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Effects of enalapril and eprosartan on the renal vascular nitric oxide system in human essential hypertension[1]

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Effects of enalapril and eprosartan on the renal vascular nitric oxide system in human essential hypertension.

Background: Experimental data in humans on the contribution of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor blockers to the nitric oxide system of the renal vasculature are inconsistent. Enalapril and eprosartan, alone and in combination, were used to determine their short-term effects on the renal nitric oxide system and renal hemodynamics of human subjects with essential hypertension.

Methods: Twenty male, white patients (27 ± 1 years) with mild essential hypertension (143 ± 11/95 ± 6 mm Hg) were included in a double-blind, randomized, placebo-controlled, fourfold cross-over study with placebo, enalapril (20 mg/day), eprosartan (600 mg/day), or combination of both drugs (10 and 300 mg/day, respectively) each over a one week period followed by a two-week washout phase. After each study phase the glomerular filtration rate (GFR) and renal plasma flow (RPF) were determined. Basal nitric oxide synthesis of the renal vasculature was assessed by the decrease in RPF after inhibition of nitric oxide synthase with N^G-monomethyl-L-arginine (L-NMMA; 4.25 mg/kg).

Results: After one week of therapy, the combination therapy decreased casual blood pressure by 5 ± 2/3 ± 1 mm Hg versus placebo ($P < 0.01$). Neither enalapril alone (−2 ± 2/1 ± 2 mm Hg, NS vs. placebo) nor eprosartan alone (−1 ± 1/0 ± 2 mm Hg, NS vs. placebo) had a clear-cut significant effect on casual blood pressure. In the combination phase, RPF increased by 123 ± 36 mL/min ($P < 0.01$). Neither enalapril alone (+59 ± 46 mL/min, $P = 0.21$) nor eprosartan alone (+113 ± 51 mL/min, $P = 0.06$) had a clear-cut significant effect on RPF. Changes of RPF induced by treatment correlated with the L-NMMA induced decrease in RPF in the combination ($r = 0.70$, $P < 0.01$) and eprosartan phase ($r = 0.86$, $P < 0.001$), but not in the enalapril phase ($r = -0.44$, $P = 0.10$). Renal vascular resistance was reduced by each active treatment with the most prominent reduction in the combination phase. GFR was unaffected by any treatment.

Conclusions: In contrast to the effects of either substance alone, a combination of half the dose of eprosartan with half the dose of enalapril had a prominent effect on renal perfusion. The effects of eprosartan on RPF are mediated, at least in part, by an increased bioavailability of nitric oxide in the renal vasculature.

Angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II type 1 receptor blockers (ARB) have been found to preserve renal function in various clinical conditions [1]. Both classes of agents inhibit the vasoconstrictive effects of angiotensin II at the efferent arteriole either by reducing the concentration of angiotensin II (in the case of ACEI) or by blocking its receptor (in the case of ARB). This specific site of action explains in part why ACEI and ARB have superior "nephroprotective" properties compared to other antihypertensive drugs. However, apart from reducing blood pressure and glomerular hypertension, other intrarenal actions such as improving endothelial function in the renal vasculature are thought to contribute to the effects of ACEI and ARB in the kidney [2].

Treatment with ACEI increases the level of bradykinin, which is degraded by angiotensin-converting enzyme. Bradykinin stimulates nitric oxide synthesis. Consequently, ACEI have been found to increase nitric oxide production in vitro and in various vasculatures, particularly in the coronary vasculature [3]. Blockade of the angiotensin II type 1 (AT_1) receptor with an ARB causes increased stimulation of the AT_2-receptor by angiotensin II whose concentrations are elevated due to the ARB medication [4]. Stimulation of nitric oxide synthesis via AT_2-receptor stimulation has been found in rats [5] and is believed to play a role in nephroprotection in humans, too [6].

On the basis of these studies, it was reasonable to assume that therapy with ACEI and ARB combined might have additive effects on nitric oxide bioavailability due to their different sites of action. In fact, combination therapy with both an ACEI and an ARB was found to increase cardiac output and ejection fraction in patients

[1] **See Editorial by Noris and Remuzzi, p. 1545.**

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with heart failure [7, 8] and to reduce proteinuria in patients with glomerulonephropathy (abstract; Russo D, *J Am Soc Nephrol* 11:76A, 2000; abstract; Kincaid-Smith PS, *J Am Soc Nephrol* 11:349A, 2000).

