 months of age and maintained on tap water (OFFCAP, n=21). At 3 months of age, 6 mating cages of OFFCAP were assigned, and their offspring (second generation, 2ndG, n=18) were used at 4 months of age. Control breeders were mated at 3 months of age, and their pups were given tap water until experimentation (CON, n=16). Every breeding cage had one male and three female rats. Four additional groups of male rats were used for basal MAP measurement at 9 months of age. In addition to male rats tested, three female rats were included in the SHRCAP group. The rats were housed at constant temperature (24°C) and humidity (60±5%) and a 12-hour light/dark cycle. Standard laboratory rat chow was provided ad libitum. The majority of litters of our rats were not significantly different.

Surgical Preparation

Before blood pressure studies, all rats were anesthetized with a mixture of chloral hydrate (150 mg/kg) and pentobarbital sodium (35 mg/kg) (Equithesin, 3 mL/kg IP, Jensen-Salisbury Laboratories, Kansas City, Mo). Chronic catheters made of PE-50 and silicone elastomer (Silastic) tubing were placed into the abdominal aorta and inferior vena cava via the femoral artery and vein for measurement of blood pressure and administration of drugs, respectively. Each rat also received a stainless-steel cannula (25 gauge) fitted with an obturator (31 gauge) that was stereotaxically implanted into the lateral ventricle (coordinates: −1.0 mm posterior to bregma, ±1.5 mm lateral to midline, −4.5 mm ventral from dura). The intracerebroventricular cannulas were fixed to the skull surface with cranioplastic cement and jewelers’ screws. After catheterization, rats were given 100 000 U penicillin G intramuscularly and allowed to recover for at least 24 hours.

Experimental Protocols

Experiments were carried out in conscious, freely moving rats in their cages after a stabilization period of 30 to 60 minutes. No food was available during the experiment. MAP was monitored with a P23DB transducer (Century Technological Co, Inglewood, Calif) and continuously recorded on a 7758B system polygraph (Hewlett-Packard Co, Palo Alto, Calif). Heart rate (HR) was monitored with a cardiotachometer (University of Alabama at Birmingham, CVRTC Electronic Shop) triggered by the arterial pressure signal and recorded on the same polygraph. Basal MAP and HR were recorded for 4 consecutive days.

All rat groups received intracerebroventricular and intravenous Ang I and Ang II. The order of drug administration was randomized, and each treatment was separated by a 24-hour interval. Intracerebroventricular injections of Ang I or Ang II were at a dose of 500 ng (Sigma Chemical Co, St Louis, Mo) in a volume of 5 μL. An injector cannula was attached to a remote 10-μL Hamilton syringe by PE-10 tubing and filled with Ang I or Ang II dissolved in sterile isotonic saline. Water intakes were measured at 15, 30, and 60 minutes and 24 hours after the injection of the peptides. Intravenous injections of Ang I or Ang II were at doses of 500 ng in a volume of 0.5 mL. Intracerebroventricular injection of saline at 5 μL or intravenous injection of saline at 0.5 mL did not produce a significant effect on HR or MAP.

Placement of the lateral cerebroventricular cannula was checked by injection of 5 μL of 1% fast green dye into the cannula. Correct placement was verified by the presence of dye in the cerebroventricular system.

Data are expressed as mean±SEM. Analysis of variance with Bonferroni’s posttest procedure was used to evaluate whether there were differences in basal MAP, HR, body weight, and pressor and drinking responses to intracerebroventricular and intravenous Ang I and Ang II among the rat groups. Statistical significance was assumed at a value of P<.05.

Results

Baseline Blood Pressure Data

Fig 1 depicts the basal MAP of all rat groups. The basal MAP values of CON, SHRCAP, OFFCAP, and 2ndG rats were 166±3, 106±2, 126±3, and 137±2 mm Hg, respectively. The basal MAP of CON rats was significantly higher than that of all other groups. SHRCAP showed a significantly lower MAP compared with OFFCAP and 2ndG rats. The basal HR values of CON, SHRCAP, OFFCAP, and 2ndG rats were 351±8, 348±8, 349±8, and 318±8 beats per minute, respectively. The body weights of SHR, SHRCAP, OFFCAP, and 2ndG rats were 309±13, 318±10, 342±10, and 329±9 g, respectively. There were no significant differences in either basal HR or body weight among all rat groups.
Central Administration of Angiotensin I and Angiotensin II

Fig 2 shows the changes in MAP in response to intracerebroventricular administration of Ang I. The CON rats showed a significantly larger increase in MAP (50±3 mm Hg) in response to Ang I administration compared with SHRCAP (25±3 mm Hg) and OFFCAP (38±2 mm Hg) rats. There was no significant difference in central Ang I–induced pressor responses between CON and 2ndG rats (43±3 mm Hg). In contrast to pressor responses, no significant differences in the HR responses to intracerebroventricular injection of Ang I were seen among the rat groups.

