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Influence of dietary salts on the cardiovascular effects of low-dose combination of ramipril and felodipine in spontaneously hypertensive rats

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1 In spontaneously hypertensive rat (SHR) we examined over a 4-week period the influence of control low sodium diet, common salt-enriched diet (sodium chloride 6% of the dry weight of the chow) and a novel mineral salt-enriched diet (potassium-, magnesium-, and l-lysine-enriched mineral salt added at a 75% higher level of 10.5% to produce the same sodium chloride concentration of 6%) on the cardiovascular effects produced by a low-dose combination of an angiotensin converting enzyme inhibitor ramipril (0.25 mg kg^{-1} day^{-1} in the food) and a calcium channel blocker felodipine (0.4 mg kg^{-1} day^{-1} subcutaneously via an osmotic minipump).

2 Common salt, but not the mineral salt, accelerated the development of hypertension and induced left ventricular and renal hypertrophy in SHR. Neither common salt nor mineral salt significantly affected heart rate.

3 The combination of ramipril and felodipine decreased systolic blood pressure and prevented the development of left ventricular hypertrophy effectively during the common salt diet without any significant effect on the heart rate. The cardiovascular effects of the drug combination were improved by the low sodium diet or by replacement of high common salt in the diet by mineral salt.

4 Responses of endothelium-intact mesenteric arterial rings *in vitro* were examined at the end of the four-week study. The combination of ramipril and felodipine markedly improved the endothelium-dependent vascular relaxation responses to acetylcholine and enhanced the endothelium-independent vascular relaxation responses to sodium nitroprusside in SHR on control and common salt diets. Replacement of common salt in the diet by mineral salt improved the endothelium-dependent vascular relaxation responses to acetylcholine. The drug combination attenuated the α-adrenoceptor-mediated vascular contractile responses to noradrenaline during the common salt diet.

5 Ramipril and felodipine in combination increased plasma renin activity by 1.9–3.2 fold without affecting serum aldosterone levels.

6 Our findings suggest that the cardiovascular effect of the low-dose combination of ramipril and felodipine was maintained during high salt intake. However, salt restriction or replacement of common salt in the diet by the potassium- and magnesium-enriched mineral salt improved the cardiovascular effects of the drug combination. In the face of a high intake of sodium, a part of the beneficial cardiovascular effects of the drug combination is apparently mediated by improved endothelium-dependent and endothelium-independent vascular relaxation responses and attenuated α-adrenoceptor-mediated vascular contractile responses.

Keywords: Ramipril; felodipine; spontaneously hypertensive rat; left ventricular hypertrophy; arterial smooth muscle; sodium; potassium; magnesium

Introduction

Angiotensin converting enzyme (ACE) inhibitors and calcium channel blockers are first-line drugs in the treatment of essential hypertension (WHO/International Society of Hypertension, 1993). We have previously found that, in the spontaneously hypertensive rats (SHR) receiving a relatively low-sodium diet, a better overall control of hypertension and of end-organ damage, without an increase in adverse effects, can be achieved by a drug combination of ramipril and felodipine given at submaximal antihypertensive doses rather than by administration of either drug alone (Mervaala *et al*, 1997a). It is known that a high intake of common salt (NaCl) plays a fundamental role in the development and maintenance of arterial hypertension (DeWardener, 1990a,b; Elliot *et al*., 1996). Unfortunately, the beneficial cardiovascular effects of ramipril, even when given at high doses, are markedly antagonized by high salt intake (Mervaala *et al*., 1994d), although the antihypertensive effect of felodipine, a calcium channel blocker with natriuretic properties (DiBona, 1985), is maintained during high salt intake, at least when given at the maximal antihypertensive dose (Mervaala *et al*., 1994b).

The aim of the present study was to examine whether dietary salts influence the cardiovascular effects of the low-dose combination of ramipril and felodipine. For this purpose the effects of the drug combination were examined in SHR both during a relatively low-sodium control diet and during a common salt-enriched, high-sodium diet. We also compared the influence of common salt on the cardiovascular effects of the drug combination with that of a novel sodium-reduced,

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potassium-, magnesium-, and l-lysine-enriched mineral salt, which in our previous animal experiments produced less acceleration of the development of hypertension and left ventricular hypertrophy than NaCl (Mervaala *et al*., 1992), and improved the therapeutic effects of angiotensin converting enzyme inhibitors (Mervaala *et al*., 1994a,d) and β-adrenoceptor blocking agents (Mervaala *et al*., 1994c).

Methods

Experimental animals and diets

Sixty eight-week old, inbred male spontaneously hypertensive rats (SHR) (Harlan Sprague Dawley, Indianapolis, IN, U.S.A.) were used in the study. The procedures and protocols of the study were in accord with our institutional guidelines and were approved by the Animal Experimentation Committee of the Institute of Biomedicine, University of Helsinki, Finland. The rats were kept in groups of five in plastic cages (55×23 cm) in conventional conditions at a room temperature of $22 \pm 1^{\circ}$C with a controlled 12 h light/dark cycle (from 07 h 00 min to 19 h 00 min). An adaptation period of two weeks was allowed after the arrival of the purchased animals from the breeding centre and before the commencement of the experiment. At the beginning of the study, the blood pressure- and body weight-matched SHR were divided into six experimental groups ($n = 10$ in each group) to receive different diet and drug regimens for four weeks. The rats received either a relatively low-sodium control diet (R3, Finnewos Aqua, Helsinki, Finland), a common salt enriched diet or a mineral salt enriched diet. The common salt enriched diet was produced by adding 6.0 g of sodium chloride (Riedel-de Haën, Seelze, Germany) to 94.0 g of the control chow and the mineral salt enriched diet, by adding 10.5 g of the commercially available mineral salt (Pansalt, Oriola Oy, Espoo, Finland) to 89.5 g of the control chow. The mineral salt had the following composition: NaCl 57%, KCl 28%, $MgSO_4.7H_2O$ 12%, l-lysine hydrochloride 2% and anticaking agents ($MgCO_3$, SiO_2) 1%. The contents of various mineral elements in the different diets used in the present study are given in Table 1. Rats had free access to tap water and chow. Ramipril was added to the chow (3.5 mg ramipril kg^{-1} dry weight of the chow) to produce an

Table 1 Contents of mineral elements in the different experimental diets used in the present study

Mineral element	*Low sodium control diet*	*Common salt enriched diet*	*Mineral salt enriched diet*
Sodium	0.3	2.6	2.6
Potassium	0.8	0.8	2.3
Magnesium	0.2	0.2	0.3
Calcium	1.0	1.0	1.0
Phosphorus	0.75	0.75	0.75

Values are expressed as percentage of the dry weight of the chow.