However, experimental data in humans concerning the contribution of the nitric oxide system of the renal vasculature to the renal effects of ACEI and ARB are inconsistent. Some investigators failed to observe an increase in basal [9] or L-arginine–stimulated [10] nitric oxide synthesis with ACEI or ARB treatment, whereas others found an increase in renal vascular nitric oxide synthase activity with ACEI treatment [11, 12].

The current study was designed to further address this issue. We examined the effects of a one-week treatment with the ACEI enalapril, the ARB eprosartan, and a combination of both drugs but with half the dose, on renal hemodynamic parameters. The response of renal plasma flow (RPF) to an inhibition of nitric oxide synthase with N^G-monomethyl-L-arginine (L-NMMA) was measured in each study phase, which permitted an assessment of the contribution of basal nitric oxide synthesis to renal perfusion in the respective study phases.

METHODS

Study participants

Subjects eligible for the study were screened for arterial hypertension at the campus of the University of Erlangen-Nürnberg. Students with a casual blood pressure ≥140 mm Hg (systolic) and/or ≥90 mm Hg (diastolic), but <180 mm Hg (systolic) and <105 mm Hg (diastolic) obtained with a standard sphygmomanometer after five minutes of rest at three independent occasions were invited to our outpatient clinic for a detailed medical examination including history taking, physical examination, direct ophthalmoscopic examination, 12-lead electrocardiography, routine laboratory examination, and 24-hour urine sampling. Echocardiography, ultrasonography of the kidneys and duplex sonography of the renal arteries were performed where indicated. Inclusion criteria were male gender, age between 18 and 35 years, and essential hypertension according to the WHO criteria as outlined above. Exclusion criteria included the presence of any form of secondary hypertension; any irreversible end-organ damage due to arterial hypertension; any significant disease other than mild essential hypertension; a history of antihypertensive drug intake within a period of three months prior to the study; cigarette smoking within a period of one year prior to the study; hypercholesterolemia (total cholesterol ≥160 mg/dL).

All volunteers gave written informed consent prior to study inclusion. Further treatment of hypertension in our outpatient clinic was offered to all subjects screened for the study, and all of them received a letter to inform their general practitioner about clinical and laboratory findings. Twenty-two subjects met all inclusion criteria in the absence of any exclusion criteria. One subject withdrew informed consent after the baseline examination, and one subject had to be excluded from the study due to a transient increase in liver enzymes that in a more detailed examination was found to be unrelated to the study medication. Baseline characteristics of the study participants are given in Table 1.

Table 1. Baseline clinical parameters

Age *years*	27 ± 1
Weight *kg*	82 ± 2
Height *m*	1.83 ± 0.02
Body surface area m^2	2.04 ± 0.04
Body mass index kg/m^2	24.3 ± 0.5
Casual systolic blood pressure *mm Hg*	143 ± 11
Casual diastolic blood pressure *mm Hg*	95 ± 6
Urinary sodium excretion *mmol/day*	223 ± 69

Study design

The study protocol was approved by the Clinical Investigation Ethics Committee of the University of Erlangen-Nürnberg.

The study consisted of four phases in a randomized double-blind order: treatment with placebo, enalapril (20 mg once daily), eprosartan (600 mg once daily), or a combination of both drugs (enalapril 10 mg and eprosartan 300 mg, once daily). Each treatment was done for seven days and was followed by a two-week washout phase before the next study phase began. Participants were asked to take the study medication at a fixed time in the morning. At day seven of each treatment phase, the participant was invited to our laboratory at 7:30 a.m. There, blood pressure was measured ("casual blood pressure" according to WHO criteria), a routine laboratory examination was performed, the participant was interviewed about any side effects of treatment, and he took his study medication for this day. Then, renal vascular endothelial function testing was performed. Study medication for the next study phase was then handed out to the participant. He was instructed to begin the following treatment phase after 14 days of wash-out phase, which was chosen as far greater than five times the half-lives of enalapril (11 hours) and eprosartan (5 to 9 hours). All analyses of the effects of treatment on hemodynamic parameters were made in comparison to the placebo phase.

