British Journal of Pharmacology (1997) 121, 503–510

Cardiovascular effects of a low-dose combination of ramipril and felodipine in spontaneously hypertensive rats

*,[1]Eero M.A. Mervaala, *,†Terttu-Liisa Teräväinen, *Lena Malmberg, *,‡Juha Laakso, *Heikki Vapaatalo & *Heikki Karppanen

*Institute of Biomedicine, Department of Pharmacology and Toxicology, P.O. BOX 8, FIN-00014 University of Helsinki, †Faculty of Veterinary Medicine, Department of Physiology, P.O. BOX 57, FIN-00014 University of Helsinki and ‡Mila Ltd., Sirrikuja 4 B, FIN-00940 Helsinki, Finland

1 Cardiovascular effects of submaximal antihypertensive doses of the angiotensin converting enzyme inhibitor, ramipril (0.25 mg kg^{-1} day^{-1} in the food), and the calcium channel blocker, felodipine (0.4 mg kg^{-1} day^{-1} subcutaneously by osmotic minipump), both alone and in combination, were examined in spontaneously hypertensive rats (SHR) in a four-week study.

2 Both ramipril and felodipine as monotherapy decreased systolic blood pressure. The antihypertensive effect of the drug combination was more than that of ramipril treatment alone, but not significantly better than that of felodipine monotherapy. Ramipril or felodipine treatments did not significantly affect the heart rate, either alone or in combination.

3 The beneficial effect of ramipril monotherapy on left ventricular hypertrophy was more prominent than that of felodipine. The cardioprotective effect of felodipine was improved when combined to ramipril. The systolic blood pressure at the end of the experimental period correlated only weakly with left ventricular hypertrophy.

4 Responses of mesenteric arterial rings *in vitro* were examined at the end of the four-week study. Ramipril and felodipine monotherapies as well as their combination markedly improved the endothelium-dependent vascular relaxation responses to acetylcholine. The combination of ramipril and felodipine slightly enhanced the endothelium-independent vascular relaxation responses to sodium nitroprusside. Ramipril treatment alone slightly diminished the vascular contractile responses to noradrenaline. Neither ramipril nor felodipine alone or in combination affected the vascular contractile responses to potassium chloride.

5 Ramipril treatment, both alone and in combination with felodipine, caused a three fold increase in plasma renin activity. Serum aldosterone, fasting blood glucose level, serum insulin and the 24 hour urinary excretions of sodium, potassium, magnesium, calcium, phosphorus or protein were not significantly affected by the drug treatments.

6 Our findings suggest that a better overall control of hypertension and end-organ damages, without an increase in adverse effects, can be achieved by the combination of submaximal antihypertensive doses of felodipine and ramipril than by monotherapy with either drug alone.

Keywords: Ramipril; felodipine; spontaneously hypertensive rat; left ventricular hypertrophy; arterial smooth muscle; renin; aldosterone

Introduction

It has been claimed that, at least in rats made hypertensive by aortic ligation, the angiotensin converting enzyme inhibitor (ACEI) ramipril is both an effective antihypertensive agent and has the ability to prevent the development of left ventricular hypertrophy (LVH) at doses so low that a fall in blood pressure is not produced (Linz *et al*., 1992; 1995). Early-onset treatment of spontaneously hypertensive rats (SHR) and stroke-prone SHR (SHRSP) with ramipril has also been shown to induce myocardial capillary growth and to improve the functional and metabolic status of the left ventricle even at nonantihypertensive doses (for a review, see Linz *et al*., 1995). The beneficial effects of ramipril as well as those of enalapril were almost completely abolished when sodium in the chow of SHRSP was increased to the high levels characteristic of human diets (Mervaala *et al*., 1994a,b). The interference of dietary sodium with the therapeutic effects of ACEIs has been documented even in human studies (MacGregor *et al*., 1987; Heeg *et al*., 1989).

Unlike ACEIs, felodipine, a dihydropyrine calcium channel blocker at high doses was able to lower blood pressure and prevent LVH effectively even in the presence of a high sodium intake (Mervaala *et al*., 1994c; 1997). Felodipine at the high effective doses causes tachycardia and oedema as adverse effects (DiBona, 1985; Nordlander *et al*., 1985; Todd & Faulds, 1992).

Felodipine exerts natriuretic effects (DiBona, 1985; Nordlander *et al*., 1985; Elmfeldt *et al*., 1992), which are likely to improve the therapeutic effects of ramipril (MacGregor *et al*., 1987; Gohlke & Unger, 1994). Ramipril, on the other hand, might decrease the activation of the sympathetic nervous system (Gohlke & Unger, 1994; review, Linz *et al*., 1995), caused by the vasodilator and blood pressure lowering effects of felodipine (Todd & Faulds, 1992). We therefore undertook this study to examine whether the therapeutic effects could be improved and, at the same time, the adverse effects diminished by simultaneous administration of submaximal antihypertensive doses of ramipril and felodipine. We also tried to elucidate the mechanisms of the observed effects by examining possible changes in vascular reactivity and a variety of hormonal and biochemical variables.

Methods

Experimental animals and diets

Forty eight-week old, inbred male spontaneously hypertensive rats (SHR) (Harlan Sprague Dawley, Indianapolis, IN,

[1] Author for correspondence.

