

Moexipril, a New Angiotensin-Converting Enzyme (ACE) Inhibitor: Pharmacological Characterization and Comparison with Enalapril

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Accepted for publication July 21, 1995

ABSTRACT

The pharmacodynamic profile of the new angiotensin-converting enzyme (ACE) inhibitor moexipril and its active diacid, moexiprilat, was studied *in vitro* and *in vivo*. *In vitro*, moexiprilat exhibited a higher inhibitory potency than enalaprilat against both plasma ACE and purified ACE from rabbit lung. Upon oral administration of moexipril (10 mg/kg/day) to spontaneously hypertensive rats, plasma angiotensin II concentration decreased to undetectable levels, plasma ACE activity was inhibited by 98% and plasma angiotensin I concentration increased 8.6-fold 1 h after dosing. At 24 h, plasma angiotensin I and angiotensin II concentrations had returned to pretreatment levels, whereas plasma ACE activity was still inhibited by 56%. Four-week oral administration of moexipril (0.1–30 mg/kg/day) to spontaneously hypertensive rats lowered blood pressure and differentially inhibited ACE activity in plasma, lung, aorta, heart and kidney in a dose-dependent fashion. Equidose treatment

(10 mg/kg/day) with moexipril and enalapril over 4 weeks led to comparable decreases in blood pressure, inhibition of plasma ACE and reduction of plasma angiotensinogen and to a similar attenuation of the pressor responses to angiotensin I and potentiation of the depressor responses to bradykinin. In contrast, ACE inhibition in aorta, heart and lung was significantly greater with moexipril than with enalapril, whereas in the kidney both drugs inhibited ACE activity to a similar extent. In summary, moexipril is an orally active ACE inhibitor that is comparable to enalapril in potency and duration of antihypertensive activity. The results of the present study demonstrate that 1) the antihypertensive potency of a given ACE inhibitor cannot be predicted from its *in vitro* characteristics and 2) the degree of blood pressure reduction does not correlate with tissue ACE inhibition.

Moexipril (2-[1-ethoxycarbonyl]-3-phenylpropyl]amino-1-oxopropyl]-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (S,S,S)) (fig. 1) belongs to the class of carboxyl-containing ACE inhibitors and is structurally related to enalapril. It has been developed as a prodrug that, after absorption, is activated to the active metabolite, moexiprilat.

In general, ACE inhibitors do not seem to differ greatly from one another with respect to their pharmacodynamic actions. However, the pharmacokinetic behavior, including rate and degree of absorption, bioavailability, protein binding, tissue distribution, metabolism and route of excretion, may vary to a large extent. Prodrug ACE inhibitors are usually 100- to 1000-fold less potent at inhibiting ACE than their active diacids, but they feature an improved bioavailability (Unger and Gohlke, 1994).

The kinetics and extent of activation of ACE inhibitors by

esterases vary from species to species; activation takes place predominantly in the liver but also in the kidneys (Unger *et al.*, 1982), in the blood and even in the cerebrospinal fluid (Gohlke *et al.*, 1989). Benazepril, cilazapril and delapril are rapidly absorbed and converted to the active compounds (Kaiser *et al.*, 1989; Williams *et al.*, 1989; Saruta and Nishikawa, 1991), whereas other ACE inhibitors, such as enalapril and perindopril, show a more delayed bioactivation (Cohen and Kurz, 1982; Mac Fayden *et al.*, 1990).

Because of differences in the lipophilicity of ACE inhibitors, penetration through biological barriers and organ-specific ACE inhibition are different (Cohen *et al.*, 1983; Unger *et al.*, 1986; Cushman *et al.*, 1989; Gohlke *et al.*, 1989; Ranadive *et al.*, 1992). However, whether tissue-specific ACE inhibition contributes to the overall antihypertensive action of these drugs in the sense of a "tissue" RAS inhibition (Unger *et al.*, 1991) is still a matter of debate. With some notable exceptions, such as the brain, the testis and the epithelial brush

Received for publication November 23, 1994

ABBREVIATIONS: ACE, angiotensin-converting enzyme; ANG I, angiotensin I; ANG II, angiotensin II; AUC, area under the curve; EDRF, endothelium-derived relaxing factor; HPLC, high-pressure liquid chromatography; MAP, mean arterial blood pressure; PRC, plasma renin concentration; RAS, renin-angiotensin system; RIA, radioimmunoassay; SHR, spontaneously hypertensive rats; SHRSP, stroke-prone spontaneously hypertensive rats.