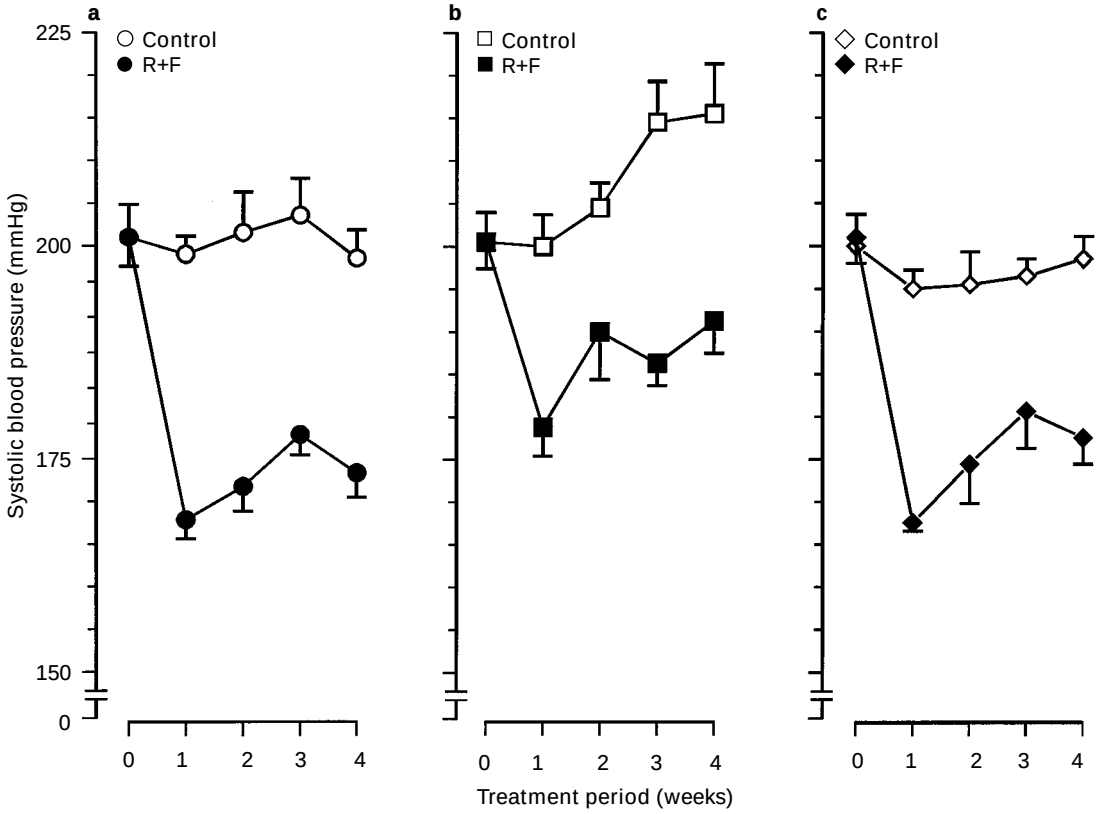

Figure 1 Line plots show development of hypertension in spontaneously hypertensive rats during different diet and drug regimens for four weeks. (a) Control group and ramipril + felodipine group (R + F) on control diet; (b) common salt group (control) and ramipril + felodipine group on common salt enriched diet; (c) mineral salt group (control) and ramipril + felodipine group on mineral salt enriched diet. The combination of ramipril and felodipine effectively decreased blood pressure irrespective of the diet. Salt restriction or replacement of common salt in the diet by mineral salt improved the antihypertensive effect of the drug combination ($P < 0.05$). The results are expressed as means with vertical lines showing s.e.mean, $n = 10$ in each group. ANOVA, analysis of variance; BS, between-subjects effect; WS, within-subject effect; IA, time-group interaction. (a) ANOVA: BS, $P < 0.001$; WS, $P = 0.73$; IA, $P < 0.001$. (b) ANOVA: BS, $P < 0.001$, WS, $P = 0.007$; IA, $P = 0.008$. (c) ANOVA: BS, $P < 0.001$; WS, $P = 0.84$; IA, $P < 0.001$.

approximate daily dose 0.25 mg kg^{-1} body weight. This dose of ramipril is submaximal antihypertensive, but exceeds the threshold antihypertensive dose by 2.5 fold (Unger *et al.*, 1984a,b). In addition, felodipine solution (1.9 mg ml^{-1}, kindly donated by Dr Margareta Nordlander, MD, from Astra Hässle AB, Mölndal, Sweden) was given subcutaneously as a continuous infusion (2.36 μl h^{-1} corresponding to approximate daily dose of 0.4 mg kg^{-1}) by using osmotic minipumps (Alzet model 2ML4, Alza Corporation, CA, U.S.A.). The selection of the dose of felodipine was based on our previous studies in SHR-SP (stroke-prone) which showed that felodipine, at doses of 6 mg kg^{-1}, s.c., and 1.2 mg kg^{-1}, s.c., caused normotension and produced a dose-dependent increase in the heart rate (Mervaala *et al.*, 1994b; 1997b).

To allow infusion of felodipine a small incision was made in the skin between the scapulae under ether anaesthesia and an osmotic minipump filled with felodipine solution was implanted. Sham-operated animals were subjected to the same procedure but the implanted pump was filled with 154 mM NaCl. Two sutures were put on the subcutanous space and the skin was closed with three metallic surgical clips. The rats were allowed to recover for 24 h in single cages after the surgical procedure.

Systolic blood pressure and heart rate were measured weekly by using a tail cuff blood pressure analyser (Apollo-2AB Blood Pressure Analyzer, Model 179-2AB, IITC Life Science, Woodland Hills, CA, U.S.A.). The analogue signals of systolic blood pressure and heart rate were automatically converted to digital values by an online microprocessor. Before the measurements the rats were warmed for 10–15 min at 28°C to make the pulsations of the tail artery detectable. Values for systolic blood pressure and heart rate were obtained by averaging readings from three to five measurements. Body weights were measured weekly during the experimental period. During the fourth week of the experiment the rats were housed individually in metabolic cages where they had free access to tap water and chow. Food consumption was recorded and urine was collected over a 24 h period. Urine volumes were measured and samples stored at −80°C until the biochemical determinations were performed. The consumption of chow and tap water was measured by weighing the chow and water bottles, respectively. At the end of the experimental period of 4 weeks the animals were decapitated and exsanguinated after they had been fasted over night. The first 3 ml of blood were taken into chilled polyethylene tubes on ice with EDTA (4.5 mM) as anticoagulant and was used for the measurements of blood glucose and plasma renin activity. Then blood was collected into glass tubes without an anticoagulant, and was used for serum aldosterone and insulin determinations. Blood samples were centrifuged (1800 × *g*) for 15 min at 4°C. One hundred microlitres of blood were required for glucose measurement, 200 μl of plasma for renin activity, 400 μl of plasma for insulin determination, and 400 μl of serum for aldosterone and insulin determinations. The heart was excised, great vessels, atria and the free wall of the right ventricle were dissected and the left ventricular mass was measured. The left ventricular wet weight-to-body weight ratio was calculated as an index of left ventricular hypertrophy. The kidneys were washed with ice-cold saline and weighed. The left + right kidney weight-to-body weight ratio was calculated as an index of renal hypertrophy.