U.S.A.) were used in the study. The rats were kept in groups of five in plastic cages (55 × 23 cm) in conventional conditions at a room temperature of 22–23°C with a controlled light/dark cycle (12/12 h). The arrival of the purchased animals was followed by an adaptation period of at least two weeks before commencement of the experiment. The animals received commercially available standard rat chow (Finnewos Aqua, Helsinki, Finland) containing 0.3% sodium, 0.8% potassium, 1.1% calcium, 0.8% phosphorus and 0.6% chloride (w/w) throughout the experimental period. Rats had free access to tap water and chow. The animals were assigned to one of four treatment groups that consisted of control, ramipril, felodipine and combination. Ramipril was added to the chow (3.5 mg ramipril kg^{-1} dry weight of the chow) to produce an approximate daily dose 0.25 mg kg^{-1} body weight. This dose of ramipril is submaximal as regards its antihypertensive effect, but exceeds the threshold antihypertensive dose by 2.5 fold (Unger *et al*., 1984a,b). Felodipine solution (1.9 mg kg^{-1}, kindly donated by Dr Margareta Nordlander, 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 osmotic minipumps (Alzet model 2ML4, Alza Corporation, CA, U.S.A.). This dose of felodipine was chosen based on our previous studies in SHRSP showing 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 heart rate (Mervaala *et al*., 1994c; 1997). A small incision was made in the skin between the scapulae under ether anaesthesia. Sham-operated animals were subjected to the same procedure with implantation of osmotic minipumps filled with 154 mM NaCl. 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 intake 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 the animals were decapitated and exsanguinated after fasting overnight. Blood samples were taken for blood glucose measurement, samples for plasma renin activity was taken into chilled tubes on ice with EDTA (4.5 mM) as anticoagulant, and those for serum aldosterone and insulin determinations into glass tubes without an anticoagulant. The heart was excised, great vessels, atria and the free wall of the right ventricle were

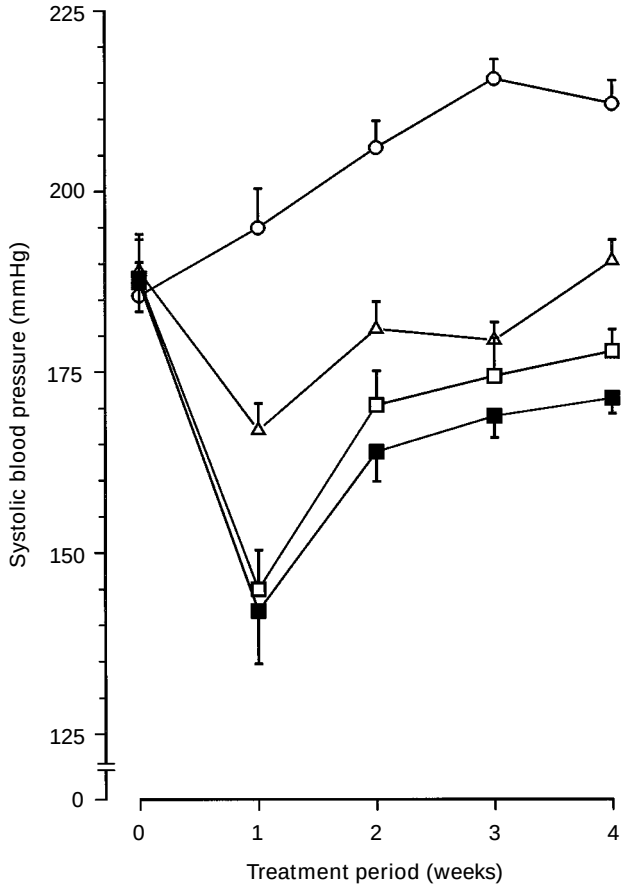

Figure 1 Blood pressure in spontaneously hypertensive rats during different drug regimens for four weeks ($n = 9–10$ in each group). (○) Control group; (□) felodipine group; (△) ramipril group; (■) ramipril + felodipine group. Ramipril and felodipine decreased blood pressure significantly ($P < 0.05$ as compared to control group). There was not any significant difference between the antihypertensive effect of ramipril and felodipine. The antihypertensive effect of the drug combination was more than that of ramipril treatment alone ($P < 0.05$), but not significantly better than that of felodipine monotherapy. The results are expressed as means and vertical lines show s.e.mean.