Renal vascular endothelial function testing

Renal plasma flow (RPF) and glomerular filtration rate (GFR) were determined by constant input clearance technique with *para*-aminohippurate (Nephrotest; Merck, Sharp & Dohme, Hertfordshire, UK) and inulin (Inutest; Fresenius, Linz, Austria), respectively, as suggested by Cole and coworkers [13] and established in our labora-

Table 2. Effect of treatment on hemodynamic parameters

Parameter	Placebo	Enalapril	Eprosartan	Combination
Casual systolic blood pressure *mm Hg*	145 ± 3	144 ± 3	144 ± 3	140 ± 2[ad,bd]
Casual diastolic blood pressure *mm Hg*	93 ± 1	92 ± 2	94 ± 1	90 ± 1[ad,ce]
Supine systolic blood pressure *mm Hg*	134 ± 3	128 ± 2[ae]	132 ± 2[bd]	125 ± 3[ae,ce]
Supine diastolic blood pressure *mm Hg*	80 ± 2	74 ± 1[ae]	78 ± 1[bd]	72 ± 2[ae,ce]
Supine mean arterial pressure *mm Hg*	96 ± 2	89 ± 2[ae]	94 ± 2[ce]	88 ± 2[ae,cd]
Heart rate min^{-1}	59 ± 1	62 ± 1[ad]	63 ± 2[ae]	62 ± 2
Renal plasma flow *mL/min*	689 ± 31	746 ± 40	797 ± 48	802 ± 38[ae]
Glomerular filtration rate *mL/min*	136 ± 4	135 ± 3	137 ± 3	138 ± 4
Filtration fraction *%*	20.5 ± 0.7	18.9 ± 0.8	18.1 ± 0.8[ad]	17.6 ± 0.6[ae]
Renal vascular resistance *mm Hg* · min^{-1} · mL^{-1}	81.3 ± 4.1	70.1 ± 3.5[ad]	68.8 ± 3.3[ad]	62.7 ± 2.7[ae,bd,cd]

[a] Significant differences of enalapril, eprosartan, or combination in comparison with placebo
[b] Significant differences of eprosartan or combination in comparison with enalapril
[c] Significant differences of combination in comparison with eprosartan
[d] $P < 0.05$
[e] $P < 0.01$

tory for many years [14, 15]. In brief, after administration of a loading dose, a steady state between infusion and renal excretion of the tracer substances was reached after 120 minutes. Blood samples for the determination of *para*-aminohippurate and inulin to assess baseline RPF and GFR values were drawn at this time and before infusion was started. Subsequently, L-NMMA (Clinalfa, Switzerland) was infused to inhibit endothelial nitric oxide synthase (3 mg/kg over 5 min, followed by constant infusion over 25 min with a rate of 3 mg/h, that is, a total dose of 4.25 mg/kg). After L-NMMA infusion, another blood sample was drawn to measure *para*-aminohippurate and inulin, and thus to assess RPF and GFR after nitric oxide synthase inhibition. Blood pressure ("supine blood pressure") was measured with an oscillometric device (Dinamap 1846 SX; Criticon, Norderstedt, Germany) in parallel with blood sampling. All participants drank 10 mL/kg of mineral water during the clearance studies.

Laboratory measurements and calculation of renal hemodynamic parameters

Blood samples were centrifuged immediately at 4°C and were stored at −21°C until measurement. Measurement of *para*-aminohippurate and inulin was performed after completion of the study with the investigators still unaware of the order of treatment phases in individual study participants. Details concerning the measurement of inulin and *para*-aminohippurate have been published previously [15]. In brief, *para*-aminohippurate was measured by the method of Smith and coworkers [16]; inulin was measured indirectly with an enzymatic method after conversion to fructose. Each blood sample was measured in duplicate with a coefficient of variation of <5%. Measurements with a coefficient of variation of 5% or greater were excluded from further analysis. Inulin and *para*-aminohippurate clearances were calculated as a metabolic clearance rate from the serum concentrations and infusion rates. Filtration fraction was calculated by dividing GFR by RPF. Renal vascular resistance (RVR) was calculated as mean arterial pressure (MAP) × (1 − hematocrit)/RPF.

Statistics

All statistical analysis was carried out using SPSS software (release 8.0; SPSS Inc., Chicago, IL, USA). Before further analysis was performed, a Kolmogorow-Smirnow test showed that all baseline data and those after each treatment phase had a normal distribution. Analysis of variance (ANOVA) was conducted to analyze differences between parameters evoked by study medication, and the Student *t* test with an α level corrected for multiple comparisons was performed for post hoc analyses. The Student *t* test was used to analyze the effects of L-NMMA in each study phase. For correlation analysis, Pearson's correlation coefficients were calculated. Comparison of correlation coefficients was performed after the Fisher z-transformation. A two-tailed *P* value <0.05 was considered to be significant. All values are expressed as mean ± standard error of the mean.