For measurements of vascular responses, the superior mesenteric artery was carefully excised and cleaned of the adherent connective tissue. A modification of the method described by Pörsti *et al.* (1991) was applied. Two successive

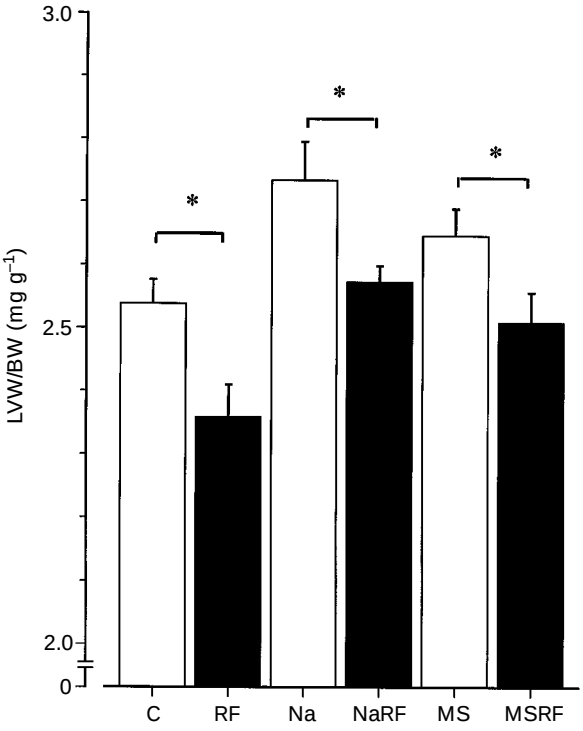

Figure 2 Left ventricular hypertrophy, expressed as left ventricular wet weight-to-body weight ratio (LVW/BW, mg kg^{-1}), of spontaneously hypertensive rats after four weeks on different diet and drug regimens. C, control group; RF, ramipril + felodipine group on control diet; Na, common salt group; NaRF, ramipril + felodipine group on common salt enriched diet; MS, mineral salt group; MSRF, ramipril + felodipine group on mineral salt enriched diet. The combination of ramipril and felodipine prevented the development of left ventricular hypertrophy irrespective of the diet. The results are expressed as means ± s.e.mean, $n = 10$ in each group. *Indicates $P < 0.05$ as compared to corresponding control group.

Table 2 Heart rate, body weight and kidney weight in different experimental groups at the end of the four-week study

Variable	*C*	*RF*	*Na*	*Na + RF*	*MS*	*MS + RF*
Heart rate (bpm)	375 ± 6	375 ± 7	384 ± 6	386 ± 5	378 ± 6	381 ± 6
Body weight (g)	334 ± 8	329 ± 3	328 ± 10	324 ± 6	318 ± 7	316 ± 9
Left + right kidney wet weight-to-body weight ratio (mg g^{-1})	6.28 ± 0.09	6.30 ± 0.10	6.80 ± 0.14[a]	6.74 ± 0.09	6.49 ± 0.10	6.55 ± 0.09

C, control group; RF, ramipril + felodipine group on control diet; Na, common salt group; Na + RF, ramipril + felodipine group on the common salt enriched diet; MS, mineral salt group; MS + RF, ramipril + felodipine group on the mineral salt enriched diet. [a]$P < 0.05$ as compared to control group. Values are mean + s.e.mean, $n = 10$ in each group.

sections (3 mm in length) of the mesenteric artery, 3 mm distally from the mesenteric artery-aorta junction, were used.

Mesenteric arterial responses in vitro

The endothelium-intact mesenteric arterial ring was placed between stainless steel hooks and mounted in an organ bath chamber in physiological salt solution (pH 7.4) of the following composition (mM): NaCl 119.0, $NaHCO_3$ 25.0, glucose 11.1, $CaCl_2$ 1.6, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2 and aerated with 95% O_2 and 5% CO_2. The ring was equilibrated for 20 min at $+37^{\circ}C$ with a resting tension of 1.5 g. The force of contraction was measured with an isometric force-displacement transducer and registered on a polygraph (FTO3C transducer, Model 7C8 Polygraph; Grass Instrument Co., Quincy, MA, U.S.A.). The presence of intact endothelium in the vascular preparations was confirmed by observing the relaxation response to 1 μM acetylcholine (ACh) in rings precontracted by 1 μM noradrenaline (NA). Cumulative concentration-contractile response curves to NA and potassium chloride (KCl), and cumulative concentration-relaxation response curves to ACh and sodium nitroprusside (SNP) were determined as described by Kähönen *et al.* (1993). The NA- and KCl-induced contractile responses were expressed in g and as percentage of the maximal response. The EC_{50} values for NA and KCl contraction in each ring were calculated as percentage of the maximal response, and for ACh and SNP relaxation as percentage of 1 μM NA-induced precontraction.

Hormonal and biochemical determinations

Plasma renin activity was determined by using a radioimmunoassay of angiotensin I (Medix Biochemica, Kauniainen, Finland). Serum aldosterone was determined by using a solid-phase radioimmunoassay specific for aldosterone (Diagnostic Products Corporation, Los Angeles, CA, U.S.A.). Serum insulin was determined by a radioimmunoassay (Incstar Corp., Stillwater, MO, U.S.A.). Fasting blood glucose was measured photometrically (Refloflux S, Boehringer Mannheim, Mannheim, Germany). Total protein concentration of urine was determined by the method of Lowry *et al.* (1951) after precipitation with 10% trichloroacetic acid. The concentrations of the elements sodium, potassium, phosphorus, magnesium and calcium in urine were determined by using Baird PS-4 inductively-coupled plasma emission spectrometer (Baird Co, Bedford, MA, U.S.A.) as described in detail elsewhere (Laakso *et al.*, 1991).

Drugs

The following drugs were used: ramipril, felodipine (Astra Hässle AB, Mölndal, Sweden), acetylcholine chloride (ACh), noradrenaline bitartrate (Sigma Chemical Co., St. Louis, MO, U.S.A.), sodium nitroprusside (SNP; F. Hoffman-La Roche AG, Basel, Switzerland). The stock solutions of the compounds used in *in vitro* studies were dissolved in distilled water. All solutions were freshly prepared before daily use and protected from light.