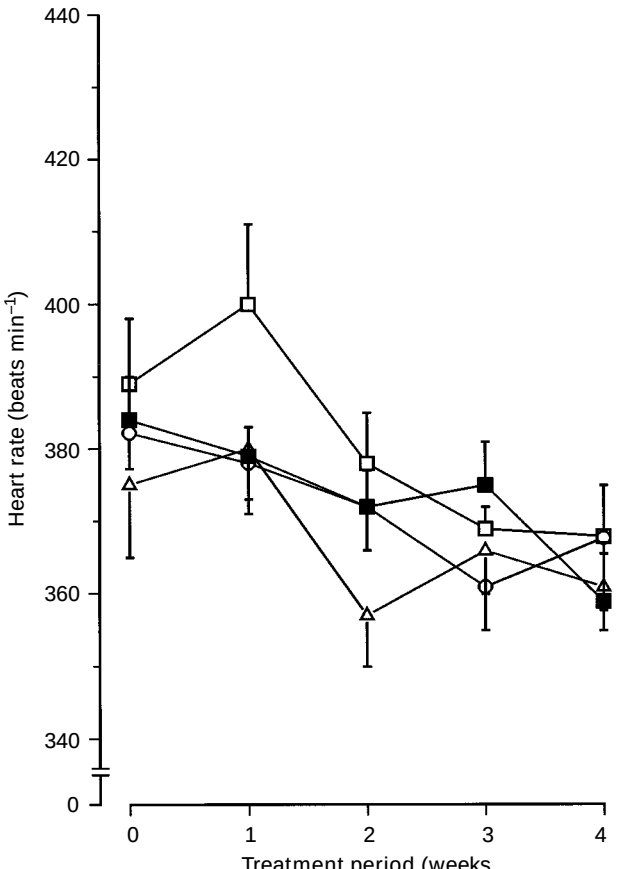

Figure 2 Heart rate of spontaneously hypertensive rats during different drug regimens for four weeks ($n = 9–10$ in each group). (○) Control group; (□) felodipine group; (△) ramipril group; (■) ramipril + felodipine group. Ramipril and felodipine monotherapies did not significantly affect the heart rate, either alone or in combination. The results are expressed as means and vertical lines show s.e.mean.

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.

For the measurements of vascular responses, the superior mesenteric artery was carefully excised and cleaned of adherent connective tissue. A modification of the method described by Pörsti *et al*. (1991) was applied. Two successive sections (3 mm in length) of the mesenteric artery, 3 mm distally from the mesenteric artery-aorta junction, were cut.

Mesenteric arterial responses in vitro

The 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°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). The concentration-contractile response curves to cumulative NA and potassium chloride (KCl), and the concentration-relaxation response curves to cumulative 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 maximal response, and for ACh and SNP relaxation as percentage of 1 μM NA-induced precontraction. All EC_{50} values were calculated with a computer programme and presented as the negative logarithm (pD_2); these values were also used in statistical analyses.

Hormonal and biochemical determinations

Plasma renin activity was determined by using a radioimmunoassay for 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 a Baird PS-4 inductively-coupled plasma emission spectrometer (Baird Co., Bedford, MA, U.S.A.) as described in detail by Laasko *et al*. (1991).

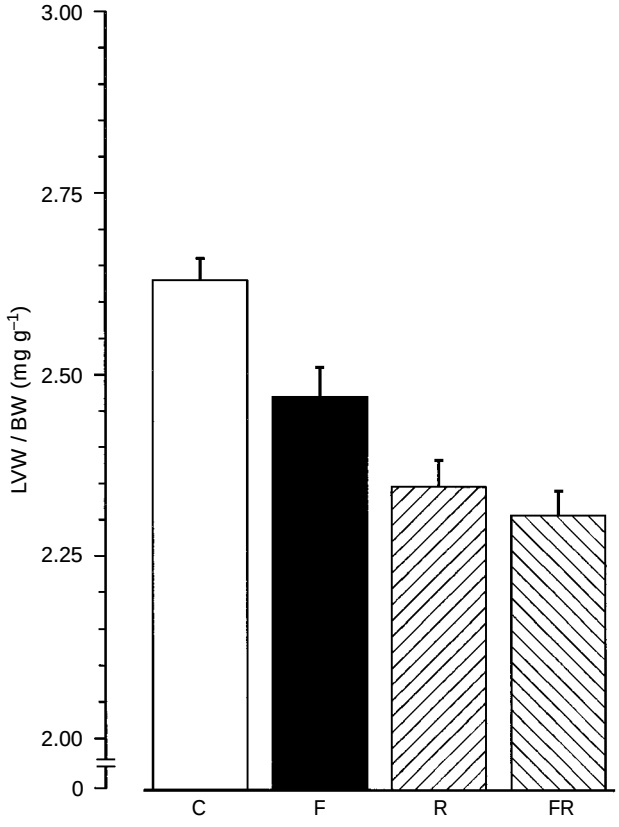

Figure 3 Left ventricular hypertrophy, expressed as left ventricular wet weight (LVW) to body weight (BW) ratio (LVW/BW), of spontaneously hypertensive rats after four weeks on different drug regimens ($n=9-10$ in each group). C, control group; F, felodipine group; R, ramipril group; FR, ramipril + felodipine group. Ramipril and felodipine monotherapies prevented the development of left ventricular hypertrophy in SHR ($P<0.05$ as compared to control group). The cardioprotective effect of felodipine was improved when combined with ramipril treatment ($P<0.05$ as compared to felodipine group). The results are expressed as means ± s.e.mean.