Peripheral Administration of Angiotensin I and Angiotensin II

We also tested the pressor responses to peripheral (intravenous) administration of Ang I and Ang II in our rat groups. Fig 6 illustrates the changes in MAP in response to intravenous administration of Ang I. The changes in blood pressure in response to intravenous injection of Ang I in CON, SHRCAP, OFFCAP, and 2ndG rats were 43±3, 29±3, 47±3, and 59±4 mm Hg, respectively.
FIG 4. Bar graph shows accumulative drinking responses to intracerebroventricular administration of angiotensin I, 500 ng in 5 μL normal saline, in four rat groups. Data are mean±SEM analyzed by analysis of variance with Bonferroni's posttest procedure. CON, control rats; SHRCAP, spontaneously hypertensive rats that received captopril; OFFCAP, SHRCAP taken off captopril at 2 months; 2NDG, first offspring of OFFCAP rats. **P<.01, *P<.05 compared with CON rats.

respectively. SHRCAP showed significantly lesser increases in blood pressure compared with all other groups. The 2ndG rats demonstrated a greater increase in blood pressure in response to intravenous Ang I than CON rats. There were no significant differences in HR responses to intravenous administration of Ang I among all rat groups. The changes in blood pressure in response to intravenous administration of Ang II in CON, SHRCAP, OFFCAP, and 2ndG rats were 51±4, 38±4, 53±4, and 59±3 mm Hg, respectively (Fig 7). The changes in blood pressure in response to intravenous injection of Ang II were statistically significant only between SHRCAP and 2ndG rats. No significant differences were found among any of the rat groups in the HR responses to intravenous injection of Ang II.

Fig 8 shows the basal MAP of the four rat groups at 9 months of age. The basal MAP values of CON, SHRCAP, OFFCAP, and 2ndG rats were 166±4, 97±6, 129±3, and 137±4 mm Hg, respectively. The average basal MAP values of the three experimental groups are significantly lower than the control group. Although three female rats were included in the SHRCAP group, we did not find differences in the basal MAP between male and female SHRCAP.

Discussion

Similar to our previous studies, lifetime oral administration of captopril prevented the development of hypertension in SHR. This antihypertensive effect of captopril persisted even after the treatment with captopril had been stopped. Months after cessation of therapy, the basal MAP of OFFCAP rats remained within a normotensive range. Additionally and surprisingly, our data also showed that 2ndG rats exhibited lower blood pressures compared with control untreated SHR. Seven
months after cessation of captopril therapy, OFFCAP rats continued to show normotensive blood pressures. Their offspring (2ndG), even at 9 months of age, had MAP values significantly lower than control SHR. These results agree well with previous studies by Giudicelli et al. and Harrap et al. which showed that prolonged administration of ACE inhibitors for a certain time period in young SHR was sufficient to prevent the full expression of genetic hypertension in this animal model. However, the differences were no greater than 25 to 30 mm Hg. In contrast to their studies, we applied ACE inhibitor treatment earlier, from the fetal stage of life, and maintained the therapy until the rats were approximately 2 months old. We found that this course of treatment prevented the development of hypertension in SHR even after the ACE inhibitor therapy had been stopped. According to our results, early application of ACE inhibitors chronically interferes with the pathogenesis of hypertension in SHR. Furthermore, our results suggest that early interference with the RAS cascade may produce permanent changes in the development and maintenance of hypertension in this animal model.