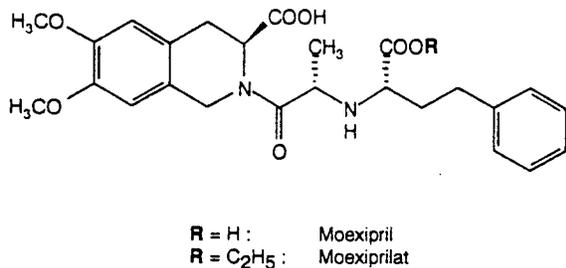


Fig. 1. Molecular structure of moexipril.

border of kidney tubules, ACE is located at the luminal site of the vascular endothelium, where it is easily accessible to ACE inhibitors circulating in the blood (Caldwell *et al.*, 1976; Ryan *et al.*, 1976; Gohlke *et al.*, 1992).

The mechanism of action of ACE inhibitors is still not fully understood. However, it is generally agreed that a repetitive reduction of circulating plasma ANG II levels by daily dosing, leading to a diminution of the effects of the peptide on vascular resistance, aldosterone release and sodium excretion, constitutes a principal mechanism of the chronic antihypertensive action of ACE inhibitors.

The aim of the present study was to characterize, *in vitro* and *in vivo*, the ACE-inhibitory and antihypertensive potency of moexipril and its active metabolite, the diacid moexiprilat, in comparison with enalapril. The pharmacodynamic features of moexipril were further characterized by comparing its effects on blood pressure in extent and time course with those on plasma parameters of the RAS.

Materials and Methods

Animals

Experiments were performed in adult male SHR (Møllegaard, Skensved, Denmark) weighing 280 to 360 g. Rats were housed individually and were kept under controlled temperature (24°C), humidity (60%) and light period (12-h light-dark cycles) and had free access to food and water throughout the experiments. Food consisted of commercial pellets (Altromin) containing approximately 100 meq/kg sodium and 210 meq/kg potassium.

Determination of Plasma and Tissue ACE Activity

Blood and tissue collection. Blood was collected from the retrobulbar venous plexus after short ether anaesthesia (45–60 s). For the determination of plasma ACE activity, blood (0.5 ml) was collected into chilled heparinized tubes. Blood samples were immediately centrifuged at $10,000 \times g$ and 4°C for 10 min, and then the plasma was separated and stored at -20°C. At the end of the treatment period and immediately after the last blood collection, animals were sacrificed by decapitation, and tissues (aorta, heart, kidney and lung) were removed and snap-frozen in liquid nitrogen. Until assayed for ACE activity, the tissues were stored at -20°C.

Homogenization. Aorta, heart, kidney and lung were homogenized on ice in 0.3% Triton-X 100 with a polytron homogenizer for 20 s, followed by sonification for 2×5 s. After centrifugation (15 min at $15,000 \times g$ at 4°C), ACE activity was assayed in the supernatant.

Determination of ACE activity. ACE activity was assayed by a modified fluorometric method using Z-Phe-His-Leu as substrate (Unger *et al.*, 1982). Plasma or tissue homogenate (50 μ l) were diluted with cold phosphate buffer (70 mM, pH 8.0, containing 300 mM sodium chloride) to a final volume of 450 μ l. The enzyme reaction was started by adding 50 μ l of a 10 mM substrate solution (Z-Phe-His-Leu) to the samples, which were then incubated at 37°C.

At the end of the incubation time (plasma and kidney 30 min, heart and aorta 60 min, lung 5 min), the reaction was terminated by transferring 100- μ l aliquots from the incubation mixture into 1 ml of 0.1 N NaOH. All subsequent steps in the assay were continued in the dark: 25 μ l of 2% ortho-phthalaldehyde solution in dimethyl sulfoxide was added to the samples. After 30 min, the reaction was terminated by addition of 1 ml of 0.8 M HCl, precipitates were spun down by a $3,000 \times g$ centrifugation step for 3 min, and fluorescence was measured within 60 min. Zero-time blank values were subtracted from the corresponding test values. All assays were performed in duplicate. Protein content of the tissue homogenates was analysed according to Lowry *et al.* (1951). The results are expressed as nmol His-Leu/ml/min (plasma) or nmol His-Leu/mg protein/min (tissues).