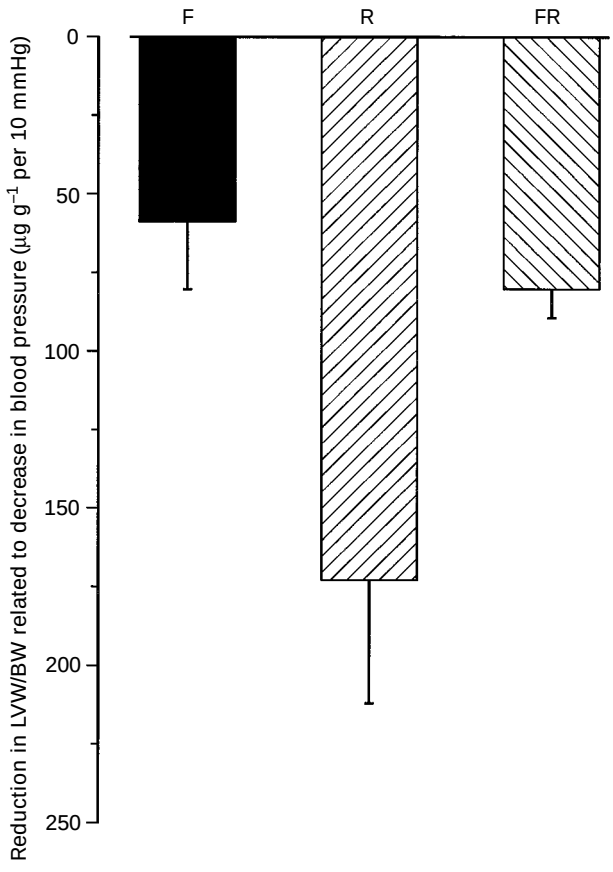

Figure 4 Reduction in left ventricular hypertrophy (LVH) related to the drug-induced decrease in systolic blood pressure, expressed as reduction in the left ventricular wet weight (LVW) to body weight (BW) ratio per 10 mmHg, of spontaneously hypertensive rats after four weeks on different drug regimens ($n=9-10$ in each group). F, felodipine group; R, ramipril group; FR, ramipril + felodipine group. The means in systolic blood pressure and LVH-index of the SHR control group were used as the baseline in the calculations. The effect of ramipril on LVH-index, when related to blood pressure, was greater than that of felodipine or the drug combination ($P<0.05$). The effect of the drug combination on LVH-index did not significantly differ from that of felodipine alone. The results are expressed as means ± s.e.mean.

Drugs

The following drugs were used: ramipril, felodipine (Astra Hässle AB, Mölndal, Sweden), acetylcholine chloride, noradrenaline bitartrate (Sigma Chemical Co., St. Louis, MO, U.S.A.), sodium nitroprusside (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.

Statistical analysis

Statistical analyses were carried out by one-way analysis of variance (ANOVA) followed by the Tukey test. Data for multiple observations over time were analysed by two-way ANOVA with repeated measures for overall treatment effect, and the Tukey 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). Regression lines were calculated by the least squares method. 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 expressed as means $\pm$ s.e.mean.

Ethics

The procedures and protocols of the study were in accordance with the guidelines of our institution and were approved by the Animal Experimentation Committee of the Institute of Biomedicine, University of Helsinki, Finland.

Results

Blood pressure and heart rate

Ramipril and felodipine alone decreased blood pressure significantly (Figure 1). Even though the antihypertensive effect of felodipine tended to be greater than that of ramipril, this difference did not quite reach statistical significance ($P=0.09$). The antihypertensive effect of the drug combination was more than that of ramipril treatment alone, but not significantly better than that of felodipine monotherapy. Ramipril or felodipine treatments did not significantly affect the heart rate, either alone or in combination (Figure 2). The slight increase seen after one week in felodipine-treated SHR did not reach statistical significance.

Left ventricular hypertrophy

Felodipine decreased the LVH-index by 5.9% and ramipril by 10.6% (Figure 3). The effect of ramipril on LVH-index was greater than that of felodipine ($P<0.05$). The combination of felodipine and ramipril decreased the LVH-index by 12.2% (Figure 3). The cardioprotective effect of felodipine was improved when combined with ramipril treatment ($P<0.05$ as compared to felodipine group). There was a weak correlation between systolic blood pressure at the end of the experimental period and LVH-index ($r=0.41$, $P=0.01$, $n=40$).

When related to the drug-induced reduction in blood pressure, the effect of ramipril on LVH-index was greater than that of felodipine or the drug combination (Figure 4).

Biochemical and hormonal variables

Ramipril treatment, both alone and in combination with felodipine, caused a three fold increase in plasma renin activity (Table 1). Serum aldosterone, fasting blood glucose and serum insulin were not significantly affected by either ramipril or felodipine monotherapy, or by the drug combination (Table 1).

Metabolic variables and indicators

There was no significant difference between different groups in the body weight gain (ANOVA, $P=0.81$) or food intake (Table 2). The 24 h urinary excretions of sodium, potassium, magnesium, phosphorus, calcium and protein were not significantly affected by either ramipril or felodipine monotherapy, or by the drug combination.

Table 1 Plasma renin activity (PRA), serum aldosterone, serum insulin and blood glucose of spontaneously hypertensive rats after four weeks on different drug regimens

	Controls	*Felodipine*	*Ramipril*	*Felodipine + ramipril*	*ANOVA* (*P* value)
PRA (ngA I ml^{-1} h^{-1})	6.6 $\pm$ 0.7	8.3 $\pm$ 1.5	17.8 $\pm$ 2.9[a]	17.6 $\pm$ 1.1[a]	0.0002
Aldosterone (ng l^{-1})	292.2 $\pm$ 44.1	291.4 $\pm$ 51.3	409.1 $\pm$ 62.1	280.1 $\pm$ 46.8	0.25
Glucose (mM)	3.8 $\pm$ 0.09	3.8 $\pm$ 0.07	3.6 $\pm$ 0.1	3.8 $\pm$ 0.1	0.83
Insulin (μ ml^{-1})	334.4 $\pm$ 24.0	316.6 $\pm$ 11.1	334.0 $\pm$ 24.9	348.0 $\pm$ 22.1	0.77

Values are means $\pm$ s.e.mean; $n=9-10$ in each group. [a]$P<0.05$ versus control group and felodipine group.