The mechanism or mechanisms underlying the chronic antihypertensive effect of early treatment of SHR with captopril that exists well beyond cessation of therapy are not known with certainty. The remodeling of the heart and the blood vessel structure may be responsible for the prolonged antihypertensive action of captopril in SHR. Ang II induces both hypertrophy and permeability to placental membrane, may contribute to the ability of circulating factors in the pregnant SHR, which are responsible for the hypertension in this animal model. It is possible that the high MAP per se in pregnant female SHR may contribute to the full expression of hypertension in their offspring or that the production of some circulating factors in the pregnant SHR, which are permeable to placental membrane, may contribute to the full development of hypertension in their progeny. In other words, the experience of high blood pressure per se and/or some specific factors in the fetal life of SHR may play a permissive role in the development of hypertension. Therefore, the lowered MAP of these female SHR under captopril therapy or the ability of captopril to affect these circulating factors may be responsible for the lack of hypertension in their 2ndG offspring. However, Ely and Turner have demonstrated that received captopril; OFFCAP, spontaneously hypertensive rats that received captopril; OFFCAP, SHRCAP taken off captopril at 2 months; 2ndG, first offspring of OFFCAP rats.

Fig 8. Bar graph shows basal mean arterial pressure (MAP) of four rat groups at 9 months of age. Basal MAP was monitored for 3 consecutive days in conscious, freely moving rats. Daily MAP values were averaged for each rat. Data are mean±SEM analyzed by analysis of variance with Bonferroni's posttest procedure. Number of animals is in parentheses. CON, control rats; SHRCAP, spontaneously hypertensive rats; OFFCAP, spontaneously hypertensive rats that received captopril; OFFCAP, SHRCAP taken off captopril at 2 months; 2ndG, first offspring of OFFCAP rats. The lower basal MAP of 2ndG rats was very surprising, given that 2ndG rats had never been directly treated with an ACE inhibitor. Their low blood pressures suggest that early long-term administration of an ACE inhibitor may permanently change some events that are responsible for the hypertension in this animal model. It is possible that the high MAP per se in pregnant female SHR may contribute to the full expression of hypertension in their offspring or that the production of some circulating factors in the pregnant SHR, which are permeable to placental membrane, may contribute to the full development of hypertension in their progeny. In other words, the experience of high blood pressure per se and/or some specific factors in the fetal life of SHR may play a permissive role in the development of hypertension. Therefore, the lowered MAP of these female SHR under captopril therapy or the ability of captopril to affect these circulating factors may be responsible for the lack of hypertension in their 2ndG offspring. However, Ely and Turner have demonstrated that...
strated that the male offspring (first generation, F₁) derived from Wistar-Kyoto mothers and SHR fathers developed hypertension. In addition, their blood pressures were significantly higher than male offspring derived from SHR mothers and Wistar-Kyoto fathers. These findings suggest that although maternal high blood pressure per se and/or some specific factors in pregnancy contribute to the development of hypertension in SHR, these factors may not be the most critical ones involved in the development of hypertension in SHR.

We found that SHR once treated with captopril during a certain period of their life showed decreased responses to central application of Ang I and Ang II. In SHRCAP, the decreased response to central Ang I injection suggests that these rats convert lower amounts of exogenous Ang I to the physiologically active form, Ang II, in the brain. These results support previous studies showing that central ACE activity is inhibited by oral administration of ACE inhibitors. In addition to an alteration in conversion of Ang I to Ang II in the brains of SHRCAP or OFFCAP groups, we also found decreased response to central Ang II injection, suggesting that there may also be an alteration or reduction of Ang II receptors in the brain. The decreased response to central administration of Ang II agrees with our previous study that long-term administration of captopril leads to a decrease in Ang II receptors in the brain. The reduced blood pressure and pressor and drinking responses to intracerebroventricular administration of Ang I and Ang II in SHRCAP support the hypothesis that an overactive central RAS is involved in the pathogenesis of hypertension in SHR.

In OFFCAP rats, the decreased responses to intracerebroventricular administration of Ang I and Ang II suggest that the inhibition of central ACE, if it occurs in a critical phase, may lead to a permanent reduction in central ACE content or activity. Consequently, the reduction in ACE activity could decrease basal Ang II production in the central nervous system of these rats and lead to a long-term prevention of hypertension through diminished activity of central angiotensinergic pathways. The persistent antihypertensive effect of early treatment of SHR with captopril may be due to a long-term alteration in the expression or function of Ang II receptors. Felix and Schelling reported an increase in septal neuronal firing rate in response to microiontophoresis of Ang II in SHR compared with Wistar-Kyoto rats. The increase in septal Ang II receptor sensitivity in SHR was abolished when the rats were placed on oral captopril during the weaning period. These data, like ours, suggest that captopril can cross the blood-brain barrier and decrease the responsiveness to Ang II by altering its central receptor number, affinity, and/or secondary messenger cascades.