Determination of PRC, Angiotensinogen and ANG I

Blood collection. For the determination of renin, angiotensinogen and ANG I, blood (2 ml) was collected from the retrobulbar venous plexus after short ether anaesthesia (45–60 s) into an ice-cold mixture of 12.5 mM EDTA; 2.6 mM 1,10-phenanthroline and 0.2 g/l neomycine sulphate (50 μ l/ml blood). Blood samples were immediately centrifuged at $10,000 \times g$ and 4°C for 10 min, and then the plasma was separated and stored at -80°C.

Determination of PRC. Plasma (100 μ l) was added to 50 μ l of an inhibitor cocktail (32 mM 8-hydroxyquinoline, 33 mM diisopropylfluorophosphate, 43 mM 2,3-dimercapto-propanol) and 600 μ l of 0.1 M Tris-maleate buffer, pH 6.5, containing an excess of purified rat renin substrate (10 mg lyophilized renin substrate per 600 μ l buffer). Immediately after mixing, an aliquot of 200 μ l was transferred into a prechilled tube containing 200 μ l of RIA-buffer (0-h value). The tube was boiled for 5 min in a water bath and centrifuged (20 min at $5,000 \times g$). The supernatant ($3 \times 50 \mu$ l) was subjected to ANG I determination. The remaining sample mixture was incubated in a water bath at 37°C. After 1 and 2 h, aliquots of 200 μ l were treated as described above. PRC was calculated by subtraction of the amount of ANG I measured at time zero from the amount of ANG I produced within 1 and 2 h of incubation, respectively. PRC is expressed as pmol ANG I/ml·h.

Determination of angiotensinogen. Plasma (50 μ l) was added to 50 μ l of an inhibitor cocktail, 600 μ l of 0.1 M Tris-maleate buffer, pH 6.5, and 50 μ l of rat kidney extract containing an excess of renin. Aliquots of 200 μ l were taken at 0, 1 and 2 h and were treated as described above. The amount of ANG I cleaved from plasma angiotensinogen was determined by ANG I RIA. Data are expressed as pmol ANG I/ml.

Determination of ANG I by RIA. Plasma (50 μ l) was incubated with 450 μ l of 0.1 M Tris-acetate buffer (RIA buffer), pH 7.4, containing 0.1% of bovine serum albumin and 0.1% Triton X-100, 50 μ l tracer 125 I-ANG I (4,000 cpm) and 50 μ l antiserum (gift from E. Hackenthal, Germany; final dilution in the assay 8.3×10^{-6}) for 20 h at 4°C. The antibody-antigen complex was separated from "free" ANG I by addition of dextran-coated charcoal and subsequent centrifugation (20 min, $3,000 \times g$). Free radioactive ANG I (pellet) was counted in a well-type gamma counter. Interference with other peptides was as follows: ANG (2–10) 100%; ANG (1–8), ANG (2–8), ANG (3–8) and ANG (4–8) less than 0.01%. Standards ranged from 3.9 to 1000 fmol/tube. The international research standard ANG I was employed, and control samples of 20, 40 and 80 fmol/tube were run in triplicate with each assay.

Determination of ANG II in Plasma

Blood collection. For the determination of ANG II in plasma, blood was collected from the retrobulbar plexus (2 ml) after short ether anaesthesia (45–60 s) into an ice-cold mixture of 12.5 mM EDTA, 2.6 mM 1,10-phenanthroline and 0.2 g/l neomycine sulphate (50 μ l/ml blood). Blood samples were immediately centrifuged at $10,000 \times g$ and 4°C for 10 min, and then the plasma was separated, snap-frozen in liquid nitrogen and stored at -80°C.