RESULTS

Effect of treatment on hemodynamic parameters

Hemodynamic parameters in the placebo, enalapril, eprosartan, and combination phase are depicted in Table 2. Compared to baseline (143 ± 11/95 ± 6 mm Hg), the placebo did not have a significant effect on blood pressure (145 ± 3/93 ± 1 mm Hg, NS). Compared to the placebo phase, effects on casual blood pressure were only found with combination treatment (decrease by 5 ± 2/3 ± 1 mm Hg; $P < 0.05$), whereas neither enalapril alone (decrease by 2 ± 2/1 ± 2 mm Hg; P = NS) nor eprosartan alone (decrease by 1 ± 1/0 ± 2 mm Hg; P = NS) had a significant effect on casual blood pressure.

In the placebo, enalapril, eprosartan, and combination phases, 17, 16, 17, and 16 sets of renal hemodynamic data

Table 3. Effect of L-NMMA on hemodynamic parameters

Parameter	Placebo	Enalapril	Eprosartan	Combination
Change of mean arterial pressure *mm Hg*	$+5 \pm 2^{a}$	$+4 \pm 2^{a}$	$+5 \pm 1^{b}$	$+4 \pm 2^{a}$
Change of heart rate *min*$^{-1}$	-3 ± 1^{a}	-3 ± 1^{a}	-4 ± 1^{c}	-4 ± 1^{b}
Change of renal plasma flow *mL/min*	-101 ± 31^{b}	-143 ± 37^{a}	-161 ± 41^{b}	-160 ± 38^{b}
Change of glomerular filtration rate *mL/min*	$+7 \pm 2^{a}$	$+15 \pm 4^{a}$	$+7 \pm 2^{c}$	$+6 \pm 3$
Change of filtration fraction *%*	$+3 \pm 1^{b}$	$+3 \pm 1^{b}$	$+4 \pm 1^{c}$	$+5 \pm 1^{c}$
Change of renal vascular resistance *mm Hg · min · L*$^{-1}$	$+14 \pm 4^{b}$	$+14 \pm 4^{b}$	$+18 \pm 3^{c}$	$+17 \pm 3^{c}$

Changes of hemodynamic parameters after administration of L-NMMA compared with baseline parameters. Blood pressure was measured with the participant in the supine position.

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$, significant effects of L-NMMA compared with baseline parameters

were available for analysis, respectively. RPF increased with combination treatment by 123 ± 36 mL/min ($P < 0.01$). No significant changes of RPF compared to the placebo phase were observed with enalapril alone (increase by 59 ± 46 mL/min, $P = 0.21$) and eprosartan alone (increase by 113 ± 51 mL/min, $P = 0.06$). GFR was not affected by any treatment. There were no significant correlations between changes in blood pressure and changes in renal hemodynamics in each study phase (data not shown).

Compared to the placebo phase (81.3 ± 4.1 mm Hg · min · L^{-1}), RVR was reduced by each treatment (enalapril, 70.1 ± 3.4 mm Hg · min · L^{-1}; eprosartan, 68.8 ± 3.1 mm Hg · min · L^{-1}; all $P < 0.05$ vs. placebo), with the most prominent effect observed in the combination phase (62.7 ± 2.7 mm Hg · min · L^{-1}; $P < 0.001$ vs. placebo, and $P < 0.05$ vs. each monotherapy).

Effect of L-NMMA on hemodynamic parameters

Administration of L-NMMA caused an increase in blood pressure, a decrease in heart rate, a decrease in RPF, and an increase in GFR in the placebo phase (Table 3). The response of hemodynamic parameters to the L-NMMA infusion was similar in the active treatment phases, although some changes did not reach our prefixed level of significance. However, L-NMMA caused a significant decrease in RPF in all study phases.

Pair-wise comparison of the effects of L-NMMA across all treatment and placebo phases revealed no statistically significant differences. For example, increases in mean arterial pressure induced by L-NMMA (+5 ± 2 mm Hg, +4 ± 2 mm Hg, +5 ± 1 mm Hg, and 4 ± 2 mm Hg) were similar in the placebo, enalapril, eprosartan, and combination phases, respectively (Table 3). However, although not statistically significant, numerically the percent decrease in RPF induced by L-NMMA seemed to be greater in the combination and eprosartan phases than in the enalapril and placebo phases (16 ± 4%, 17 ± 3%, 11 ± 4%, and 12 ± 4%, respectively).

