Replacement of Regular Salt by a Novel Salt Alternative Improves the Cardiovascular Effects of the ACE Inhibitor Enalapril

Eero M.A. Mervaala*, Juha T. Laakso**, Jaakko-Juhani Himberg***, and Heikki O. Karppanen*

The present high levels of sodium chloride (regular salt, RS) intake interfere with the therapeutic effects of angiotensin converting enzyme inhibitors. In previous studies a novel potassium-, magnesium- and l-lysine-enriched and sodium-reduced salt alternative (SA) has been virtually devoid of the hypertensive, left ventricular hypertrophy producing, and life-span shortening effects, characteristic of RS. We therefore compared the influence of SA on the cardiovascular effects of enalapril with that of RS in male stroke-prone spontaneously hypertensive rats (SHRSP). During the 28-day experiment, RS alone produced a marked rise in blood pressure, induced remarkable left ventricular hypertrophy, and caused the death of five out of 18 SHRSP. Oral enalapril treatment did not significantly affect either of the detrimental cardiovascular effects of RS but there were no deaths in the enalapril-treated group. The SA supplemented diet neither caused mortality nor induced any significant rise in blood pressure as compared to control SHRSP, and caused significantly less cardiac hypertrophy than RS. During SA, enalapril had a marked antihypertensive effect and it also completely blocked the salt-induced left ventricular hypertrophy. During SA + enalapril or SA alone, there was no tendency to hyperkalemia in any of the SHRSP. There was not any difference in the plasma renin activity (PRA) between control, RS and SA groups. Enalapril increased PRA to the same extent, approximately three-fold, in the RS and in the SA supplemented SHRSP. Hence, PRA does not explain the marked improvement of the effects of enalapril by SA in comparison to RS. Our findings suggest that replacement of regular salt by the novel salt alternative may remarkably improve the cardiovascular effects of enalapril treatment. (*Hypertens Res* 1994; 17: 59-69)

Key Words: enalapril, stroke-prone spontaneously hypertensive rats, cardiac hypertrophy, renin, sodium, potassium, magnesium

Angiotensin converting enzyme inhibitors (ACEIs) belong to the first-line drugs in the treatment of arterial hypertension and congestive heart failure. Unfortunately, the high levels of sodium chloride (regular salt) intake, typical for most industrialized societies (1), interfere with the therapeutic effects of ACEIs. Therefore, salt restriction or natriuretic diuretics are frequently used to improve the therapeutic efficacy of ACEIs (2).

Increased intake of potassium exerts antihypertensive effects and protects against stroke (3-5). Increased intake of magnesium may also, in some cases, reduce blood pressure and produce a variety of other favorable cardiovascular and metabolic effects (6-9).

Even without drug treatment, reduction in the intake of sodium chloride produces an antihypertensive effect (1, 10-12). In our recent studies, a novel

potassium-, magnesium-, and l-lysine-enriched salt alternative, even in high doses, did not raise blood pressure and produced little if any left ventricular hypertrophy in SHR, whereas such harmful cardiovascular effects were characteristic of regular salt (13). The novel salt alternative is increasingly used instead of regular salt both in home kitchens and by the food industry (14). Hypertensive patients, including those receiving antihypertensive drugs, are particularly likely to replace the use of regular salt by the salt alternative. We therefore undertook this study to examine possible beneficial or harmful interactions of the ACEI enalapril with the salt alternative. Using stroke-prone spontaneously hypertensive rats as a model, we studied the effects of enalapril during salt alternative or regular salt supplementation on blood pressure, left ventricular hypertrophy and plasma electrolyte levels. The

From the *Department of Pharmacology and Toxicology, University of Helsinki, Finland. **Mila Ltd., Helsinki, Finland.

***Department of Clinical Pharmacology, University of Helsinki, Finland.

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Address for Reprints: Eero Mervaala, M.D., Department of Pharmacology and Toxicology, University of Helsinki, P.O. BOX 8 (Siltavuorenpenger 10), FIN-00014 University of Helsinki, Finland.

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effects of the salt alternative could be due either to the lowered intake of sodium chloride or increased intakes of potassium, magnesium and L-lysine. We therefore conducted two sets of experiments. In the first experiment, regular salt and the salt alternative were supplemented at the same level. Hence, the concentration of sodium chloride in the diet was 43% lower in the salt alternative group. In the second experiment, regular salt was added at a 43% lower level than the salt alternative. Hence, in this experiment the dietary sodium chloride level was the same in both groups. Even though the present salt alternative contains considerably less potassium than salt substitutes which have produced hyperkalemia (15, 16), we paid special attention to potassium determinations.