Extraction of plasma. Plasma was extracted on phenylsilica cartridges (Bondelut, Analytichem), which were conditioned with 2 ml of methanol and then rinsed with 2 ml of water. One milliliter of cold plasma was rapidly passed through the cartridge, which was subsequently washed with water (3×1 ml). Absorbed angiotensins were eluted with methanol (3×0.5 ml) into conical polypropylene tubes. The eluate was evaporated to dryness by means of an air stream at room temperature, and the residue was stored at -80°C until HPLC separation. The recovery of the extraction was $94.7\% \pm 1.2\%$ ($n = 5$) as verified with ^{125}I -ANG II.

HPLC. Samples were reconstituted with $100 \mu\text{l}$ 1 M acetic acid, stirred and centrifuged for 5 min at $6,000 \times g$ and 4°C . The supernatant was injected directly into the HPLC system. The HPLC system (Waters, Eschborn, Germany) consisted of two pumps driven by a programmable control unit (Waters 600), an automatic injection unit (WISP 712), an integrated column heater and a fraction collector.

Angiotensins were separated on a reverse-phase column (Nucleosil 100-5-C18, 125×3 mm, Macherey & Nagel, Düren, Germany) using a linear gradient from 25% to 50% methanol containing 0.056% ortho-phosphoric acid over 30 min starting 4 min after injection. A good base-line separation of angiotensin peptides was achieved (fig. 2). Before separating small quantities of plasma angiotensins, each column was loaded with 100 to 200 ng of angiotensin I, II and III to check retention times by UV detection at 220 nm and to avoid loss of peptide occurring after use of unloaded columns. After loading, the columns were thoroughly rinsed, and blank runs after the injection of $100 \mu\text{l}$ of 1 M acetic acid were performed until no angiotensin was detectable by radioimmunoassay.

The temperature of the column was kept at 35°C . Consecutive fractions of 18 s ($150 \mu\text{l}$), collected into tubes containing $350 \mu\text{l}$ of RIA buffer were directly subjected to RIA. A standard mixture of ANG I, ANG II and ANG III was run daily to verify retention times and recoveries of the respective peptides. In addition, $100 \mu\text{l}$ of 1 M acetic acid (blank run) was injected daily to control whether carry-over between samples occurred.

Recovery of peptides on the HPLC column was determined by injection of ^{125}I -ANG II. The average recovery of ^{125}I -ANG II was $98.6\% \pm 2\%$ ($n = 5$).

Determination of ANG II by RIA. HPLC fractions with RIA buffer ($500 \mu\text{l}$) were incubated with $50 \mu\text{l}$ tracer ^{125}I -ANG II (4,000 cpm) and $50 \mu\text{l}$ antiserum (gift of D. Ganten, Berlin, Germany; final dilution in the assay 8.3×10^{-6}) for 20 h at 4°C . The antibody-

antigen complex was separated from "free" ANG II by addition of dextran-coated charcoal and subsequent centrifugation. Free radioactive ANG II (pellet) was counted in a well-type gamma counter. The evaluation of the samples was carried out by computer analysis, and standard curves were smoothed with spline functions. Standards ranged from 1 to 250 fmol/tube, and the detection limit was 2 pg ANG II/tube. Interference with other angiotensin peptides was as follows: ANG (2-8), ANG (3-8) and ANG (4-8) 100%; ANG (1-10) and ANG (2-10) $\leq 1\%$. The international research standard ANG II was employed and control samples of 10, 20, and 40 fmol/tube were run in triplicate with each assay.

Blood Pressure Measurements

Systolic blood pressure was measured under light ether anesthesia by tail plethysmography. MAP was measured directly by use of a Statham PC3Dh pressure transducer, amplified by a Gould Brush pressure computer (Gould Inc. Osham, CA) and recorded by an IBM-compatible PC-AT computer with specifically developed software (Stauss *et al.*, 1990). One day before experiments, all rats were instrumented with catheters placed in the abdominal aorta (PP-10 in PP-50, Portex Corp., Hythe, Kent, U.K.) and inferior vena cava (PP25) *via* the right femoral artery and vein for recording MAP and for i.v. administration of ANG I, ANG II and bradykinin. Catheters were brought to the exterior at the nape of the neck. On the day of the experiment, the rats were attached to the monitoring equipment. Experiments were started after a stabilization period of 60 min. Bradykinin (10, 100, 300 and 100 ng), ANG I (100 ng) and ANG II (100 ng) were dissolved in 0.9% NaCl and given as i.v. bolus injections at 5-min intervals. Depressor responses to bradykinin and pressor responses to ANG I and ANG II are expressed as area under the curve (AUC) (mmHg/min) of the blood pressure response.