Ang II receptors in brain may not be downregulated by increased ligand concentrations, the typical response with Ang II receptors in the periphery. Moreover, recent evidence suggests that regulation of the Ang II receptor in the SHR may be abnormal in a number of tissues, including the brain. We have previously given evidence that captopril treatment causes a downregulation in the Ang II receptors in brain tissue and primary neuronal cell cultures from SHR but not in normotensive rat strains such as Wistar-Kyoto and Sprague-Dawley. These findings complement studies of Sumners and Raizada, who reported that captopril blocked the increase in Ang II binding induced by α-methylparatyrosine in neuronal cultures of SHR, and the finding of Nazarali et al. that oral administration of enalapril to SHR produced a significant decrease in Ang II binding sites in the subfornical organ of the brain. In the current study, the decreased response to intracerebroventricular Ang II induced by ACE inhibition strongly indicates that a direct effect of captopril on Ang II receptors may lead to a permanent downregulation of central Ang II receptors in SHR. This would explain why OFFCAP rats showed normotensive blood pressures and attenuated responses to intracerebroventricular administrations of Ang I and Ang II compared with CON rats. The 2ndG rats showed smaller blood pressure responses to intracerebroventricular injections of Ang I and Ang II compared with CON rats, but these responses were not significantly different from CON rats. These rats did show diminished drinking responses to Ang I and Ang II, suggesting an alteration in Ang II binding in the brains of these rats.

In contrast to central responsiveness to Ang peptides in our rat groups, we did not find any significant differences in blood pressure responses to peripheral administration of Ang I and Ang II among the rat groups with the exception that SHRCAP showed a significantly lesser increase in blood pressure in response to intravenous injection of Ang I. It is well documented that a decrease in plasma Ang II levels produces an increase in vascular Ang II receptors, whereas an elevation of Ang II levels is accompanied by a decrease in receptor number. Long-term treatment with captopril could cause an increase in vascular Ang II receptors of SHR. However, there were no increases in responsiveness to intravenous injection of Ang II in SHRCAP or OFFCAP rats. Captopril enhanced the baroreceptor reflex of SHR in our previous study, so it is possible that the increment of vascular Ang II receptors induced by captopril may be overcome by its enhancing effects on the central baroreceptor reflex system. Hence, we found no net increase in the pressor response to intravenous injection of Ang II. Our data indicate that the peripheral and central Ang II receptors of SHR may respond differently to long-term treatment of an ACE inhibitor and that alterations in the vascular Ang II receptor may not play an important role in the maintenance of normotensive blood pressures in OFFCAP and 2ndG rats.

From our experimental data, we believe that the inhibition of the central RAS is responsible in part for the antihypertensive effect of long-term oral administration of ACE inhibitors. Different ACE inhibitors, however, are distinct in affecting the central and peripheral RASs in terms of their magnitude and duration of action. All of the ACE inhibitors, even the most hydrophilic agents, however, produce a decrease in ACE activity in the circumventricular organs such as the subfornical organ and organum vasculosum of the lamina terminalis. These are sites where Ang II evokes central actions of vasopressin release and drinking and pressor responses. In addition, Ang II—containing pathways from the circumventricular organs to the paracerebroventricular nucleus and other areas are involved in cardio-
vascular regulation. These data suggest that all ACE inhibitors have effects in the central nervous system.

In conclusion, administration of an ACE inhibitor, captopril, in a critical phase can prevent the development of hypertension in SHR. The prolonged antihypertensive effect with an ACE inhibitor may be due to remodeling of the cardiovascular system and to an alteration in the central RAS.

Acknowledgments

These studies were supported by US Public Health Service grants HL-31515 and HL-46554. We thank Sal J. Lucania from the Bristol-Myers Squibb Pharmaceutical Research Institute for providing us with captopril. We also thank Sharon C. Francis, Douglas Moeller, Darrell Tjepkes, David Edwards, and Dr Lin Zhang for assistance in these studies.

References


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Hypertension. 1993;22:139-146
doi: 10.1161/01.HYP.22.2.139

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1993 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

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