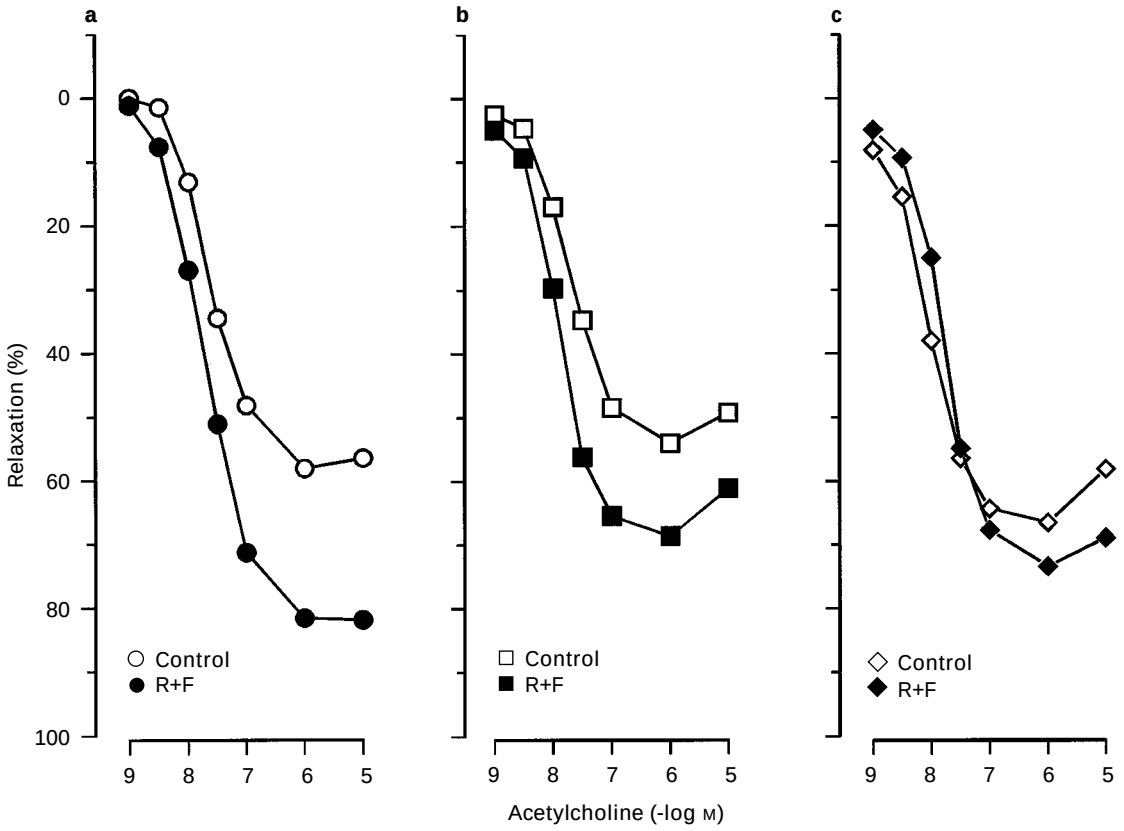

Figure 3 Relaxation responses to acetylcholine (ACh) of isolated endothelium-intact mesenteric arterial rings precontracted with noradrenaline from SHR after four weeks on different diet and drug regimens: (a) control diet, (b) common salt diet and (c) mineral salt diet. The combination of ramipril and felodipine (R + F) improved the vascular relaxation responses to ACh in endothelium-intact mesenteric arterial rings during control and common salt enriched diets. The results are expressed as means, $n = 10$ in each group. ANOVA, analysis of variance; BS, between-subjects effect; WS, within-subject effect; IA, time-group interaction. (a) ANOVA: BS, $P = 0.04$; WS, $P < 0.0001$; IA, $P = 0.09$. (b) ANOVA: BS, $P = 0.004$; WS, $P < 0.0001$; IA, $P = 0.009$; ANOVA: BS, $P = 0.92$; WS, $P < 0.001$; IA, $P = 0.04$.

Statistical analysis

Statistical analyses were carried out by one-way analysis of variance (ANOVA) followed by Tukey's test. Data for multiple observations over time were analysed by two-way ANOVA with repeated measures for overall treatment effect, and Tukey's test was used for multiple paired comparisons of treatment groups at different times. The area under the curve (AUC) was calculated mathematically by the method outlined by Matthews *et al.* (1990). To test differences between the drug-treated group and corresponding control group unpaired t test was used. Differences between the means that had $P<0.05$ were considered significant. The data were analysed by use of SYSTAT statistical software (SYSTAT Inc, Evanston, IL, U.S.A.). The results are presented as means $\pm$ s.e.mean.

Results

Blood pressure, heart rate and left ventricular hypertrophy

The combination of ramipril and felodipine decreased systolic blood pressures measured during the low sodium control diet by 25 mmHg (from 201 ± 3 to 173 ± 3 mmHg) ($P<0.001$) (Figure 1). Although the drug combination also had a good antihypertensive effect during the common salt-enriched diet, the systolic blood pressure (191 ± 4 mmHg) remained higher than in the ramipril + felodipine-treated SHR group that received the control diet (173 ± 3 mmHg) ($P<0.05$) (Figure 1). During the mineral salt enriched diet the combination of ramipril and felodipine lowered systolic blood pressure to the same extent and to the same level as during the control diet (Figure 1). At the end of the experimental period, the systolic blood pressure of the ramipril + felodipine-treated SHR that received the mineral salt-enriched diet (178 ± 3 mmHg) was lower than that of ramipril + felodipine-treated SHR receiving the common diet ($P<0.05$). The drug combination did not significantly affect the heart rate irrespective of the diet (Table 2).

The combination of ramipril and felodipine prevented the development of left ventricular hypertrophy (LVH) irrespective of the diet (Figure 2).

The common salt-enriched diet alone accelerated the development of both hypertension and left ventricular hypertrophy in SHR as compared to SHR group on control diet (Figures 1 and 2) (ANOVA $P<0.001$; $P<0.05$). By contrast, the mineral salt-enriched diet neither raised systolic blood pressure nor induced LVH as compared to the SHR control group (Figures 1 and 2). Neither common salt nor mineral salt significantly affected the heart rate (Table 2).

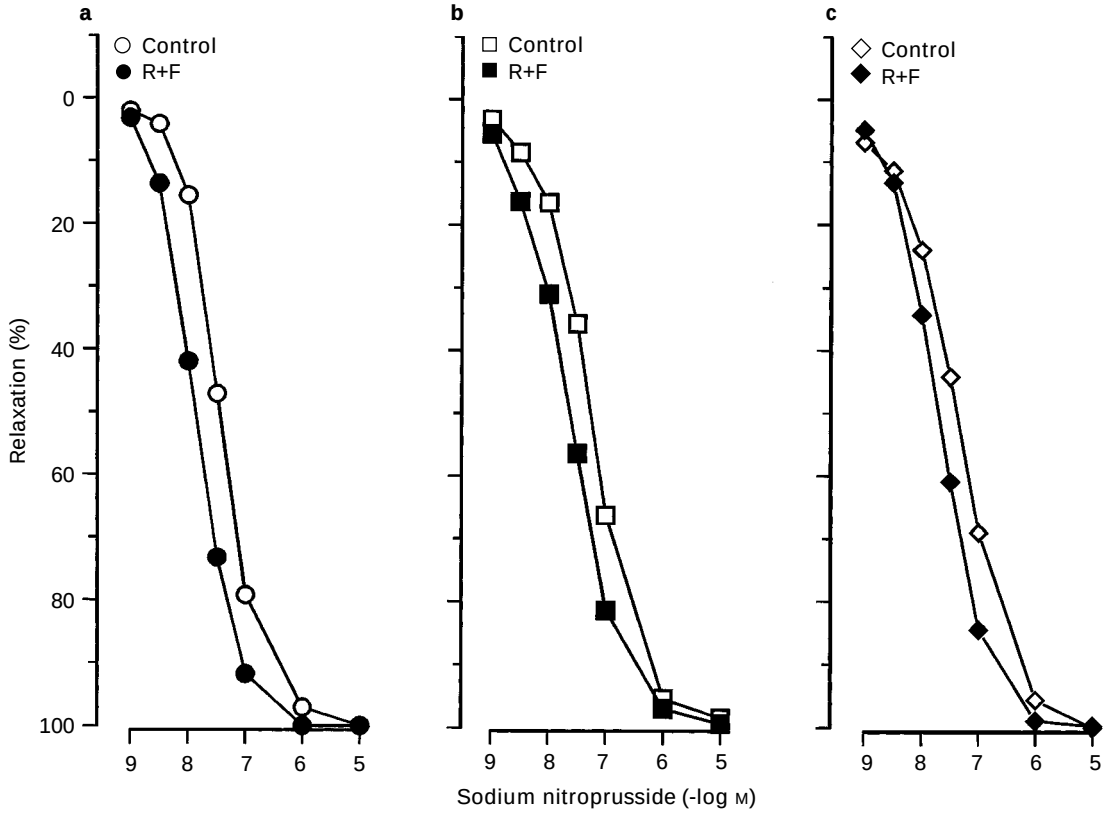

Figure 4 Relaxation responses to sodium nitroprusside (SNP) of isolated endothelium-intact mesenteric arterial rings precontracted with noradrenaline from SHR after four weeks on different diet and drug regimens: (a) control diet, (b) common salt diet and (c) mineral salt diet. The combination of ramipril and felodipine (R + F) improved the vascular relaxation responses to SNP in endothelium-intact mesenteric arterial rings during control and common salt enriched diets. The results are expressed as means, $n=10$ in each group. ANOVA, analysis of variance; BS, between-subjects effect; WS, within-subject effect; IA, time-group interaction. (a) ANOVA: BS, $P=0.001$; WS, $P<0.001$; IA, $P<0.001$. (b) ANOVA: BS, $P=0.002$; WS, $P<0.001$; IA, $P<0.001$. ANOVA: BS, $P=0.15$; WS, $P<0.001$; IA, $P=0.004$.