Experimental Protocols

Experiment 1: *In vitro* inhibition of purified ACE from rabbit lung and of ACE activity in rat plasma by moexipril, enalapril and their active diacids. Purified ACE from rabbit lung was prediluted to yield an ACE activity of 101 to 105 nmol His-Leu/min/ml enzyme solution, which was comparable to plasma ACE activity in the rat (Unger *et al.*, 1985; Gohlke *et al.*, 1989).

A solution ($50 \mu\text{l}$) of either ACE purified from rabbit lung or rat plasma was diluted with ice-cold phosphate buffer (70 mM, pH 8, containing 300 mM sodium chloride) to a final volume of $400 \mu\text{l}$.

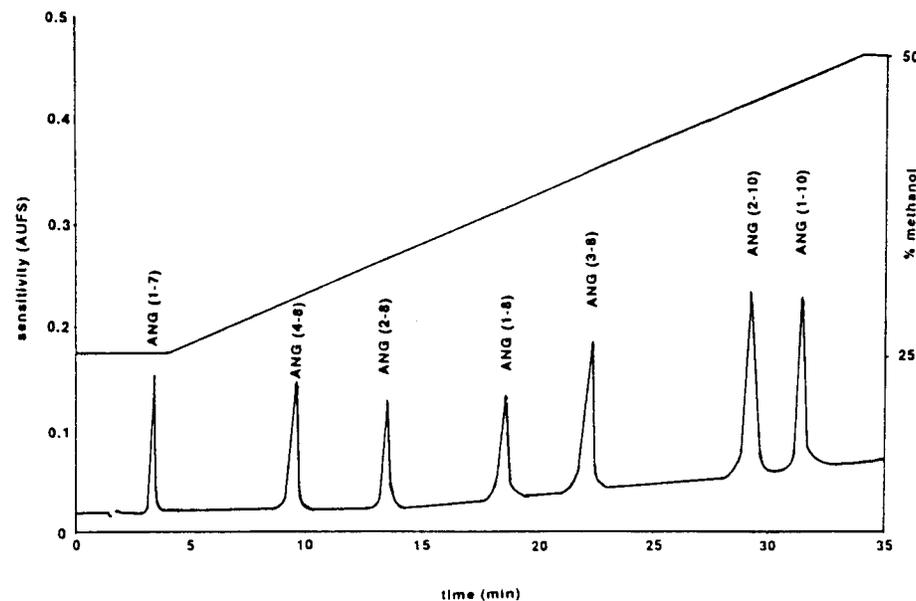


Fig. 2. HPLC separation of angiotensin peptides using a linear gradient from 25% to 50% methanol containing ortho-phosphoric acid over 30 min starting 4 min after injection; 1000 ng ANG I (ANG 1-10) (30:48 min) and 1000 ng ANG (2-10) (28:48 min), 500 ng ANG (1-7) (3:21 min), 500 ng ANG II (ANG 1-8) (18:12 min), 500 ng ANG III (ANG 2-8) (13:23 min), 500 ng ANG (3-8) (21:42 min), 500 ng ANG (4-8) (9:18 min). $\lambda = 220$ nm, temperature 35°C , flow 0.5 ml/min.

Vehicle (buffer) or drugs were added to the enzyme-buffer solution in a volume of 50 μ l to yield final assay concentrations ranging from 10^{-4} M to 10^{-11} M. The enzyme reaction was started by adding 50 μ l of a 10 mM substrate solution. Incubation time was 30 min. All assays were performed in duplicate.

Experiment 2: Time course of changes in the plasma RAS of SHR after oral treatment with moexipril for 3 days. Sixty SHR were randomly allocated into six groups ($n = 10$ per group). Five groups were given a dose of 10 mg/kg/day moexipril, and one group received vehicle (distilled water) by gavage for 3 days. At 1, 2, 4, 8 and 24 h after the last dose, the animals were sacrificed (moexipril-treated rats, $n = 10$ per time-point; vehicle-treated rats, $n = 2$ per time-point), and blood was collected for determination of plasma angiotensin peptides and ACE activity. Data for vehicle-treated rats were pooled.

