Enalapril Prevents Stroke and Kidney Dysfunction in Salt-Loaded Stroke-Prone Spontaneously Hypertensive Rats

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The influence of chronic treatment with the angiotensin I converting enzyme (ACE) inhibitor enalapril on blood pressure, kidney function, and survival was examined in stroke-prone spontaneously hypertensive rats (SHRSP). Male SHRSP that were fed a Japanese rat chow plus a 1% NaCl drinking solution beginning at 7–8 weeks of age developed severe hypertension and stroke; 14 of 18 untreated control SHRSP died by 14 weeks of age and exhibited evidence of cerebrovascular lesions. When enalapril (15 mg/kg/day) was included in the drinking solution of 15 SHRSP, blood pressure was initially reduced by only a slight degree, whereas survival improved markedly; only one of 10 SHRSP died before the rest were killed at 18 to 21 weeks. The remaining five enalapril-treated SHRSP lived beyond 36 weeks and on histological examination exhibited no evidence of cerebrovascular lesions. Chronic enalapril treatment also prevented the greater urinary excretion of protein and severe renal lesions observed in untreated SHRSP but did not affect urinary salt and water excretion. In anesthetized rats, glomerular filtration rate and tubular reabsorption of water were lower in untreated control SHRSP when compared with enalapril-treated SHRSP. Mean arterial pressure was comparable in both groups. These data support a possible role for ACE inhibition in the prevention of stroke and maintenance of kidney function independent of any marked change in blood pressure of SHRSP. Whether the protective effects of ACE inhibition relate to reduced angiotensin II formation, increased tissue kinins, or another mechanism remains to be determined. (Hypertension 1989;13:115-121)

In 1974 Okamoto and coworkers1 reported the development of a stroke-prone substrain of the spontaneously hypertensive rat (SHRSP). These animals developed severe hypertension and a high incidence (80%) of cerebrovascular hemorrhage or infarction after 15 weeks of age. The pial arteries of SHRSP showed cellular hyperplasia or proliferation of adventitial cells and fibrinoid necrosis,1 which resulted in microinfarction.2 Parenchymal brain lesions were noted that were thought to be the result of vascular leakage.3,4 Similar abnormalities were observed in the microvasculature of the heart, kidney, and other organs. SHRSP fed a Japanese rat chow exhibited a higher incidence of stroke than SHRSP fed an American rat chow (88% vs. 30% by 9 months of age).5 Elevation of sodium intake (by replacing normal drinking water with a 1% NaCl solution) also increased blood pressure and accelerated the onset of stroke.6,7 Angiotensin I converting enzyme (ACE) inhibitors may lower blood pressure of SHR8-10 and SHRSP11-14 maintained on normal or low-sodium diets and lessen proteinuria and glomerular sclerosis in experimental models of diabetes mellitus15 and remnant chronic renal failure.16 In SHRSP maintained on a 1% NaCl drinking solution, plasma renin activity was initially suppressed followed by marked elevations.17 In the present study, we examined a possible pathophysiological role for the renin-angiotensin system by chronically administering the ACE inhibitor enalapril in SHRSP maintained on a high sodium intake.

Materials and Methods

SHRSP derived from the Okamoto-Aoki strain of SHR (obtained from Dr. William Watson of the

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National Institutes of Health, Bethesda, Maryland) were bred locally. Male SHRSP (generations F-56 and F-57) were weaned at 4–5 weeks of age. All rats were given 1% NaCl, instead of water, as drinking fluid and fed Japanese Stroke-Probe Rodent Diet 39-288 (Zeigler Brothers Inc., Gardnens, Pennsylvania) starting at 7–8 weeks of age. Analysis of the Japanese Stroke-Probe Rodent Diet (mean of four batches) fed to our rats and the usual diet (Purina Lab Chow 5001, Ralston Purina Inc., St. Louis, Missouri; mean of two batches) revealed, respectively, the following dietary composition: potassium (0.71% vs. 1.23% by weight), protein (16.95% vs. 22.15% by weight), and sodium (0.38% vs. 0.35% by weight). Lower content of potassium and protein in the Japanese diet has been previously reported by Yamori and coworkers. The Japanese diet was associated with a higher incidence of stroke and therefore this special diet was used. At 8–9 weeks of age, enalapril maleate was added to the drinking solution of 15 SHRSP at 41–78 mg/l, approximately 15 mg/kg/day for the remainder of the study. The control group of SHRSP (n=18) were littermates that were treated identically, except that no enalapril was added to the drinking solution. The brains of nine untreated and 10 enalapril-treated SHRSP that died or were killed were removed and fixed in phosphate-buffered formaldehyde in preparation for pathologic examination. Brains were sliced transversely at five to seven levels and examined for gross abnormalities. The entire brain was then embedded in paraffin blocks and histological sections (5–7 μm) from each were stained with hematoxylin and eosin and examined microscopically for lesions. Kidneys obtained at death or when killed from nine untreated and five enalapril-treated SHRSP were similarly prepared for histological examination.