Table 2 Body weight, 24 h food and water intake, urine volume and urinary excretion rates of various mineral elements and proteinuria of spontaneously hypertensive rats after four weeks on different drug regimens

	Controls	*Felodipine*	*Ramipril*	*Felodipine + ramipril*	*ANOVA* (*P* value)
Body weight (g)	322 $\pm$ 9	324 $\pm$ 6	314 $\pm$ 6	322 $\pm$ 9	0.81
Food intake (g d^{-1})	18.7 $\pm$ 0.4	18.1 $\pm$ 0.6	20.5 $\pm$ 0.9	19.2 $\pm$ 0.6	0.08
Water intake (ml d^{-1})	21.7 $\pm$ 2.5	20.5 $\pm$ 1.6	21.0 $\pm$ 1.2	24.0 $\pm$ 1.9	0.54
Urine volume (ml d^{-1})	18.4 $\pm$ 2.2	18.2 $\pm$ 1.3	18.6 $\pm$ 1.4	21.4 $\pm$ 2.2	0.54
sodium (mmol d^{-1})	1.7 $\pm$ 0.1	1.6 $\pm$ 0.09	1.7 $\pm$ 0.08	1.7 $\pm$ 0.08	0.73
potassium (mmol d^{-1})	2.6 $\pm$ 0.1	2.7 $\pm$ 0.1	2.7 $\pm$ 0.1	2.8 $\pm$ 0.1	0.65
magnesium (μmol d^{-1})	107.8 $\pm$ 9.9	118.3 $\pm$ 19.3	113.5 $\pm$ 17.7	138.4 $\pm$ 19.6	0.63
phosphorus (μmol d^{-1})	353.3 $\pm$ 29.7	389.3 $\pm$ 31.2	301.3 $\pm$ 32.6	292.4 $\pm$ 30.0	0.11
calcium (μmol d^{-1})	24.7 $\pm$ 4.0	27.0 $\pm$ 4.4	17.2 $\pm$ 1.5	19.7 $\pm$ 2.0	0.13
Proteinuria (mg d^{-1})	17.3 $\pm$ 1.4	17.8 $\pm$ 1.0	15.9 $\pm$ 1.0	19.1 $\pm$ 1.1	0.24

Values are means $\pm$ s.e.mean; $n=9-10$ in each group.

Mesenteric arterial responses in vitro

Ramipril and felodipine monotherapies as well as the drug combination improved the endothelium-dependent vascular relaxation responses to acetylcholine equally (Figure 5, Table 3). The maximal relaxation response to acetylcholine correlated significantly with the systolic blood pressure at the end of the experiment ($r=-0.36$, $P=0.02$, $n=39$).

The combination ramipril and felodipine enhanced the endothelium-independent vascular relaxation responses to sodium nitroprusside (Figure 5, Table 3). Also either drug as a monotherapy tended to have this effect, but the changes did not reach statistical significance.

Ramipril treatment alone, but not in combination with felodipine, antagonized the vascular contractile responses to noradrenaline (Figure 6, Table 3). Whereas, neither ramipril