Materials and Methods

Experimental Animals and Diets

Experiment I

Fifty-seven male stroke-prone spontaneously hypertensive rats (SHRSP) purchased from Møllegaards Breeding Centre, LI. Skensved, Denmark, were used in this study. At the beginning of the study, the nine-week-old rats were randomized into five subgroups to receive different diets for four weeks: 1) a control group ($n = 10$) receiving standard rat chow (Finnewos Aqua, Helsinki, Finland; Na 0.3%), 2) a high sodium chloride group without enalapril ($n = 18$) (6.0 g of NaCl, Merck, Darmstadt, Germany, added to 94.0 g of the chow; NaCl 6%), 3) a salt alternative group without enalapril ($n = 10$) (6.0 g of the commercially available salt alternative Pansalt®, Oriola Oy, Espoo, Finland added to 94.0 g of the chow; NaCl 3.4%), 4) a high sodium chloride group with enalapril treatment ($n = 10$) (NaCl 6%), and 5) a salt alternative group with enalapril ($n = 10$) (NaCl 3.4%). The salt alternative used in the present study has the following composition: NaCl 57%, KCl 28%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 12%, L-lysine hydrochloride 2%, and anticaking agents (MgCO_3 , SiO_2) 1%. Therefore, the sodium chloride concentration was 43% lower in the SA groups than in the regular salt groups. Enalapril (enalapril maleate, kindly donated by Merck, Sharp and Dohme Research Laboratories, Rahway, N.J., USA) was added at the level of 350 mg enalapril per kg of dry weight of the chow to produce an average daily dose of approximately 30 mg per kg body weight. The contents of sodium, potassium, magnesium and other nutrients in the different diets are given in Table 1. The rats had free access to tap water and chow. During the fourth week of the experiment, the rats were housed individually in metabolic cages. Feed intake was recorded and urine was collected over a 24-hour period. Rats were examined daily for survival and signs of stroke throughout the study. Assessment of stroke was based on the presence of evident and stable hemiplegia, akinesia, lethargy, and hyporesponsiveness according to the symptomatological classification described by Yamori *et al.* (17). The study was approved by the Animal Experimentation Commit-

tee of the University of Helsinki, Finland.

Experiment II

Sixteen male SHRSP were used in this study. At the beginning of the study, the nine-week-old rats were randomized into two subgroups: 1) a moderately high sodium chloride group with enalapril ($n = 8$) (3.4 g of NaCl added to 96.6 g of the chow; NaCl 3.4%), and 2) a salt alternative group with enalapril ($n = 8$) (6.0 g of the salt alternative added to 94.0 g of the chow; NaCl 3.4%). The content of sodium chloride in the chow was thereby adjusted to the same level in the two groups. Enalapril maleate was added at the level of 350 mg enalapril per kg of dry weight of the chow to produce an average daily dose of approximately 30 mg per kg body weight. Otherwise, Experiment II was performed in the same way as Experiment I.

Measurement of Blood Pressure

Systolic blood pressure of the pretrained rats was measured weekly using a tail cuff method (Blood pressure recorder, model no. 8002e, W+W electronics Inc., Basel, Switzerland). The same technician made all blood pressure measurements. Details of the procedure have been described earlier (13).

Sample Preparation

After the four-week experimental period, the rats were anesthetized with sodium pentobarbital (65 mg/kg i.p.), the carotid artery was cannulated, and blood samples were drawn into chilled tubes on ice using EDTA as anticoagulant. The hearts were excised, the great vessels, atria and the free wall of the right ventricle were dissected, and the left ventricular mass (LVM) was measured. The left ventricular wet weight-to-body weight ratio was calculated as an index of left ventricular hypertrophy.

Determination of Plasma Renin Activity

Plasma renin activity was determined by using a radioimmunoassay (RIA) of angiotensin I, modified for rat plasma (18).