Balance Studies
Rats were housed individually in metabolic cages (Nalgene, Rochester, New York) and allowed free access to Japanese rat chow and 1% NaCl drinking water. Studies were conducted in 10 of the enalapril-treated and 13 of the control SHRSP. Measurements of food and saline intake, body weight, urine volume, and the excretion of sodium, potassium, and protein were made over consecutive 2- to 3-day periods until the rats were 12 weeks of age. Systolic blood pressure was measured by tail-cuff plethysmography at weekly intervals in conscious, restrained rats using a Natsume manometer-tachometer system (model KN-210, Peninsula Laboratories Inc., Belmont, California). Analyses
Renal Clearance Studies
Experiments were performed to determine the renal clearance of tritiated inulin for estimation of glomerular filtration rate (GFR) by using techniques previously described. For these studies, additional groups of control SHRSP (n=5) and enalapril-treated SHRSP (at 14.6 weeks of age, n=8) were used. Four of the enalapril-treated SHRSP were also used for clearance studies at 20 weeks of age. Rats were anesthetized with sodium pentobarbital (65 mg/kg i.p.), and body temperatures were maintained at 37°C by placing the rats on a heating table. Tracheae were cannulated and the rats allowed to respire spontaneously. Blood pressure was monitored via a femoral arterial catheter with a pressure transducer (model PZ3Db, Gould Statham Instr., Hato Rey, Puerto Rico) and polygraph recorder (model 7D, Grass Instruments Co., Quincy, Massachusetts). Three cannulae were inserted into a jugular vein: one for administration of supplemental doses of anesthetic, one for infusion of tritiated inulin (ICN Chem. & Radioisotopes, Irvine, California; 0.2–0.4 μCi/100 g body wt/hr at 40 μl/min), and one for infusion of saline (16 μl/min). A femoral vein was cannulated for infusion of saline (23 μl/min). The bladder was catheterized with PE-90 tubing inserted through a small abdominal incision; the wound was sutured and the free end of the catheter exteriorized for collection of urine samples. Clearance studies started after a 1-hour equilibration period. Urine was collected for three consecutive 20-minute periods and output determined gravimetrically. Femoral arterial blood samples were taken at the midpoint of each clearance period for measurement of plasma tritiated inulin concentration and hematocrit. At the end of each experiment, the kidneys were excised, decapsulated, and weighed immediately.

Tritium radioactivity in plasma and urine samples was measured with a Tri-Carb liquid scintillation counter (model 460C, Packard Instrument Company, Inc., Downers Grove, Illinois). GFR was calculated from the clearance of tritiated inulin. Sodium and potassium concentrations in the urine were determined with a flame photometer (model 343, Instrumentation Laboratory, Inc., Lexington, Massachusetts). Urinary protein concentration was determined by the sulfosalicylic acid turbidity method. Urinary excretion of both electrolytes and proteins were calculated as the products of urinary concentrations and urine flow rates.

Renal clearance data were analyzed statistically by using a one-way analysis of variance and Dunnet’s test. Data from chronic studies were analyzed with the software package SAS (Statistical Analysis System, Cary, North Carolina). Statistical methods included repeated-measures analysis of variance for overall treatment effects and the Bonferroni method for comparison of treatment groups at different times. Differences between means that had a p<0.05 were considered statistically significant. Data are reported as the mean±SEM.

Results
Figure 1 illustrates the percentage of survival among 18 untreated control and 15 enalapril-treated SHRSP. Mortality occurred precipitously from 12
to 14 weeks of age in untreated rats. In contrast, only one animal died among the 15 receiving chronic enalapril treatment. Nine of these animals were killed at 19–21 weeks of age for purposes of additional studies. Among the remaining five enalapril-treated SHRSP, no additional deaths were noted up to 36 weeks of age. Gross abnormalities (i.e., hemorrhage) were observed in five of nine brains from untreated control SHRSP, whereas no macroscopic lesions were observed in brains from 10 enalapril-treated SHRSP. Histological examination revealed evidence of cerebrovascular lesions in all except one of the brains from control SHRSP. As previously observed, these lesions included hematomas, hemorrhagic and anemic infarcts, edema, and rarefaction of the cerebral cortex, and fibrinoid necrosis of vessels. There was no evidence of any of these lesions in the brains of enalapril-treated SHRSP. There was no initial difference in systolic blood pressure between the control group and the group treated with enalapril (Figure 2). Thereafter, blood pressure rose in both groups, though at a slightly lesser rate in enalapril-treated SHRSP. After strokes began to occur at 12 weeks of age in untreated rats, blood pressure became variable and no longer differed significantly between the untreated and enalapril-treated groups. Beyond that time, blood pressure continued to increase, but without strokes, in enalapril-treated rats and eventually reached levels greater than had been observed in the control group.