Contribution of the renal vascular nitric oxide system to the effects of treatment on RPF

To examine whether the renal vascular nitric oxide system contributes to the effects of enalapril and/or eprosartan treatment on RPF, correlations between the increase in RPF compared to the placebo phase and the response of RPF to L-NMMA were calculated for the respective treatment phases. In the enalapril phase, there was no significant correlation between the increase in RPF by enalapril and the L-NMMA–induced decrease in RPF ($r = -0.44$, $P = 0.10$). In contrast, highly significant correlations between the effects of treatment and the response of RPF to L-NMMA were found in the eprosartan ($r = -0.86$, $P < 0.001$) and combination phases ($r = -0.70$, $P < 0.01$), with these correlations not being statistically different (Fig. 1). The greater basal nitric oxide synthesis and release in the renal vasculature (that is, the greater the vasoconstriction), the more marked was the increase in RPF after one week of treatment.

DISCUSSION

The present study examined the effects of a series of one-week treatments with the ACEI, enalapril, and the ARB, eprosartan, alone or in combination on renal hemodynamic parameters. Neither substance alone had a clear-cut effect on RPF over the observation period, whereas an increase in RPF was found with the combination therapy. Similarly, RVR was more reduced with the combination therapy than with enalapril or eprosartan therapy alone.

One week of treatment with eprosartan alone did not significantly reduce blood pressure. The absence of a blood pressure lowering effect after short-term treatment with an ARB is not uncommon, as the response rate of blood pressure has been found greater after 26 weeks than after 12 weeks of treatment with eprosartan [17]. Our data indicate an effect of eprosartan on renal hemodynamics that is independent of blood pressure. However, the effects of enalapril and combination therapy on renal hemodynamics also were independent of blood pressure changes, as shown by the lack of significant correlations between blood pressure changes and changes in RPF and GFR.

We observed a trend toward an increase of RPF after the one-week treatment with eprosartan alone ($P = 0.06$). This principal effect of eprosartan on RPF in our