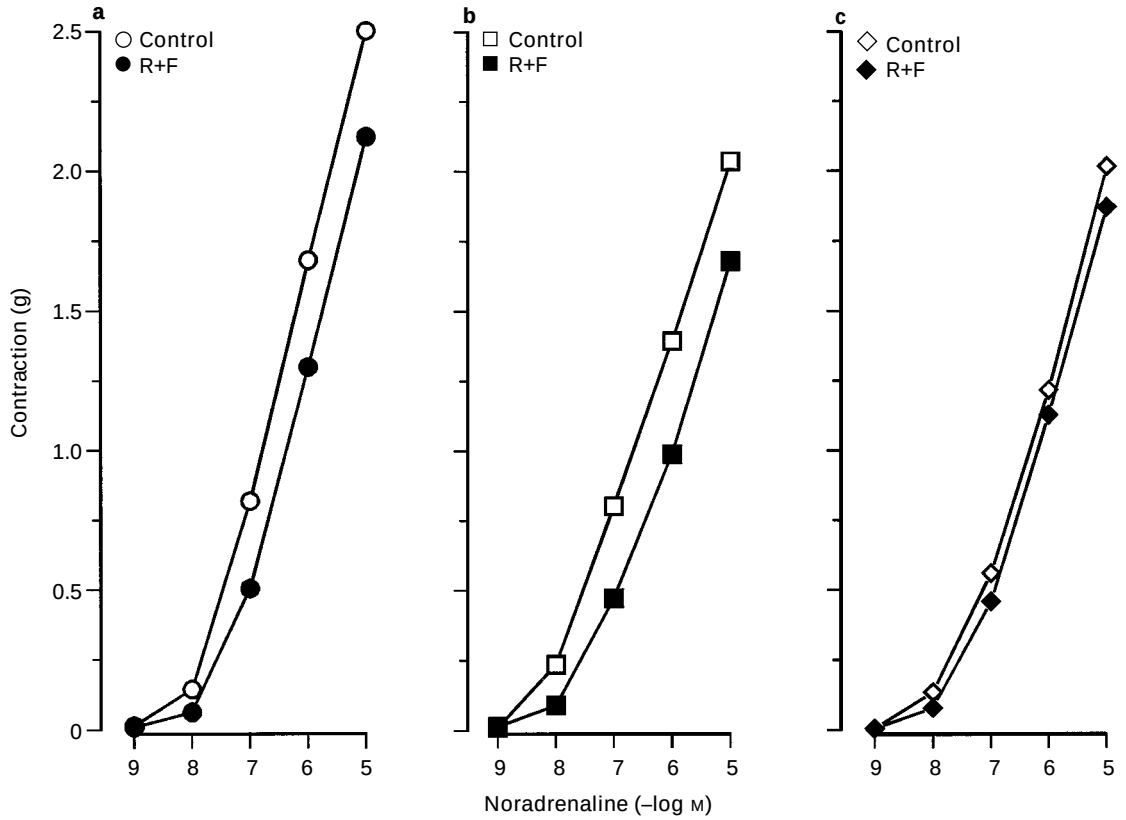

Figure 5 Contractile responses to noradrenaline of isolated endothelium-intact mesenteric arterial rings from SHR after four weeks on different diet and drug regimens: (a) control diet, (b) common salt diet and (c) mineral salt diet. The combination of ramipril and felodipine (R + F) antagonized the contractile responses to noradrenaline during common salt enriched diet. The results are expressed as means, $n = 10$ in each group. ANOVA, analysis of variance; BS, between-subjects effect; WS, within-subject effect; IA, time-group interaction. (a) ANOVA: BS, $P = 0.08$; WS, $P < 0.001$; IA, $P = 0.10$. (b) ANOVA: BS, $P = 0.005$; WS, $P < 0.001$; IA, $P = 0.01$. (c) ANOVA: BS, $P = 0.30$; WS, $P < 0.001$; IA, $P = 0.80$.

Mesenteric artery responses in vitro

The combination of ramipril and felodipine markedly improved the vascular relaxation responses to ACh and SNP during both the control diet and the common salt-enriched diet (Figures 3 and 4). The mineral salt-enriched diet slightly, but significantly, improved the vascular relaxation responses to ACh at concentrations 0.1 μM and 1.0 μM as compared to common salt-enriched diet ($P < 0.05$) (Figure 3). Neither of the salt diets significantly affected the vascular relaxation responses to SNP (Figure 4).

The combination of ramipril and felodipine attenuated the concentration-dependent contractile responses to NA during the common salt-enriched diet, but not during the control or mineral salt enriched diets (Figure 5). Replacement of common salt in the diet by mineral salt reduced the sensitivity of the mesenteric arterial rings to NA (ANOVA, $P = 0.009$, EC_{50} 0.35 ± 0.05 μM in the control group, 0.23 ± 0.03 μM in the common salt group and 0.48 ± 0.08 μM in the mineral salt group; common salt group *versus* mineral salt group, $P < 0.05$). Neither drug treatment nor salt supplementations significantly affected the concentration-dependent contractile responses to KCl (Figure 6).

Factors associated with renal functions

The common salt-enriched diet produced renal hypertrophy as indicated by a marked increase in the left + right kidney weight-to-body weight ratio (Table 2). The increase in the renal hypertrophy-index induced by mineral salt-enriched diet did not quite reach statistical significance (Table 2). The common salt-induced changes in the renal hypertrophy were not affected by the combination of ramipril and felodipine (Table 2).

Both common salt and the mineral salt increased 24 h urinary excretion of protein (Table 3). The drug combination had no effect on 24 h urinary protein excretion (Table 3).

The common salt-enriched diet decreased plasma renin activity, and both common salt- and mineral salt-enriched diets decreased serum aldosterone level (Table 3). The drug combination increased plasma renin activity, without changing serum aldosterone level, irrespective of the diet (Table 3).

Both the common salt- and the mineral salt-enriched diet increased the water consumption and urine volume 3 fold. The daily urinary sodium excretion in the common salt and mineral salt groups was approximately 15 to 16 fold that of the control group. The excretion of magnesium was 2 fold greater in the common salt group without drug treatment and 3.5 fold greater in the mineral salt group than in the control group (Table 4). Mineral salt increased the urinary excretion of potassium by 4 fold. The urinary excretion of calcium was increased 10 to 15 fold by both common salt and mineral salt. Ramipril and felodipine in combination increased urine volume during the common salt-enriched diet and urinary sodium excretion during the control diet (Table 4).