The enalapril-treated group fared well and gained weight; in untreated SHRSP body weight began to decline after 12 weeks of age as the rats became debilitated (Figure 3). The average body weight at death was 187±7 g in the 18 control SHRSP. Enalapril treatment prevented the decline in food intake and body weight that was observed in untreated control SHRSP. Saline intake was not significantly different between the groups but tended to be greater in enalapril-treated SHRSP. Measurements of 24-hour urinary water and electrolyte excretion are shown in Figure 4. There were no consistent significant differences in urine volume, sodium excretion, or potassium excretion between control and enalapril-treated SHRSP. Urinary excretion of protein in seven enalapril-treated and 11 untreated control SHRSP are compared in Figure 5. Urinary protein excretion increased in both groups as the animals grew older but to a much greater extent in untreated animals. Proteinuria was marked for a short period in three of the seven enalapril-treated SHRSP, which temporarily skewed the average results. However, urinary protein excretion in these enalapril-treated rats was not maintained at a high level and the average excretion returned toward baseline by the 12th week, although systolic blood pressure continued to climb. Histological examination of all kidneys from untreated control SHRSP showed mild-to-severe segmental glomerular, arterial, and arteriolar necrosis, proliferative arteriopathy, and tubular dilatation. The sections of kidney from enalapril-treated SHRSP showed no lesions or...
FIGURE 3. Line graphs showing body weight and food and saline intake of untreated control stroke-prone spontaneously hypertensive rats (SHRSP) and SHRSP given enalapril (15 mg/kg/day in their drinking solution). Rats were maintained on 1% NaCl drinking solution and Japanese rat chow starting at 7–8 weeks of age. Body weight data are for 18 untreated control and 15 enalapril-treated SHRSP, and food and saline intake data are for 13 control and 10 enalapril-treated SHRSP. Values are mean±SEM. Numbers in parentheses indicate the number of rats for which data were obtained.

FIGURE 4. Line graphs showing urine volume, sodium excretion, and potassium excretion of untreated control stroke-prone spontaneously hypertensive rats (SHRSP) and SHRSP treated with enalapril. Values represent mean±SEM for 24-hour excretions (collections made over 2–3-day periods). Numbers in parentheses represent the number of rats for which collections were obtained.

Discussion
These data demonstrate that enalapril treatment decreases stroke-related mortality and protects against renal dysfunction in SHRSP maintained on high-sodium intake. There were no lesions in the brains of enalapril-treated SHRSP, including the one animal that died spontaneously. This animal probably died from some cause other than stroke. In contrast, all except one of the untreated control SHRSP exhibited severe cerebrovascular lesions consistent with the occurrence of stroke. The minor effect of enalapril on the blood pressure elevation of these animals suggests that its beneficial effects may occur by a mechanism other than blood pressure reduction. A similar dissociation between blood pressure effects and survival has been reported in studies of SHRSP on a high sodium diet that were receiving an increased potassium intake. Increased potassium (either KCl or KHCO₃) lowered blood pressure in some of these rats; however, potassium reduced the incidence of stroke by 98% compared with a group of control rats with similar blood pressure levels. It may be relevant that plasma renin activity, and presumably angiotensin II (Ang II), has been reported markedly decreased in...
rats given a high-potassium diet. In a previous study with the thromboxane A2 (TXA2) synthase inhibitor UK-38,485, we observed a contrasting dissociation between blood pressure level and stroke in SHRSP. Despite lowering blood pressure by 15 to 29 mm Hg at 10 and 11 weeks of age during TXA2 synthase inhibition, there was no effect on mortality; 70% of the animals with or without treatment died by 14 weeks of age. The ability of TXA2 synthase inhibition to lower blood pressure without affecting mortality suggests that factors in addition to blood pressure may be important in causing stroke in these rats.

Enalapril treatment also prevented the greater proteinuria and marked reductions in GFR observed in untreated SHRSP. A lowering of hematocrit, which usually occurs in association with renal failure, was also prevented in SHRSP treated with enalapril. Urine flow was similar in treated and untreated animals. However, the maintenance of salt and water excretion in untreated SHRSP was probably secondary to reduced tubular reabsorption since the GFR decreased. This difference in fractional excretion of water would be consistent with renal tubular damage in the untreated versus enalapril-treated rats. These findings are further supported by the absence of severe renal damage observed on microscopic examination of kidneys from enalapril-treated SHRSP. In other studies, chronic enalapril treatment lowered systemic blood pressure and was thought to exert beneficial renal effects by decreasing glomerular hypertension. Also, in rats with passive Heymann nephritis, enalapril and captopril both prevented the increased urinary albumin excretion. In the present study mean arterial pressure was not altered; however, we cannot rule out an effect of enalapril to reduce glomerular capillary pressure or permselectivity.