Determination of Electrolyte Concentrations

The concentrations of the elements sodium, potassium, phosphorus, magnesium, calcium and zinc in urine were determined by using a Baird PS-4 inductively-coupled plasma emission spectrometer (Baird Co, Bedford, M.A., USA) as described in detail elsewhere (19). For magnesium the intra-assay imprecision was 1.5% and the interassay imprecision was 0.5%. The intra-assay imprecision for the other elements was better than 3% and the interassay imprecision was not more than 5%. The mean levels obtained for the NIST (National Institute of Standards, Washington, USA) 1577b reference material were within 3.5% of NIST certified values. The concentrations of sodium and potassium in plasma were determined employing a standard ion selective electrode method using a Cobas Fara II random access analyzer (F.Hoffman-La Roche, Basel, Switzerland) according to the instructions of the manufacturer. The concentration of magnesium in plasma was determined by a spectroscopic

Table 1. Contents of Sodium, Potassium, Magnesium and Other Nutrients in the Different Diets

		Control diet	Common salt diet	Salt alternative diet
Mineral element				
Sodium				
Experiment I		0.3*	2.6*	1.6*
Experiment II			1.6*	1.6*
Potassium		0.8*	0.8*	1.7*
Magnesium		0.2*	0.2*	0.27*
Other nutrients (contents common for all diets)				
Mineral elements			B ₁	3 mg/kg
Calcium	1.0*		B ₂	12 mg/kg
Phosphorus	0.75*		B ₆	5 mg/kg
Iron	178 mg/kg		B ₁₂	0.02 mg/kg
Zinc	115 mg/kg		Calcium pantothenate	10 mg/kg
Manganese	89 mg/kg		Niacin	40 mg/kg
Copper	18 mg/kg		Choline chloride	1,000 mg/kg
Iodine	2 mg/kg		Major constituents	
Cobalt	1 mg/kg		Water	11.7*
Vitamins			Crude fat	5.3*
A	12,000 iu/kg		Crude protein	21.0*
D ₃	1,500 iu/kg		Fiber	2.8*
E	63 mg/kg		Carbohydrate	52.2*
K ₁	0.25 mg/kg		Ash	7.0*
K ₃	10 mg/kg		Metabolizable energy	13.0 MJ/kg

*Values are expressed as % (g/100 g) of the dry weight of the pellets.

method using metallochromic dye, Camalgite (Sherwood Medical, St Louis, M.O., USA), and the Cobas Fara II. The intra-assay imprecision was 0.2% for sodium, 0.4% for potassium and 1.5% for magnesium, and the interassay imprecision was 0.5% for sodium, 0.65% for potassium and 4.5% for magnesium.

Statistical Analysis

Statistical analysis was carried out by one-way analysis of variance (ANOVA) supported by the Scheffe's test. Data for multiple observations over time were analyzed by two-way ANOVA with repeated measures for overall treatment effect, and the Scheffe's test was used for multiple pairwise comparisons of treatment groups at different times. The area under the curve (AUC) (blood pressure *vs.* time) of each individual rat in different treatment groups was also calculated mathematically by the methods outlined by Matthews *et al.* (20). The AUCs were then tested by ANOVA, supported by the Scheffe's test. Differences between means that had $p < 0.05$ were considered significant. Statistical analysis of the mortality during the 28-day follow-up period was calculated by a 2×2 Table using Fisher exact test (2-tail). The data were analyzed using BMDP Statistical Software (Los Angeles, CA, USA). The results are expressed as means $\pm$ SEM.

Results

Experiment I

Death-rate and Stroke

Five of the 18 SHRSP which were supplemented with regular salt died during the experiment. All of these rats suffered from clinically diagnosed stroke before death. By contrast, none of the 10 SHRSP receiving the salt alternative died or suffered from stroke. In the presence of the enalapril treatment, no deaths occurred and there was no evidence of stroke in the SHRSP supplemented either with regular salt or the salt alternative. The mortality in the regular salt group was significant as compared to the other groups (mortality within 28 days, 2×2 Table, high sodium chloride group *vs.* other groups combined, Fisher exact two-tail test, $p = 0.002$).

Blood Pressure and Left Ventricular Hypertrophy

Supplementation of the diet with regular salt produced a marked further elevation of blood pressure as compared to the age-related increase in blood pressure of the control SHRSP (Fig. 1). Enalapril did not significantly affect the rise of blood pressure during supplementation with regular salt. The rats receiving the salt alternative diet showed no significant increase in blood pressure when compared to the control SHRSP. In the salt alternative supplemented rats with enalapril treatment the blood pressure was markedly decreased from the third study week onwards, and during the overall 4-week

follow-up period, as indicated by decreased AUC (Fig. 1).