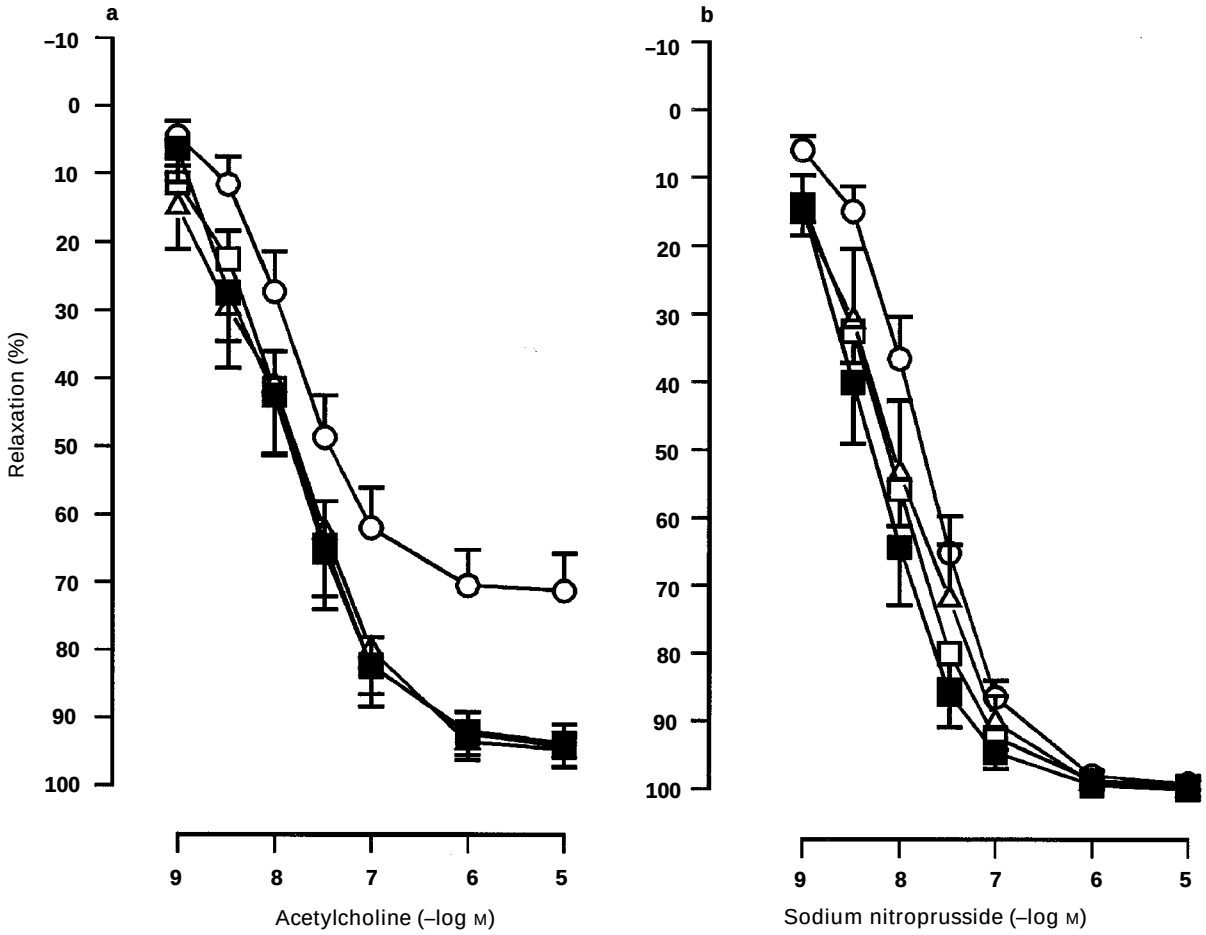

Figure 5 Relaxation responses to acetylcholine (ACh) (a) and to sodium nitroprusside (SNP) (b) of isolated endothelium-intact mesenteric arterial rings precontracted with noradrenaline from SHR after four weeks on different drug regimens: (○) control group; (□) felodipine group; (△) ramipril group; (■) ramipril + felodipine group. Ramipril and felodipine monotherapies as well as the drug combination improved the vascular relaxation responses to ACh equally in endothelium-intact mesenteric arterial rings ($P<0.05$ as compared to control group). The combination of ramipril and felodipine enhanced the vascular relaxation responses to SNP ($P<0.05$ as compared to control group). The results are expressed as means, $n=9-10$ in each group; vertical lines show

Table 3 Parameters of contractile and relaxation responses of isolated endothelium-intact arterial rings of spontaneously hypertensive rats after four weeks on different drug regimens

	Controls	*Felodipine*	*Ramipril*	*Felodipine + ramipril*	*ANOVA* (*P* value)
		Relaxation responses (pD_2)			
Acetylcholine	7.44 ± 0.17	7.82 ± 0.12	7.86 ± 0.25	7.85 ± 0.20	0.27
Sodium nitroprusside	7.77 ± 0.10	8.12 ± 0.10	8.08 ± 0.20	8.27 ± 0.14[a]	0.04
Maximal relaxation (% of 1 μM NA-induced precontraction)					
Acetylcholine	71 ± 6	94 ± 3[a]	95 ± 3[a]	94 ± 3[a]	0.0003
Sodium nitroprusside	99 ± 0.5	100 ± 0.5	100 ± 0	100 ± 0	0.33
		Contractile responses (pD_2)			
Noradrenaline	6.43 ± 0.06	6.29 ± 0.07	6.04 ± 0.09[a]	6.25 ± 0.10	0.006
KCl	1.41 ± 0.008	1.38 ± 0.02	1.37 ± 0.01	1.39 ± 0.009	0.21
		Maximal force (g)			
Noradrenaline	2.53 ± 0.10	2.43 ± 0.13	2.28 ± 0.13	2.24 ± 0.11	0.24
KCl	2.34 ± 0.09	2.23 ± 0.17	2.28 ± 0.12	2.03 ± 0.13	0.33

Values are mean ± s.e.mean; $n=9-10$ in each group. EC_{50} values are presented as the negative logarithm (pD_2) of concentration of the agonist. [a]$P<0.05$ versus control group.

nor felodipine significantly affected the vascular contractile responses to potassium chloride, either alone or in combination (Figure 6, Table 3).

Discussion

In addition to a good control of blood pressure, prevention and regression of left ventricular hypertrophy (LVH) and other end-organ effects is important in the overall control of hypertension (Joint National Committee, 1993). In the present study, at submaximal antihypertensive doses, ramipril alone had a relatively weak antihypertensive effect and felodipine, in spite of a much better control of blood pressure, had a small effect only on LVH. The combination of the two drugs produced both a very effective control of blood pressure and a remarkable decrease in LVH index without any tendency to tachycardia or any evidence of other types of adverse effects. Our findings are thus in good accordance with those of previous studies demonstrating a beneficial interaction between angiotensin converting enzyme inhibitors and calcium channel blockers in man (Osswald & Mühlbauer, 1995; Waeber & Brunner, 1995).