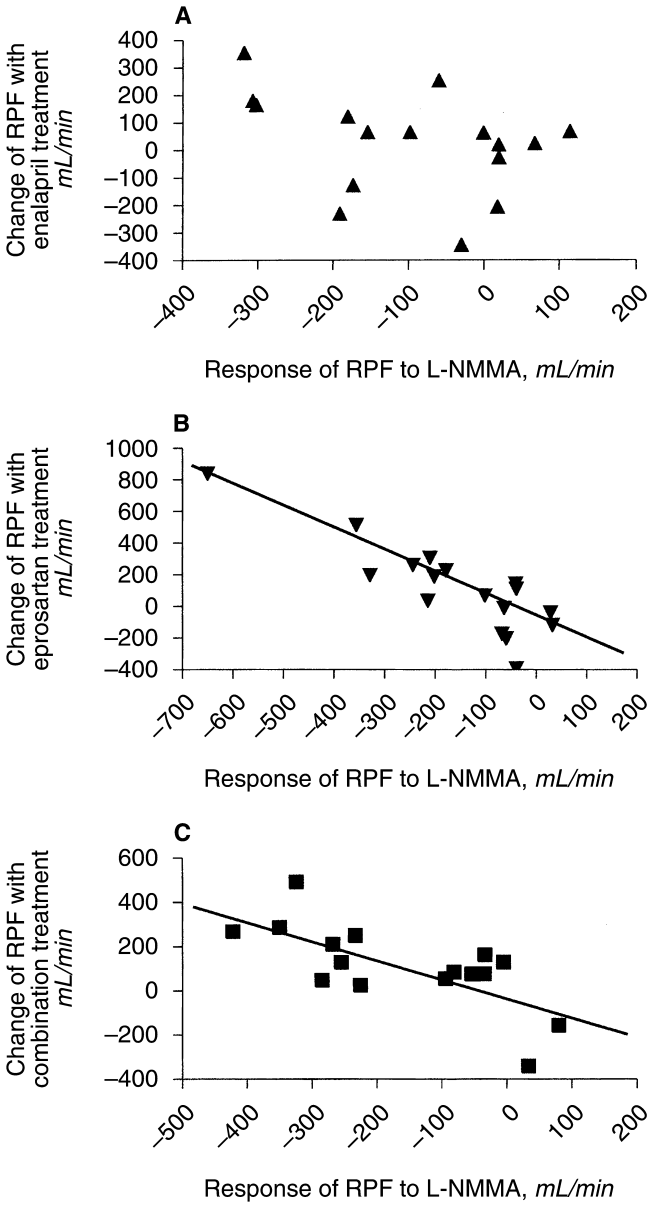

Fig. 1. Contribution of the renal vascular nitric oxide system to the effects of treatment on renal plasma flow. The response of renal plasma flow (RPF) on inhibition of nitric oxide with N^G-monomethyl-L-arginine (L-NMMA; that is, the change in RPF) in the respective study phases is shown on the x-axis. Changes of basal RPF with enalapril, eprosartan, and combination therapy are shown on the y-axis of panels *A* ($r = -0.44$, P = NS), *B* ($r = -0.86$, $P < 0.001$), and *C* ($r = -0.70$, $P = 0.002$), respectively.

study is in accordance with data from other investigators who also found an increase in RPF after treatment with several ARB drugs as well as with ACEI [10, 11, 18–21]. Similarly, other investigators have found non-significant effects of ACEI and ARB on GFR [10, 18–21]. Other parameters such as filtration fraction and RVR might be superior to estimate the effects of ACEI and ARB on renal hemodynamics, since they integrate changes in both GFR and RPF and both RPF and MAP, respectively, evoked by the study drugs. In accordance with the study of Schmitt and coworkers [19], we found a decrease in filtration fraction with combination therapy (and, in addition, with eprosartan alone), and a decrease in RVR in every active treatment phase.

In contrast to therapy with either enalapril or eprosartan alone, our study results show a clear-cut effect of the combination therapy on RPF even after a relatively short treatment period. This finding supports data from other investigators who found additive effects of such combination therapy in heart failure [7, 8] and proteinuria (abstract; Russo D, *J Am Soc Nephrol* 11:76A, 2000; abstract; Kincaid-Smith PS, *J Am Soc Nephrol* 11:349A, 2000) that were independent of blood pressure. The mechanism of these additive effects is not yet clear, although theoretical considerations and experimental data help explain them [22]. The different sites of action of ARB and ACEI cause a complete blockade of angiotensin II effects combined with enhanced bradykinin production. Thereby, nitric oxide bioavailability is increased directly via AT_2-receptor stimulation and indirectly via bradykinin [3, 5]. Of note, we did not add an ARB on top of maximum dose ACEI, but instead used half the dose of both substances. Nevertheless, a clear-cut effect on casual blood pressure, RPF, and RVR was found in the combination phase, but not in phases with the maximum recommended dose of enalapril and eprosartan alone. We are convinced that this finding also suggests a specific effect of combining two therapeutic principles and is not the mere result of elevated tissue concentrations of drugs interfering with the actions of angiotensin II.

From in vitro experiments and animal models, an improvement of renal vascular endothelial function is believed to play a role in the effects of ARB, but also of ACEI, on renal hemodynamics [2]. However, experimental data in humans do not uniformly show a stimulation of the renal vascular nitric oxide system after treatment with ARB or ACEI. Likewise, Dijkhorst-Oei et al did not find an improvement of renal vascular endothelial function with enalapril treatment in hypertensive patients [9], and Komers et al found the same result with ramipril and losartan in healthy subjects [10]. In contrast, Higashi et al found an improvement of renal vascular endothelial function with the ACEI imidapril in hypertensive patients [11], a result also found by Mimran et al in patients treated with an ACEI for more than two years [12]. Some of these discrepancies can be explained by pharmacological differences between the study drugs and by differences between the study cohorts. Also, different treatment periods ranging from three [9, 10] and over 12 weeks [11], to more than two years [12] might have effects on the results reported here and by other investigators. Our relatively short treatment period of

one week was chosen according to data from experiments with ACEI indicating that an effect on endothelial dysfunction exists in other vasculatures even after short treatment periods [reviewed in 23], and to reduce any potential harm due to the combination therapy, which was and still is not established in subjects with mild essential hypertension who are otherwise healthy. Another factor for the different results found in this and other studies might play an important role: to date there is no consensus on how to measure or how to define renal vascular endothelial function in humans.