Regular salt produced left ventricular hypertrophy as indicated by a marked increase in the left ventricular wet weight-to-body weight ratio (Fig. 2). In the salt alternative supplemented rats, the left ventricular hypertrophy index was increased when compared to the control group, but decreased as

Fig. 1. Line plots show the blood pressure of stroke-prone spontaneously hypertensive rats during different diet and drug regimens. Open circles, controls on normal diet (C, $n = 10$); open squares, supplementation with regular salt (Na, $n = 18-13$); open triangles, supplementation with the salt alternative (SA, $n = 10$); solid squares, regular salt with enalapril (E + Na, $n = 10$); solid triangles, salt alternative with enalapril (E + SA, $n = 10$). Repeated analysis of variance: between-subject effects, $p < 0.0001$; within-subject effects, time $p < 0.0001$, time-group interaction $p < 0.0001$. $a = \text{Na vs. C}$ $p < 0.01$, Na vs. E+SA $p < 0.001$, SA vs. E+SA $p < 0.01$, E+Na vs. E+SA $p < 0.01$. $b = \text{Na vs. C}$, SA and E+SA $p < 0.001$, $\text{E+Na vs. C and E+SA}$ $p < 0.001$, SA vs. E+SA $p < 0.001$. $c = \text{Na vs. C}$, SA and E+SA $p < 0.001$, E+Na vs. E+SA $p < 0.001$. Bar graphs show area under the curve (AUC) (blood pressure versus time) in different treatment groups. Analysis of variance, $p < 0.0001$. $d = \text{Na vs. C and E+SA}$ $p < 0.001$, Na vs. SA $p < 0.05$. $e = \text{E+SA vs. C}$ $p < 0.05$, $\text{E+SA vs. SA and E+Na}$ $p < 0.001$. Vertical bars indicate SEM.

compared to the regular salt group. Enalapril treatment did not inhibit the salt-induced left ventricular hypertrophy in the regular salt group. By contrast, in the enalapril-treated SHRSP of the salt alternative group, the left ventricular hypertrophy index did not differ from that of the control SHRSP.

Metabolic Variables and Indicators

There were no significant differences between the groups in the feed intake during the fourth study week (Table 2). All groups with salt supplementation had an increased 24-hour urine output. The 24-hour urine output in the salt alternative group with or without enalapril, was smaller than that in the regular salt group. At the end of the follow-up period, the body weight of the rats in the regular salt group was slightly smaller than that in the other groups (Fig. 3). However, overall during the 4-week follow-up period no differences in the body weight gains between the different groups were found (Fig. 3). The calculated average daily dose of enalapril was 31.3 ± 1.1 mg/kg body weight in the regular salt group and 34.6 ± 3.3 mg/kg body weight in the salt alternative group ($p = 0.364$).

Fig. 2. Bar graphs show left ventricular hypertrophy index expressed as left ventricular wet weight (LVWW)-to-body weight (BW) ratio of stroke-prone spontaneously hypertensive rats after four weeks on the different diet and drug regimens. C, controls on normal diet ($n = 10$); Na, supplementation with regular salt ($n = 13$); SA, supplementation with the salt alternative ($n = 10$); E + Na, regular salt with enalapril ($n = 10$); E + SA, salt alternative with enalapril ($n = 10$). Analysis of variance $p < 0.0001$. $a = \text{Na vs. C and E+SA}$ $p < 0.001$, Na vs. SA $p < 0.01$. $b = \text{SA vs. C}$ $p < 0.01$, SA vs. E+SA $p < 0.05$. $c = \text{E+Na vs. C and E+SA}$ $p < 0.001$. Vertical bars indicate SEM.

Urine Electrolytes

The 24-hour urinary excretion of sodium was increased in all groups with regular salt or salt alternative supplementation when compared to the control group (Table 2). In enalapril-treated SHRSP receiving the salt alternative, sodium excretion was smaller than in the corresponding regular salt group. The level of potassium in urine was increased in the salt alternative supplemented rats both with and without enalapril treatment. In the regular salt supplemented rats, both with and without enalapril, the level of potassium was decreased when compared to the controls. Magnesium excretion was increased in the salt alternative groups. Calcium excretion was markedly increased in all groups with salt supplementation. The excretion of phosphorus and zinc was markedly increased in the regular salt supplemented rats, both with and without enalapril treatment.

Plasma Electrolytes and Renin Activity

There were no differences between the groups in

the plasma sodium and magnesium levels. The potassium level was decreased in the regular salt supplemented, enalapril-treated rats. The plasma renin activity was similar in the controls, in the regular salt supplemented and in the salt alternative supplemented groups without enalapril treatment (Table 3). Enalapril treatment increased the plasma renin activity to the same extent, approximately three-fold, in the regular salt and the salt alternative groups.