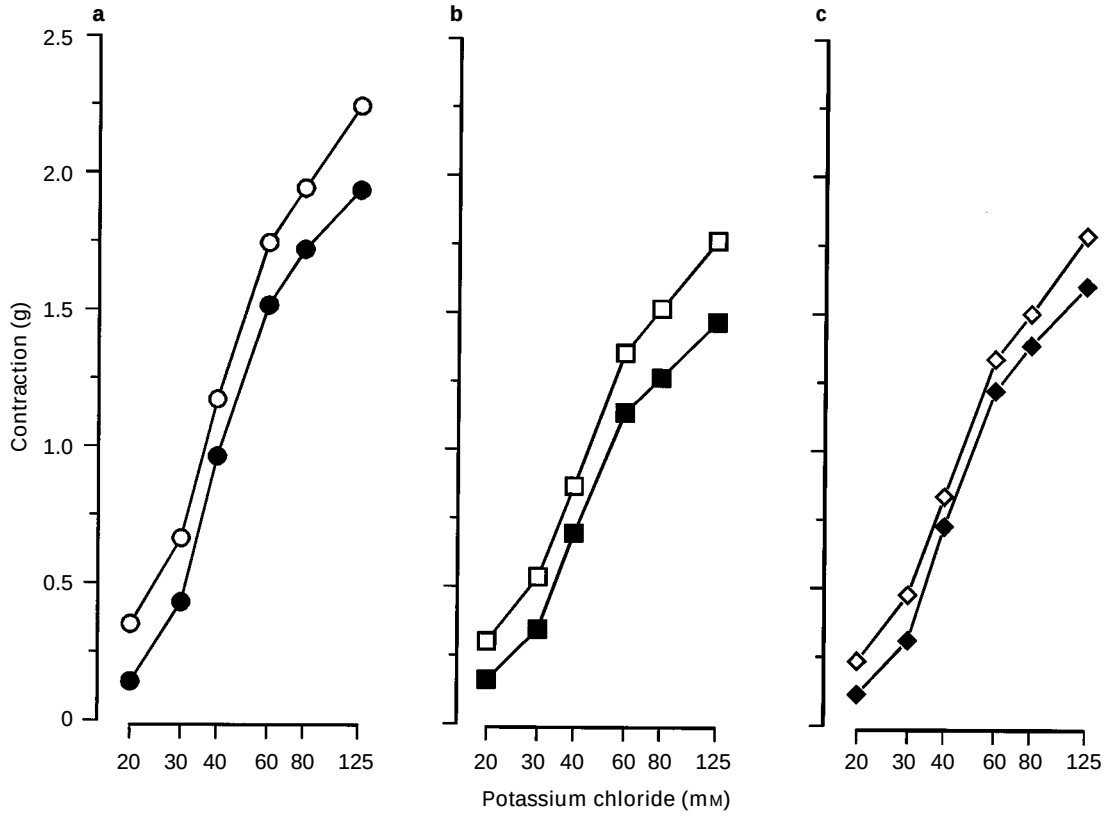

Figure 6 Contractile responses to potassium chloride of isolated endothelium-intact mesenteric arterial rings from SHR after four weeks on different diet and drug regimens: (a) Control diet, (b) common salt diet and (c) mineral salt diet. Neither the combination of ramipril and felodipine (R + F) nor salt supplementations significantly affected the contractile responses to potassium chloride. The results are expressed as means, $n = 10$ in each group. ANOVA, analysis of variance; BS, between-subjects effect; WS, within-subject effect; IA, time-group interaction. (a) ANOVA: BS, $P = 0.19$; WS, $P < 0.001$; IA, 0.98. (b) ANOVA: BS, $P = 0.08$; WS, $P < 0.001$; IA, 0.65. (c) ANOVA: BS, $P = 0.20$; WS, $P < 0.001$; IA, 0.98.

Table 3 Hormonal and biochemical variables in different experimental groups at the end of the four-week study

Variable	*C*	*RF*	*Na*	*Na + RF*	*MS*	*MS + RF*
Plasma renin activity (ng AI ml^{-1} h^{-1})	5.3 ± 0.5	16.7 ± 1.7[a]	2.3 ± 0.6[b]	4.8 ± 0.9[a]	5.1 ± 0.6	9.5 ± 1.4[a]
Serum aldosterone (ng ml^{-1})	215.5 ± 34.8	289.1 ± 41.5	113.4 ± 8.7[c]	134.1 ± 18.4	121.0 ± 14.6[c]	102.5 ± 17.9
Serum insulin (iu ml^{-1})	6.1 ± 0.5	7.3 ± 0.3	5.5 ± 0.5	6.2 ± 0.3	5.9 ± 0.8	5.9 ± 0.2
Blood glucose (mmol l^{-1})	4.2 ± 0.2	4.2 ± 0.2	4.1 ± 0.1	4.0 ± 0.1	4.1 ± 0.2	3.9 ± 0.1
Urinary protein excretion (mg d^{-1})	15.7 ± 0.8	15.6 ± 1.9	21.5 ± 2.0[c]	21.5 ± 1.5	22.7 ± 2.1[c]	26.7 ± 5.0

C, control group; RF, ramipril + felodipine group on control diet; Na, common salt group; Na + RF, ramipril + felodipine group on the common salt enriched diet; MS, mineral salt group; MS + RF, ramipril + felodipine group on the mineral salt enriched diet. [a]$P < 0.05$ as compared to corresponding group without drug treatment; [b]$P < 0.05$ as compared to control group and mineral salt group; [c]$P < 0.05$ as compared to control group. Values are means ± s.e.mean, $n = 10$ in each group.

Table 4 Twenty four hour food and water consumption, urine volume and urinary excretion rates of various mineral elements in different experimental groups at the end of the four-week study

Variable	*C*	*RF*	*Na*	*Na + RF*	*MS*	*MS + RF*
Food consumption (g d^{-1})	13.5 ± 0.6	17.1 ± 0.8[b]	17.4 ± 0.7[a]	18.2 ± 0.6	17.9 ± 0.7[a]	18.7 ± 0.9
Water consumption (ml d^{-1})	22.0 ± 2.0	19.9 ± 1.5	59.0 ± 3.4[a]	64.4 ± 3.1	75.0 ± 3.8[a,c]	72.8 ± 4.5
Urine volume (ml d^{-1})	16.5 ± 1.7	18.4 ± 1.6	50.8 ± 2.6[a]	61.5 ± 3.2[b]	67.3 ± 3.0[a,c]	69.3 ± 3.1
Sodium (mmol d^{-1})	1.1 ± 0.1	1.9 ± 0.2[b]	15.2 ± 0.6[a]	16.9 ± 1.1	16.3 ± 1.2[a]	16.6 ± 0.6
Potassium (mmol d^{-1})	2.1 ± 0.1	2.4 ± 0.1	2.2 ± 0.1	2.7 ± 0.2[b]	8.4 ± 0.5[a,c]	8.7 ± 0.2
Magnesium (μmol d^{-1})	131 ± 20	162 ± 17	226 ± 33[a]	218 ± 42	444 ± 76[a,c]	475 ± 45
Calcium (μmol d^{-1})	15 ± 2	24 ± 5	165 ± 14[a]	181 ± 17	227 ± 25[a,c]	219 ± 13

C, control group; RF, ramipril + felodipine group on control diet; Na, common salt group; Na + RF, ramipril + felodipine group on the common salt enriched diet; MS, mineral salt group; MS + RF, ramipril + felodipine group in the mineral salt enriched diet. [a]$P < 0.05$ as compared to control group; [b]$P < 0.05$ as compared to corresponding group without drug treatment; [c]$P < 0.05$ as compared to common salt group. Values are means ± s.e.mean, $n = 10$ in each group.

Blood glucose and serum insulin

Neither common salt, mineral salt nor the drug treatment affected fasting blood glucose or serum insulin levels (Table 3).

Discussion

The main finding of the present study conducted in spontaneously hypertensive rats (SHR) was that a low-dose combination of the angiotensin converting enzyme inhibitor, ramipril, and a calcium channel blocker, felodipine, maintained its antihypertensive effect in face of a high intake of sodium. When estimated as the absolute decrease in systolic blood pressure in mmHg, a four-week treatment with the drug combination lowered systolic blood pressure in SHR equally during the common salt-enriched and the low-sodium control diets. However, it should be emphasized, that even though the combination of ramipril and felodipine decreased systolic blood pressure very effectively in face of a high salt intake, the systolic blood pressure of the drug-treated SHR receiving the low-sodium control diet was still significantly lower than that of drug-treated SHR on the common salt-enriched diet. The present study also showed that the antihypertensive effect of the drug combination can be markedly improved by replacing common salt in the diet by a potassium- and magnesium-enriched mineral salt.