The "golden standard" to examine endothelium-dependent vasodilation, that is, the administration of endothelium-dependent vasodilators such as acetylcholine, is associated with a considerable risk for study participants when the renal vasculature must be examined, since a renal artery catheter has to be invasively inserted [24]. Therefore, non-invasive methods have been developed to examine renal vascular endothelial function including systemic administration of L-arginine, the substrate for nitric oxide synthesis, and L-NMMA, a competitive inhibitor of endothelial nitric oxide synthase. However, while some authors directly interpret changes of renal hemodynamics in response to L-arginine or L-NMMA as endothelium-dependent vasodilation or vasoconstriction, respectively [9, 12], other authors in addition measure changes of plasma and urinary concentrations of nitrite and nitrate or urinary cyclic guanosine 3′,5′-monophosphate concentration as indicators of nitric oxide activity to conclude about changes of renal vascular endothelial function [8, 11]. However, these parameters should be interpreted cautiously, since nitrate and nitrite might derive from vasculatures other than the renal vasculature, and cyclic guanosine 3′,5′-monophosphate also serves as a second messenger for atrial natriuretic peptide and does not specifically reflect nitric oxide activity. Thus, while such parameters are helpful for the assessment of nitric oxide-dependent vasodilation, in analyzing endothelial function of the renal vasculature they cannot substitute for direct measurement of RPF in response to inhibition (or stimulation) of nitric oxide synthase.

In our laboratory, we have established the administration of L-arginine (100 mg/kg) and L-NMMA (total dose, 4.25 mg/kg) to analyze renal vascular endothelial function [25–27]. For the present study we chose to administer L-NMMA, since we expected that both enalapril and eprosartan would increase basal nitric oxide synthesis [3, 5]. In fact, there was an increase in RPF compared to baseline values with eprosartan therapy, whereas changes of RPF with enalapril alone and eprosartan alone were not statistically significant. Administration of L-NMMA decreased RPF in all study phases including the placebo phase by about the same percentage. These results are in accordance with two other studies [28, 29], and, at first glance, rule out any change in endothelial function through the study medication. However, keeping in mind that there is an individually different response of RPF to the study medication between study participants, one is forced to use another approach to analyze the effect of L-NMMA on renal vascular tone.

Therefore, we calculated the correlation coefficients between the response of RPF to nitric oxide synthase inhibition with L-NMMA after seven days of treatment and the increase in RPF with a particular treatment (enalapril, eprosartan, or combination). Thus, our study used a more sensitive tool to focus more on the individual subject's reaction on both changes of RPF due to the study medication and to L-NMMA–induced vasoconstriction during the respective medication. With this approach, significant correlations were found between the increase in RPF through the study medication and the response of RPF to L-NMMA (as a measure of basal nitric oxide production and release) in the eprosartan and combination phases, but not in the enalapril phase. In other words, the improvement of RPF by eprosartan alone or in combination with enalapril is at least in part nitric oxide-dependent, whereas this is not the case to the same extent for enalapril. This interpretation is supported by the equality of the correlation coefficients in the eprosartan and combination phases.

Our study has several limitations. First, oral salt intake is a factor known to influence renal hemodynamics considerably [30], and our study participants were not kept on a metabolic ward for strictly controlling sodium intake by a standardized diet. However, they were advised to maintain their usual diet throughout the whole study period to reduce the probability that sodium intake changed during study periods. Besides, the relatively high salt intake that is common in Bavaria to some extent might be responsible for the relatively low blood pressure changes with ACEI and ARB treatment in our study participants [31]. Second, fluid load during a clearance study can influence renal hemodynamics markedly [32]. Therefore, the same well-defined volume was administered to our study participants at each examination. Third, differences in the time-course of drug action might be responsible for different effects on renal hemodynamics. For eprosartan, a once-daily dosage has been found to be as effective as a twice-daily dosage [33]. For enalapril, different responses of renal hemodynamics have been found 4, 12, and 24 hours after drug intake [20]. We decided to advise our participants to take their study medication for day 7 immediately before the *para*-aminohippurate and inulin infusions were started, so that exactly the same time interval between drug intake and administration of L-NMMA in all study participants could be guaranteed.

In conclusion, a one-week treatment with the combined eprosartan/enalapril therapy given at half the dose of each drug increases RPF via a nitric oxide-dependent

mechanism. Enalapril administered alone had no effect on renal vascular endothelial function nor did it increase RPF. Although the one-week treatment with eprosartan alone had no clear-cut effect on RPF, this substance appears to increase nitric oxide bioavailability in the renal vasculature.

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