Experiment II

Death Rate and Stroke

None of the enalapril-treated SHRSP in experiment II died or showed any evidence of stroke during the 4-week follow-up period.

Blood Pressure and Left Ventricular Hypertrophy

The blood pressure in the moderately high sodium chloride group was higher than in the salt alternative group from the first to the third study week, and during the overall 4-week follow-up period

Table 2. 24-Hour Feed Consumption, Urine Volume and Urinary Excretion Rates of Various Mineral Elements of Stroke-Prone Spontaneously Hypertensive Rats after Three Weeks on the Different Diets and Drug Regimens

	C	Na	E+Na	SA	E+SA	ANOVA	Difference between groups at $p < 0.05$
Feed intake (g/day)	25.6 ± 1.7	20.1 ± 1.1	22.5 ± 0.9	22.3 ± 1.3	24.8 ± 2.0	0.045	—
Urine volume (ml/day)	16.3 ± 1.9	65.2 ± 2.8	71.9 ± 5.0	47.1 ± 1.9	39.4 ± 2.3	<0.0001	C vs. Na, SA, E + Na and E + SA Na and E + Na vs. SA and E + SA
sodium (mmol/day)	1.5 ± 0.09	13.1 ± 0.7	14.9 ± 1.1	11.1 ± 0.8	10.6 ± 0.3	<0.0001	C vs. Na, SA, E + Na and E + SA E + Na vs. SA and E + SA
potassium (mmol/day)	2.81 ± 0.18	1.76 ± 0.10	1.83 ± 0.13	5.73 ± 0.35	5.72 ± 0.20	<0.0001	C vs. Na, SA, E + Na and E + SA Na vs. SA and E + SA E + Na vs. SA and E + SA
magnesium (mmol/day)	0.24 ± 0.04	0.27 ± 0.01	0.35 ± 0.03	0.41 ± 0.03	0.40 ± 0.02	0.0003	C and Na vs. SA and E + SA
phosphorus (mmol/day)	0.94 ± 0.06	1.10 ± 0.05	1.09 ± 0.08	0.80 ± 0.05	0.80 ± 0.03	0.0007	Na and E + Na vs. SA and E + SA
calcium (mmol/day)	0.034 ± 0.005	0.17 ± 0.009	0.25 ± 0.02	0.16 ± 0.02	0.16 ± 0.01	<0.0001	C vs. Na, SA, E + Na and E + SA E + Na vs. Na, SA and E + SA
zinc (μmol/day)	0.19 ± 0.03	0.48 ± 0.08	0.55 ± 0.06	0.41 ± 0.02	0.37 ± 0.03	0.001	C vs. Na and E + Na

The control group (C, $n = 10$) received standard rat chow (Na 0.3%). The chow of the high sodium chloride group (Na, $n = 13$) and that of the high sodium chloride + enalapril group (E + Na, $n = 10$; enalapril mixed in the chow to produce an approximate daily dose of 30 mg/kg) was supplemented with regular salt (6% sodium chloride). The chow of the salt alternative group (SA, $n = 10$) and that of the salt alternative + enalapril group (E + SA, $n = 10$; enalapril as above) was supplemented with the salt alternative (6% Pansalt®).

Fig. 3. Line plots show body weight of stroke-prone spontaneously hypertensive rats during different diet and drug regimens. Open circles, controls on normal diet (C, $n = 10$); open squares, supplementation with regular salt (Na, $n = 18$ –13); open triangles, supplementation with the salt alternative (SA, $n = 10$); solid squares, regular salt with enalapril (E + Na, $n = 10$); solid triangles, salt alternative with enalapril (E + SA, $n = 10$). Repeated analysis of variance: between-subject effects, $p = 0.1068$; within-subject effects, time $p < 0.0001$, time-group interaction $p < 0.0001$. $a = \text{Na}$ vs. all other test groups, $p < 0.001$. Vertical bars indicate SEM.

Fig. 4. Line plots show blood pressure of stroke-prone spontaneously hypertensive rats during different diet and drug regimens. Solid squares, moderate regular salt supplementation (NaCl 3.4%) with enalapril (E + Na, $n = 8$); solid triangles, salt alternative (SA 6%, corresponding to 3.4% NaCl) with enalapril (E + SA, $n = 8$). Repeated analysis of variance between-subject effects, $p = 0.0099$; within-subject effects, time $p < 0.001$, time-group interaction $p = 0.0071$. * $p < 0.05$, ** $p < 0.01$. Area under the curve (AUC) (blood pressure vs. time) $p = 0.005$. Vertical bars indicate SEM.