A combination of two antihypertensive drugs acting by different mechanisms permits improved efficacy and broadens the fraction of responders (Waeber & Brunner, 1995). Even though the sodium concentration of the standard rat chow used in the present study was rather low (Na 0.3%), it still exceeded by six fold the dietary sodium concentration considered as adequate for the normal growth of a laboratory rat (Na 0.05%) (Ganguli *et al*., 1969). The sodium in the chow might thus have attenuated, at least in part, the antihypertensive effect of ramipril, as demonstrated in our previous studies (Mervaala *et al*., 1994a,b). On the other hand, the natriuretic effect of felodipine (DiBona, 1985; Nordlander *et al*., 1985; Elmfeldt *et al*., 1992) might have sensitized the animals to the therapeutic effects of ramipril. One of the main advantages of the use of a combination of two low-dose antihypertensive drugs acting by different mechanisms is to avoid dose-related side effects. We have previously found that, in SHR, high-dose felodipine treatment causes marked dose-dependent tachycardia throughout the four week experimental period (Mervaala *et al*., 1994c; 1997). The increase in heart rate is apparently due to a reflex activation of the sympathetic nervous system, caused by the marked vasodilator effect of felodipine (Nordlander, 1985; Todd & Faulds, 1992). In the present study even the relatively low dose of felodipine tended to increase the heart rate in the beginning of the drug treatment. On the other hand, the absence of tachycardia, in spite of the pronounced fall in blood pressure, suggests that ramipril was able to block the reflex activation of the sympathetic nervous system. Our suggestion is further supported by the finding that, ramipril in the present study diminished the vascular contractile responses of the mesenteric artery to noradrenaline.

High blood pressure is one of the most powerful determinants of the development of LVH (Frochlich *et al*., 1992). Therefore, the pronounced antihypertensive effects of felodi-

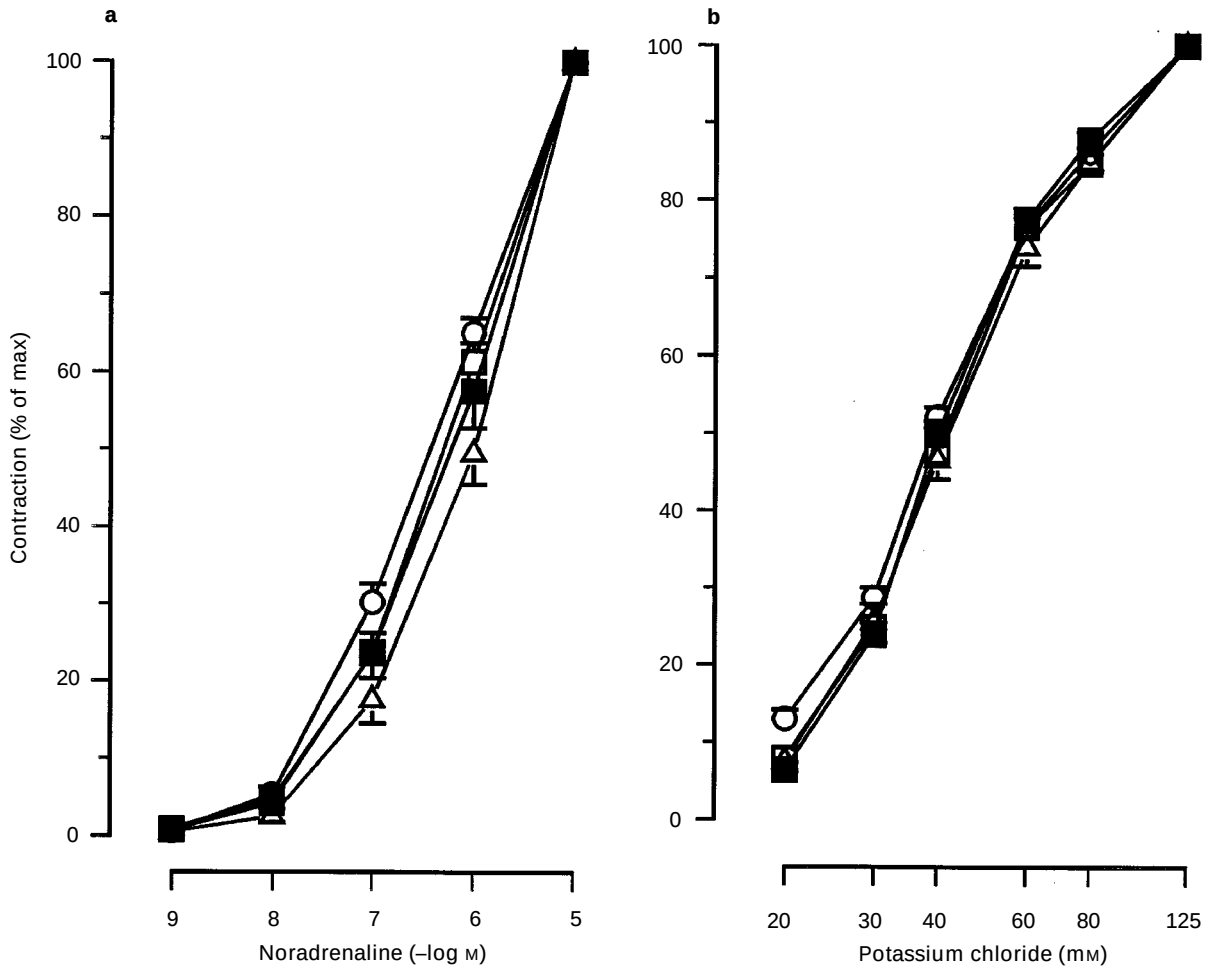

Figure 6 Contractile responses to noradrenaline (a) and to potassium chloride (b) of isolated endothelium-intact mesenteric arterial rings from SHR after four weeks on different drug regimens: (○) control group; (□) felodipine group; (△) ramipril group; (■) ramipril + felodipine group. Ramipril antagonized the vascular contractile responses to noradrenaline ($P<0.05$ as compared to control group). Drug treatments did not significantly affect the contractile responses to potassium chloride. The results are expressed as means, $n=9-10$ in each group; vertical lines show s.e.mean.