Mechanisms for the protective actions of enalapril may include reductions in detrimental effects of Ang II or enhancement of beneficial effects of kinins. When the hypertensive state is associated with high dietary salt intake, plasma renin activity initially tends to be low because increases in body sodium, blood volume, and blood pressure depress the renal release of renin. Thus, enalapril has been reported relatively ineffective for antihypertensive therapy in SHR given saline drinking solution. However, plasma renin activity may increase as vascular damage occurs. It has been reported that placement of SHRSP on 1% NaCl drinking solution results in an initial lowering of plasma renin activity at 1 week followed by a marked elevation at 3 to 4 weeks. These observations are consistent with present physiological data in that blood pressure was not markedly affected during the early stage of
chronic enalapril treatment in SHRSP given 1% NaCl solution to drink. However, recent studies have demonstrated a gradual restoration of Ang II levels in the circulation of animals treated chronically with enalapril and may account for the lack of blood pressure lowering in the late stage of hypertension in salt-loaded SHRSP. In other forms of experimental hypertension, such as animals with ligation of the aorta between the origin of the renal arteries, plasma levels of renin are markedly increased (greater than 10-fold) and ACE inhibitors, Ang II antagonists, or specific antibodies to Ang II markedly decrease blood pressure. However, even in situations where plasma renin activity is low, such as the rat remnant kidney model and experimental diabetes, inhibition of the circulating renin-angiotensin system alone cannot explain the effectiveness of ACE inhibitors. Thus, Ang II has been demonstrated to be generated by vascular tissue and the inhibition of Ang II in specific tissues may be of importance. Likewise, enalapril may increase levels of kinins by reducing kinase activity. Kinins are potent dilators of the cerebral and renal circulations and may exert local effects during ACE inhibition to maintain flow without altering systemic blood pressure. Of significance in this regard, ACE inhibitors have been found to increase the autoregulatory range of cerebral blood flow in hypertensive patients. Finally, altered prostaglandin levels may also be a factor in the effects of enalapril since kinins are potent stimulators of prostaglandin formation.

In addition to its direct effect to constrict blood vessels, Ang II has been reported to amplify vascular responses to vasoconstrictor substances (such as norepinephrine), promote norepinephrine release from nerve terminals, enhance sympathetic outflow from the central nervous system, release aldosterone from the adrenal gland, and alter sodium reabsorption by the kidney via a direct tubular action; all of which may play a detrimental role in the hypertensive state. Our data do not provide information concerning many of these effects to explain the benefits of enalapril. However, it would appear that the enhanced excretion of water and electrolytes secondary to enalapril administration may not be involved since we did not find differences between treated and untreated groups. Likewise, it does not appear that the benefits of enalapril relate to a reduced intake of saline by SHRSP. Although central nervous system Ang II is reported to stimulate water intake and salt consumption, saline intake was as great or greater in our enalapril-treated rats as the untreated control rats. Enalapril prevented weight loss, which appeared to be related to the maintenance of food intake. Additionally, the vascular detrimental influence of the renin-angiotensin system may not be limited to its effects on vascular resistance and salt and water excretion. Neutrophil adhesion to endothelium is the first step of leukocyte-mediated vascular injury. Recent reports indicate an increased adherence of granulocytes to the luminal surface of aortic and cerebral vascular endothelium and subendothelial space from hypertensive animals and SHRSP. Ang II may influence neutrophil accumulation via production of neutrophil chemotactic activity in vascular endothelial cells. In addition, Ang II has been reported to stimulate hypertrophy (i.e., protein synthesis) in cultured vascular smooth muscle. These findings raise the possibility of additional benefits of enalapril through prevention of various direct vasculotoxic effects of Ang II. However, since the dose of enalapril used in the present study is considerably higher than that used clinically, extrapolation of our results to hypertensive patients predisposed to stroke must be guarded. The results of the Medical Research Council trial of treatment of mild hypertension indicate a lower incidence of stroke in those patients treated with bendrofluazide compared with those treated with propranolol and has led some investigators to hypothesize that Ang II may protect against stroke. This suggestion is based on the interpretation that the effect of the thiazide was due to stimulation of Ang II production, whereas the beta blocker had an opposite effect on the renin-angiotensin system. ACE inhibitors were not examined in that study and our findings call attention to the need for further clinical investigations in this area.

In conclusion, ACE inhibition with enalapril ameliorated kidney dysfunction and diminished stroke-related mortality and the occurrence of brain lesions in SHRSP but did not prevent the development of severe hypertension. The beneficial effects of enalapril could not be related to alterations in water and electrolyte excretion. Whether the protective effects of enalapril relate to reduced Ang II formation and its vasculotoxic effects, increased tissue kinins, or another mechanism remains to be determined.

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