(Fig. 4). The left ventricular hypertrophy index of the moderately high sodium chloride group was higher than that of the salt alternative group (Fig. 5).

The moderately high sodium chloride diet (NaCl 3.4%) attenuated the cardiovascular effects of enalapril less than the high sodium chloride diet (NaCl 6.0%) (area under the curve: blood pressure versus time 673 ± 12 vs. 707 ± 9 , $p = 0.031$; left ventricular hypertrophy: 3.064 ± 0.027 mg/g vs. 3.563 ± 0.059 mg/g, $p < 0.001$).

Metabolic Variables and Indicators

There were no differences between the groups in the feed intake (Table 4) or in the body weight gain (repeated ANOVA between-subject effects $p = 0.4699$; within-subject effects, time $p < 0.001$, time-group interaction $p = 0.4062$). There was no difference between the groups in the 24-hour urine out-

put (Table 4). The calculated average daily dose of enalapril was 34.6 ± 0.9 mg/kg body weight in the regular salt group and 31.9 ± 1.8 mg/kg body weight in the salt alternative group ($p = 0.203$).

Urine Electrolytes

There were no differences between the groups in the 24-hour urinary excretion of sodium, magnesium, calcium or zinc. In the salt alternative group, the excretion of potassium was higher and that of phosphorus was lower than in the regular salt group (Table 4).

Plasma Electrolytes and Renin Activity

The plasma renin activity was markedly higher and plasma potassium was slightly higher in the salt alternative group than in the regular salt group (Table 3).

Table 3. The Plasma Levels of Sodium, Potassium and Magnesium and Plasma Renin Activity of Stroke-Prone Spontaneously Hypertensive Rats at the End of the Study

Experiment I	C	Na	E+Na	SA	E+SA	ANOVA	Difference between groups at $p < 0.05$
Sodium (mmol/l)	138.8±0.4	136.8±0.9	137.7±0.3	137.7±0.6	138.5±0.4	0.1448	—
Potassium (mmol/l)	3.49±0.06	3.31±0.08	3.08±0.04	3.41±0.11	3.33±0.08	0.0225	C vs. E+Na
Magnesium (mmol/l)	0.85±0.008	0.82±0.02	0.81±0.02	0.82±0.02	0.83±0.01	0.4547	—
Renin activity (ng Ang I/ml/h)	7.2±1.1	7.3±1.0	20.7±1.0	7.1±1.4	19.9±3.0	<0.0001	C, Na and SA vs. E+Na and E+SA
Experiment II	E+Na	E+SA	<i>t</i> -Test (<i>p</i> -value)				
Sodium (mmol/l)	138.6±0.3	137.6±0.4	0.096				
Potassium (mmol/l)	3.44±0.03	3.60±0.05	0.022				
Magnesium (mmol/l)	0.82±0.02	0.82±0.02	0.799				
Renin activity (ng Ang I/ml/h)	13.6±3.6	31.2±4.2	0.006				

In Experiment I, the control group (C, $n=10$) received standard rat chow (Na 0.3%). The chow of the high sodium chloride group (Na, $n=13$) and that of the high sodium chloride+enalapril group (E+Na, $n=10$; enalapril mixed in the chow to produce an approximate daily dose of 30 mg/kg) was supplemented with regular salt (6% sodium chloride). The chow of the salt alternative group (SA, $n=10$) and that of the salt alternative+enalapril group (E+SA, $n=10$; enalapril as above) was supplemented with 6% of the salt alternative (Pansalt®).

In Experiment II, both groups received enalapril orally, at a dose of approximately 30 mg/kg/day, for four weeks. The chow of one group (E+Na, $n=8$) was supplemented with regular salt (3.4% sodium chloride), and the chow of the other group (E+SA, $n=8$) with the salt alternative (6% Pansalt®, corresponding to 3.4% sodium chloride).