pine and ramipril leading to decreased pressure load on the myocardium are likely to explain, to a great extent, the drug-induced decreases in the LVH-index. However, in the present study there was only a weak correlation between systolic blood pressure and the degree of LVH suggesting that blood pressure is not the sole determinant of LVH in SHR. Interestingly the beneficial effect of submaximal antihypertensive dose of ramipril on LVH was more prominent than that of felodipine, even though ramipril decreased blood pressure to a lesser extent than felodipine. The weak effect of felodipine on LVH could be explained, at least in part, by a modest activation of the sympathetic nervous system, as suggested previously (Nyborg & Mulvany, 1985; Leenen & Holliwell, 1992). On the other hand, our finding that ramipril has a prominent cardioprotective effect lends further support to the previous suggestion that ACE inhibitors exert cardioprotective effects beyond their antihypertensive properties (Linz *et al*., 1992; 1995).

It has been shown previously that long-term treatment with ACE inhibitors restores the impaired endothelial function in SHR (Arvola *et al*., 1993; Mervaala *et al*., 1994c; Linz *et al*., 1995). Treatment with ACE inhibitors improves the endothelium-dependent vascular relaxation mainly by preventing the degradation of bradykinin liberated from the endothelial cells (Gohlke & Unger, 1994; Linz *et al*., 1995). In the present study, the submaximal antihypertensive dose of ramipril markedly improved the arterial relaxation responses to acetylcholine, suggesting the involvement of endothelium-dependent mechanisms. Interestingly, treatment with felodipine, at a submaximal antihypertensive dose, also improved endothelium-dependent vascular relaxation to the same extent as ramipril. The increased endothelium-dependent relaxation of the blood vessels in the drug-treated groups might have contributed to the antihypertensive effects. However, it is also possible that, in the presence of a lower blood pressure, the structure and function of the endothelial cells are better maintained. Hence, the increased relaxation might have been the consequence rather than the cause of the lower blood pressure.

In conclusion, our findings suggest that a better overall control of hypertension and end-organ damage, without an increase in adverse effects, can be achieved by the combination of felodipine and ramipril rather than by monotherapy with either drug alone.

This study was supported by grants from the Academy of Finland, the University of Helsinki and the Sigrid Jusélius Foundation. We thank Ms Remi Hakama, Ms Marja-Liisa Räsänen and Ms Toini Siiskonen for providing excellent technical assistance.

References

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.

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

ELMFELDT, D., NORDLANDER, M. & EDGAR, B. (1992). Renal effects of felodipine – A review. *Kidney Int*., **41** (suppl. 36), S54–S60.

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.

GANGULI, M., SMITH, J. & HANSON, L. (1969). Sodium metabolism and requirements in lactating rats. *J. Nutrition*, **99,** 395–400.

GOHLKE, P. & UNGER, T. (1994). Angiotensin-converting enzyme inhibitors. In *Textbook of Hypertension*, ed. Swales, J.D. pp. 1115–1127. (Oxford: Blackwell Scientific Publications).

HEEG, J., DE JONG, P., VAN DER HEM, K. & DE ZEEUW, D. (1989). Efficacy and variability of the antiproteinuric effect of ACE inhibition by lisinopril. *Kidney Int*., **36,** 272–279.

JOINT NATIONAL COMMITTEE. (1993). The Fifth Report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure (JNC V). *Arch. Int. Med*., **153,** 154–183.

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.

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

MERVAALA, E.M.A., LAAKSO, J., HIMBERG, J.-J. & KARPPANEN, H. (1994b). 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., PAAKKARI, I., LAAKSO, J., NEVALA, R., TERÄVÄINEN, T.-L., FYHRQUIST, F., VAPAATALO, H. & KARPPANEN, H. (1994c). 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. (1997). 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).

NORDLANDER, M. (1985). Haemodynamic effects of short and long term administration of felodipine in spontaneously hypertensive rats. *Drugs*, **29** (suppl. 2), 90–101.

NORDLANDER, M., DIBONA, G., LJUNG, B. & THOREN, P. (1985). Renal and cardiovascular effects of acute and chronic administration of felodipine to SHR. *Eur. J. Pharmacol*., **113,** 25–36.

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.

OSSWALD, H. & MÜHLBAUER, B. (1995). The pharmacological basis for the combination of calcium channel antagonists and converting enzyme inhibitors in the treatment of hypertension. *J. Hypertens*., **13** (suppl. 2), S21–S28.

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.

TODD, P. & FAULDS, D. (1992). Felodipine. A review of the pharmacology and therapeutic use of the extended release formulation in cardiovascular disorders. *Drugs*, **44,** 251–277.

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,** 872–880.

WAEBER, B. & BRUNNER, H.R. (1995). Main objectives and new aspects of combination treatment of hypertension. *J. Hypertens*., **13** (suppl. 2), S15–S19.

(Received September 20, 1996
Revised February 4, 1997
Accepted March 5, 1997)