To study further the cardiovascular effects of the combination of ramipril and felodipine during different salt diets, responses of mesenteric arterial rings *in vitro* were examined at the end of the follow-up period. The present study showed that the vascular relaxation responses to ACh and SNP of isolated endothelium-intact mesenteric arterial rings were markedly improved by treatment of the donor animals with the drug combination, not only during a relatively low-sodium diet but also in face of a high intake of common salt. This suggests that a part of the beneficial cardiovascular effects of the combination of ramipril and felodipine is mediated by improved endothelium-dependent and endothelium-independent vascular relaxations. Our findings are consistent with the idea that long-term treatment with ACE inhibitors restores the impaired endothelial function of SHR mainly by preventing the degradation of bradykinin liberated from the endothelial cells (Linz *et al*., 1992; 1995; Arvola *et al*., 1993; Mervaala *et al*., 1994a). The present study also revealed that the vascular relaxation responses to ACh of isolated endothelium-intact mesenteric arterial rings can be improved by potassium- and magnesium supplementation of the diet of the animals, suggesting that the beneficial cardiovascular effects of mineral salt are also mediated, at least in part, by improved endothelium-dependent vascular relaxations.

The combination of ramipril and felodipine attenuated the concentration-dependent vascular contractile responses to noradrenaline in SHR during high sodium intake. This finding is in good agreement with data from previous studies showing that, long-term treatment with ACE inhibitors decreases the α-adrenoceptor-mediated vascular responses in SHR (Arvola *et al*., 1993; Kähönen *et al*., 1995), and supports the idea that the NaCl-induced hypertension in SHR is partly mediated by increased activity of the sympathetic nervous system (Oparil *et al*., 1988)

Kidneys play a central role in the regulation of blood pressure as well as in electrolyte and fluid balance. It has been shown previously that, in SHR, the renal pressure-natriuresis response is blunted and reset toward higher pressures (Roman & Cowley, 1985). The present study was able to confirm previous findings (Aoki *et al*., 1972; Oparil *et al*., 1988; Mervaala *et al*., 1992; 1994a, b, c, d) that the addition of common salt to a relatively low-sodium control diet markedly accelerated the development of hypertension in SHR. Our findings of the suppression of the renin-angiotensin-aldosterone system, as well as the increase in the renal hypertrophy-index by the high sodium intake, suggest that the sodium-induced hypertension in SHR is mediated, at least in part, by increased volume load. Interestingly the high sodium enriched diet also induced mild renal damage in the present study as indicated by an increase in the urinary protein excretion.

In the present study, the combination of ramipril and felodipine markedly improved the renal pressure-natriuresis mechanism in SHR irrespective of the diet. Also the results showed that the urinary excretion of both sodium and water in the drug-treated SHR were the same or even greater than those of the corresponding SHR without the drug treatment, although the systolic blood pressure of the drug-treated SHR was significantly lower. In SHR receiving the high sodium enriched diet, the natriuretic effect of felodipine (DiBona, 1985; Mervaala *et al*., 1994b; 1997b) is likely to explain the improvement of the pressure-natriuresis response by the drug treatment. This suggestion is supported by our recent finding that the cardiovascular effects of even a maximal antihypertensive dose of ramipril (3 mg kg^{-1}) is almost completely abolished in stroke-prone SHR (SHRSP) when sodium in the chow is increased to the same level as used in the present study (Mervaala *et al*., 1994d). However, it should be emphasized, that, in the present study, the drug combination was not able to normalize completely the renal functions in SHR during high intake of sodium, as indicated by moderate increases in systolic blood pressure, renal hypertrophy and urinary protein excretion.

The antihypertensive effect of the drug combination was improved when common salt in the diet was replaced by the mineral salt and the intake of potassium and magnesium were increased by 200% and 50%, respectively. We have previously shown that the beneficial effect of the mineral salt on blood pressure is mediated, to a great extent, by the prevention of sodium chloride-induced volume overload (Mervaala *et al*., 1992). Consistent with this finding, mineral salt did not significantly induce renal hypertrophy in the present study.

High blood pressure is one of the most powerful determinants of the development of left ventricular hypertrophy (LVH) (Frochlich *et al*., 1992). Therefore, in the present study, the marked antihypertensive effect of the low-dose drug combination is likely to explain to a great extent the prevention of LVH. Even though previous studies have demonstrated that high-dose felodipine treatment exerts only a weak influence on LVH because of the drug-induced activation of the sympathetic nervous system (Nyborg & Mulvany, 1985; Leenen & Holliwell, 1992; Mervaala *et al*., 1994b), the low-dose felodipine treatment, at least in combination with ramipril, effectively prevented the development of LVH. However, we have recently found that the beneficial effects of a submaximal antihypertensive dose of ramipril on LVH was more prominent than that of felodipine (Mervaala *et al*., 1997a). The present study also confirmed our recent finding (Mervaala *et al*., 1997b) that, felodipine, at the dose used in the present study, does not significantly affect the activity of the sympathetic nervous system, as indicated by lack of effect of the combination therapy on heart rate. It is also noteworthy that, unlike high-dose ramipril (Mervaala *et al*., 1994d) or high-dose metoprolol treatment (Mervaala *et al*., 1994c), the low-dose combination of ramipril and felodipine also

effectively prevented the development of LVH during high salt intake.

It has been shown previously that SHR have impaired glucose tolerance and insulin resistance (Reaven & Chang, 1991). In the present study neither common salt, mineral salt or the drug treatment affected fasting blood glucose or serum insulin levels, indicating the lack of any significant effect on glucose metabolism in SHR.

In conclusion, our findings suggest that the beneficial cardiovascular effects of the low-dose combination of ramipril and felodipine in SHR are maintained during high salt intake. However, salt restriction, or replacement of common salt in the diet by a potassium- and magnesium-enriched mineral salt enhances the cardiovascular effects of the drug combination. In the face of a high intake of sodium, a part of the beneficial cardiovascular effects of the drug combination is apparently mediated by improved endothelium-dependent and endothelium-independent vascular relaxation responses and by attenuated α-adrenoceptor-mediated vascular contractile responses.

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References

AOKI, K., YAMORI, Y., OOSHIMA, A. & OKAMOTO, K. (1972). Effects of high or low sodium intake in spontaneously hypertensive rats. *Jpn. Circ. J.*, **36,** 539 – 545.

ARVOLA, P., RUSKOAHO, H., WUORELA, H., PEKKI, A. & VAPAATALO, H. (1993). Quinapril treatment and arterial smooth muscle responses in spontaneously hypertensive rats. *Br. J. Pharmacol.*, **108,** 980 – 990.

DEWARDENER, H.E. (1990a). The primary role of the kidney and salt intake in the aethiology of essential hypertension: part I. *Clin. Sci.*, **79,** 193 – 200.

DEWARDENER, H.E. (1990b). The primary role of the kidney and salt intake in the aethiology of essential hypertension: part II. *Clin. Sci.*, **79,** 289 – 296.

DIBONA, G. (1985). Effects of felodipine on renal function in animals. *Drugs*, **29,** (suppl 2), 168 – 175.