Discussion

An increased intake of sodium chloride (regular salt) produced a marked further elevation of blood pressure and increase in left ventricular hypertrophy as compared to the age-related increases in control SHRSP. The increased intake of sodium chloride also produced significant mortality of SHRSP during the four-week experiment. These effects of regular salt are in agreement with previous findings in SHR and SHRSP (3, 13, 14, 21). By contrast, the sodium-reduced, potassium-, magnesium-, and *l*-lysine-enriched salt alternative, at the same intake level as regular salt, neither caused any mortality nor induced any significant rise in blood pressure. It also caused significantly less left ventricular hypertrophy than regular salt did. Our previous studies (13, 14), using higher levels of the salt alternative, demonstrated that the increased intakes of potassium and/or magnesium and *l*-lysine from the salt alternative protected against the harmful effects of the increased intakes of sodium chloride. Tobian (22) has reported a protective effect of potassium supplementation against stroke and mortality in salt-loaded rats.

Previous studies have suggested that the detrimental effects of a high intake of sodium chloride could have been mediated mainly by elevated blood volume and cardiac output and by elevated sympathetic nervous system activity (23). Normalization

of sodium and water balance and sympathetic nervous activity appeared to explain, at least in part, the protection against the harmful effects of the increased intake of sodium chloride by the increased intakes of potassium and/or magnesium and *l*-lysine from the salt alternative (13, 14). Potassium supplementation also protected against dysfunction of endothelial cells in stroke-prone spontaneously hypertensive rats (24) and preserved endothelial function in Dahl rats during a high sodium chloride diet (25). Moreover, Watson *et al.* have shown (26) that magnesium sulfate amplifies the release of prostacyclin by human umbilical vein endothelial cells in a dose-dependent manner. However, the smaller intake of sodium chloride in the salt alternative group may also have contributed to the favorable effect.

Enalapril treatment did not produce any significant lowering of blood pressure and it did not decrease the left ventricular hypertrophy in SHRSP receiving the high sodium chloride diet, while the moderately high sodium chloride diet antagonized slightly less the cardiovascular effects of enalapril. However, in spite of the lack of effect on these cardiovascular variables, enalapril protected against stroke and mortality. In agreement with our observation, Stier and coworkers (27) also found that during a high intake of sodium chloride, enalapril treatment decreased SHRSP mortality. The mechanism of this protective effect of ACEI is not

clear.

In the present study chronic treatment with enalapril produced a remarkable antihypertensive effect in SHRSP receiving the salt alternative supplementation. The left ventricular hypertrophy also remained at the same level as in control SHRSP. Pre-

Fig. 5. Bar graphs show left ventricular hypertrophy index expressed as left ventricular wet weight (LVWW)-to-body weight (BW) ratio of stroke-prone spontaneously hypertensive rats after four weeks on the different diet and drug regimens. E+Na, moderate regular salt supplementation (NaCl 3.4%) with enalapril ($n = 8$); E+SA, salt alternative (SA 6%, corresponding to 3.4% NaCl) with enalapril ($n=8$). Vertical bars indicate SEM.

vious studies have shown that ACEIs lower blood pressure and decrease left ventricular hypertrophy during a relatively low-sodium diet (2, 27-29). The diuretic effect of potassium supplementation (30) may have contributed to the beneficial effect of the salt alternative during enalapril treatment. ACEI treatment has been shown to improve the endothelium-dependent vascular relaxation in SHR by increasing the production of endothelium-derived relaxing factor(s) and by preventing the degradation of bradykinin liberated from the endothelial cells (31-33). In agreement with these studies, we found recently (34) that in SHRSP ramipril improved vascular relaxation during a low-sodium diet. The vascular relaxation enhancing effect of ramipril was blocked by regular salt supplementation (34). However, during the salt alternative supplemented diet (sodium chloride intake adjusted to the same level) the vascular relaxation enhancing effect of ramipril was only slightly attenuated (34). Hence, changes in vascular reactivity may also explain the hypertensive effect of sodium chloride and the beneficial effects of potassium, magnesium and *l*-lysine.

It has been suggested that enalapril treatment might increase the risk of hyperkalemia, at least in patients taking supplemental potassium or a potassium-sparing diuretic (2). However, the present salt alternative did not cause hyperkalemia, neither alone nor in combination with enalapril treatment.

The inhibitory effect of a high intake of sodium chloride on the antihypertensive effect of ACEI is consistent with previous observations (2, 29, 35). It has been suggested that the resistance towards the effects of ACEI results from salt-induced inhibition in the activity of the renin-angiotensin-aldosterone system (2). However, neither in the regular salt nor in the salt alternative supplemented SHRSP the increased intake of sodium chloride affected plasma renin activity (PRA). Furthermore, in the presence of enalapril treatment, PRA was increased to the

Table 4. 24-Hour Feed Consumption, Urine Volume and Urinary Excretion Rates of Various Mineral Elements of Stroke-Prone Spontaneously Hypertensive Rats after Three Weeks on the Different Diet and Drug Regimens.

	E+Na	E+SA	<i>t</i> -Test (<i>p</i> -value)
Feed intake (g/day)	23.9 ± 0.6	22.4 ± 1.2	0.271
Urine volume (ml/day)	41.9 ± 1.1	36.9 ± 3.5	0.214
sodium (mmol/day)	11.6 ± 0.9	10.6 ± 0.4	0.384
potassium (mmol/day)	2.82 ± 0.23	5.87 ± 0.23	<0.001
magnesium (mmol/day)	0.38 ± 0.03	0.45 ± 0.03	0.119
phosphorus (mmol/day)	1.50 ± 0.07	0.99 ± 0.06	<0.001
calcium (mmol/day)	0.16 ± 0.02	0.15 ± 0.01	0.842
zinc (μmol/day)	0.30 ± 0.03	0.33 ± 0.02	0.285

Both groups received enalapril orally, at a dose of approximately 30 mg/kg/day, for four weeks. The chow of one group (E+Na, $n=8$) was supplemented with regular salt (3.4% sodium chloride), and the chow of the other group (E+SA, $n=8$) with the salt alternative (6% Pansalt®, corresponding to 3.4% sodium chloride).

same extent in the regular salt and in the salt alternative supplemented SHRSP. Hence, PRA does not appear to explain the marked improvement of the effects of enalapril by the salt alternative in comparison to regular salt.

The lack of suppression in PRA by the increased intake of sodium chloride would, at first sight, appear rather surprising. However, Leenen and Toal (36) found that in SHR the maximum suppression of PRA that could be attained by dietary salt, was produced at the level of 101 μmol (0.23%) sodium per gram rat chow. The control diet in our experiment contained sodium at the level of 0.3% which corresponds to 130 $\mu\text{mol/g}$ rat chow. At corresponding dietary sodium levels, and Toal (36) found similar low PRA in SHR as we did in SHRSP. At lower dietary sodium levels, Leenen and Toal (36) found a progressive rise in PRA with decreasing sodium intake. Moreover, at the low dietary sodium level of 9 $\mu\text{mol/g}$ rat chow, the development of spontaneous hypertension was prevented. These findings indicate that both SHR and SHRSP are very sensitive both to the PRA suppressing and the hypertensive effects of sodium.

The lack of suppression of PRA by regular salt supplementation could also result from malignant hypertension and renal lesions associated with a paradoxical rise in PRA, as suggested by Volpe *et al.* (37). However, we did not find any pathologically high PRA levels in the regular salt group, and there was not any difference in PRA between the regular salt and the salt alternative groups in the present study.

While effects on the circulating renin-angiotensin-aldosterone system do not explain the beneficial influence of the salt alternative on the effects of enalapril, our findings do not exclude the possibility that there may have been differences between the regular salt and the salt alternative groups in renin-angiotensin or kinin systems in the myocardium and other tissues. There is a lot of evidence to suggest that angiotensin II is involved in the production of myocardial (38-44) and vascular wall hypertrophy (45). Reduction of myocardial tissue angiotensin II by chronic treatment with ACEI in SHR has been reported (46). Enalapril treatment also suppressed the accumulation of elastin and collagen in the cardiovascular tissues of growing rats (47). ACE inhibition also increases the levels of bradykinin which appears to give protection against the development of cardiovascular hypertrophy (32).

Supplementation of SHRSP with regular salt or the salt alternative produced a five-fold increase in the urinary calcium excretion, both in the absence and in the presence of enalapril treatment. This finding confirms the results of previous studies demonstrating a rise in urinary calcium excretion with increasing intake of sodium chloride both in man and animals (48). Since the calciuric effect was similar in the regular salt and the salt alternative supplemented groups, it does not explain the difference in the enalapril-induced cardiovascular effects between the groups. However, our finding lends further

support to the suggestion that a high intake of salt may contribute to the development of osteoporosis (48).

In conclusion, the present sodium-reduced, potassium- and magnesium-enriched salt alternative was virtually devoid of the hypertensive and cardiac hypertrophy producing effects, typical of regular salt. Replacement of regular salt in the diet by the salt alternative remarkably improved the cardiovascular effects of enalapril. This effect was mainly due to increased intake of potassium and/or magnesium and *L*-lysine. The present salt alternative with a moderate content of potassium did not produce hyperkalemia, even when combined with ACEI treatment.

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