ELLIOT, P., STAMLER, J., NICHOLS, R., DYER, A.R., STAMLER, R., KESTELOOT, H. & MARMOT, M. (1996). Intersalt revisited: further analyses of 24 hour sodium excretion and blood pressure within and across populations. *Br. Med. J.*, **312,** 1249 – 1253.

FROCHLICH, E., APSTEIN, C., CHOBANIAN, A., DEVEREUX, R., DUSTAN, H., DZAU, V., FAUAD-TARAZI, F., HORAN, M., MARCUS, M., MASSIE, B., PFEFER, M., RE, R., ROCCELLA, E., SAVAGE, D. & SHUB, C. (1993). The heart in hypertension. *N. Engl. J. Med.*, **327,** 998 – 1008.

KÄHÖNEN, M., ARVOLA, P., VAPAATALO, H. & PÖRSTI, I. (1993). Comparison of cumulative and non-cumulative administration of vasoactive agents in arterial smooth muscle responses in vitro. *Pharmacol. Toxicol.*, **73,** 142 – 145.

KÄHÖNEN, M., MÄKYNEN, H., WU, X., ARVOLA, P. & PÖRSTI, I. (1995). Endothelial function in spontaneously hypertensive rats: influence of quinapril treatment. *Br. J. Pharmacol.*, **115,** 859 – 867.

LAAKSO, J.T., TIKKANEN, H. & MICHELSSON, J.-E. (1991). Element concentrations in normal and immobilization-induced necrotic rabbit muscles. *Trace Elem. Med.*, **8,** 34 – 42.

LEENEN, F.H. & HOLLIWELL, D.L. (1992). Antihypertensive effect of felodipine associated with persistent sympathetic activation and minimal regression of left ventricular hypertrophy. *Am. J. Cardiol.*, **69,** 639 – 645.

LINZ, W., SCHAPER, J., WIEMER, G., ALBUS, U. & SCHÖLKENS, B.A. (1992). Ramipril prevents left ventricular hypertrophy with myocardial fibrosis without blood pressure reduction: a one year study in rats. *Br. J. Pharmacol.* **107,** 970 – 975.

LINZ, W., WIEMER, G., GOHLKE, P., UNGER, T. & SCHÖLKENS, B. (1995). Contribution of kinins to the cardiovascular actions of angiotensin-converting enzyme inhibitors. *Pharmacol. Rev.*, **47,** 25 – 49.

LOWRY, O.H., ROSENBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, **193,** 265 – 275.

MACGREGOR, G.A., MARKANDU, N.D., SINGER, D.R., CAPPUCCIO, F.P. & SHORE, A.C. (1987). Moderate sodium restriction with angiotensin converting enzyme inhibitor in essential hypertension: a double blind study. *Br. Med. J.*, **294,** 531 – 534.

MATTHEWS, J.N.S., ALTMAN, D.G., CAMPBELL, M.J. & ROYSTON, P. (1990). Analysis of serial measurements in medical research. *Br. Med. J.*, **300,** 230 – 235.

MERVAALA, E.M.A., HIMBERG, J.-J., LAAKSO, J., TUOMAINEN, P. & KARPPANEN, H. (1992). Beneficial effects of a potassium- and magnesium-enriched salt alternative. *Hypertension*, **19,** 535 – 540.

MERVAALA, E.M.A., LAAKSO, J., HIMBERG, J.-J., KARPPANEN, H. (1994a). Replacement of regular salt by a novel salt alternative improves the cardiovascular effects of the ACE inhibitor enalapril. *Hypertens. Res.*, **17,** 59 – 69.

MERVAALA, E.M.A., LAAKSO, J. & KARPPANEN, H. (1994b). Cardiovascular effects of felodipine are not antagonized by dietary salt. *Eur. J. Pharmacol.*, **255,** 73 – 79.

MERVAALA, E.M.A., LAAKSO, J., VAPAATALO, H. & KARPPANEN, H. (1994c). Improvement of cardiovascular effects of metoprolol by replacement of common salt with a potassium- and magnesium-enriched salt alternative. *Br. J. Pharmacol.*, **112,** 640 – 648.

MERVAALA, E.M.A., PAAKKARI, I., LAAKSO, J., NEVALA, R., TERÄVÄINEN, T.-L., FYHRQUIST, F., VAPAATALO, H. & KARPPANEN, H. (1994d). Replacement of salt by a novel potassium- and magnesium-enriched salt alternative improves the cardiovascular effects of ramipril. *Br. J. Pharmacol.*, **111,** 1189 – 1197.

MERVAALA, E.M.A., TERÄVÄINEN, T.-L., MALMBERG, L., LAAKSO, J., PÖRSTI, I., VAPAATALO, H. & KARPPANEN, H. (1997a). Cardiovascular effects of a low-dose combination of ramipril and felodipine in the spontaneously hypertensive rats. *Br. J. Pharmacol.*, (in press).

MERVAALA, E.M.A., TERÄVÄINEN, T.-L., MALMBERG, L., LAAKSO, J., VAPAATALO, H. & KARPPANEN, H. (1997b). Cardiovascular and renal effects of the combination of felodipine and metoprolol during a high-salt and a moderate-salt diet. *Jpn. Circ. J.*, (in press).

NYBORG, N.C. & MULVANY, M.J. (1985). Lack of effect of antihypertensive treatment with felodipine on cardiovascular structure of young spontaneously hypertensive rats. *Cardiovasc. Res.*, **19,** 528 – 536.

OPARIL, S., MENG, Q.C., CHEN, Y.-F., YANG, R.-H., JIN, H. & WYSS, J.M. (1988). Genetic basis of NaCl-sensitive hypertension. *J. Cardiovasc. Pharmacol.*, **12,** S56 – S69.

PÖRSTI, I., ARVOLA, P., WUORELA, H., ILKKA, M., SÄYNÄVÄLAMMI, P., HUHTALA, H., METSÄ-KETELÄ, T. & VAPAATALO, H. (1991). Effects of a high calcium diet and deoxycorticosterone on vascular smooth muscle responses in spontaneously hypertensive rats. *J. Hypertens.*, **8,** 835 – 841.

REAVEN, G.M. & CHANG, H. (1991). Relationship between blood pressure, plasma insulin and triglyceride concentration, and insulin action in spontaneously hypertensive and Wistar-Kyoto rats. *Am. J. Hypertens.*, **4,** 34 – 38.

ROMAN, R.J. & COWLEY, A.W. Jr. (1985). Abnormal pressure-diuresis-natriuresis response in spontaneously hypertensive rats. *Am. J. Physiol.*, **248,** F199 – F205.

UNGER, T., FLECK, T., GANTEN, D., LANG, R.E. & RETTIG, R. (1984a). 2-[N-[(s)-1-ethoxycarbonyl-3-phenylpropyl-L-alanyl]-(1S.3S.5S)2-azabicyclo [3.3.0] octane-3-carboxylic acid (HOE 498): antihypertensive action and persistent inhibition of tissue converting enzyme activity in spontaneously hypertensive rats. *Drug. Res.*, **34,** 1426 – 1430.

UNGER, T., GANTEN, D., LANG, R.F. & SCHOLKENS, B.A. (1984b). Is tissue converting enzyme inhibition a determinant of the antihypertensive efficacy of converting enzyme inhibitors? Studies with two different compounds HOE 498 and MK 421 in spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol.*, **6,** 872.

WHO/INTERNATIONAL SOCIETY OF HYPERTENSION. (1993). Guidelines for the management of mild hypertension: memorandum from the World Health Organization/International Society of Hypertension meeting. *J. Hypertens.*, **11,** 905–918.

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