Experiment 3: Effect of 4-week oral treatment with different doses of the ACE inhibitor moexipril on blood pressure and on plasma and tissue ACE activity in SHR. Fifty SHR were divided randomly into six groups and treated with moexipril at doses of 0.1, 1, 3, 10 or 30 mg/kg/day ($n = 8$ each). The ACE inhibitor moexipril was dissolved in distilled water and added to the overnight drinking fluid. The concentrations of the drug solutions were adjusted to the individual drinking habits of the rats to ensure appropriate dosing. Control animals ($n = 10$) were given tap water instead of the drug solution but otherwise were kept under identical conditions. Body weight and fluid intake were measured at 2 to 3-day intervals. Duration of treatment was 4 weeks.

Systolic blood pressure was measured (always between 9 and 11 A.M.) before treatment and after 2, 3 and 4 weeks of treatment. Plasma ACE activity was determined before treatment and after 3 days and 2 and 4 weeks of treatment. At the end of the treatment period, the animals were sacrificed, and ACE activity was determined in aorta, heart, kidney and lung.

Experiment 4: Effect of 4-week oral equidose treatment with moexipril and enalapril on blood pressure and on parameters of the plasma RAS. Thirty SHR were divided randomly into three groups ($n = 10$ each). Group 1 received moexipril (10 mg/kg/day), group 2 received enalapril (10 mg/kg/day) and group 3 served as control receiving vehicle (distilled water). Drugs or vehicle were administered once daily by gavage. Duration of treatment was 4 weeks. All measurements were performed at maximum plasma ACE inhibition, i.e., 2.5 h after drug administration. Systolic blood pressure was measured before treatment and after 1, 2, 3 and 4 weeks of treatment. Parameters of the plasma RAS (ACE activity, ANG I, renin and angiotensinogen) were measured before treatment and after 3 days and 2 and 4 weeks of treatment. At the end of the treatment period, the animals were sacrificed, and ACE activity was determined in aorta, lung, heart and kidney.

Experiment 5: Effect of 4-week oral equidose treatment with moexipril and enalapril on MAP, ANG I pressor responses and bradykinin depressor responses in conscious SHR. Thirty SHR were divided randomly into three groups ($n = 10$ each). Group 1 received moexipril (10 mg/kg/day), group 2 received enalapril (10 mg/kg/day) and group 3 served as control receiving vehicle (distilled water). Drugs or vehicle were administered once daily by gavage. After 4 weeks of treatment, blood pressure was measured directly in conscious, unrestrained animals through chronically implanted femoral artery catheters. The measurements were performed at maximum plasma ACE inhibition, i.e., 2.5 h after the last drug application. Rats were then challenged with iv. injections of ANG I, ANG II and bradykinin in order to test the degree of peripheral ACE inhibition *in vivo*.

Drugs

Bradykinin, ANG I and ANG II were purchased from Serva Laboratories, Heidelberg, Germany. Moexipril and moexiprilat were supplied by Schwarz Pharma AG (Monheim, Germany). Enalapril and enalaprilat were supplied by the Merck Institute for Therapeutic

Research (West Point, PA). Purified ACE from rabbit lung was a gift from P. Bünning (Frankfurt, Germany).

Statistics

Means \pm SEM are reported. Data were subjected to analysis of variance (ANOVA) followed by *post hoc* tests between groups (Bonferroni) when appropriate. Analysis of blood pressure data and of ACE activity data in experiments 3 and 4 was performed by ANOVA with repeated measures using the SYSTAT statistical software. When a significant difference was indicated between groups, univariate *F* test was used for analysis of differences between the control group and groups receiving various drug doses. A significance level of $P < .05$ was accepted.

Results

In vitro inhibition of purified ACE from rabbit lung.

Full concentration-response curves were recorded for the prodrug ACE inhibitors moexipril and enalapril and their respective active diacid forms (fig. 3). All ACE inhibitors concentration-dependently inhibited purified ACE from rabbit lung with comparable slopes of concentration-response curves. The prodrugs were generally much less potent than the diacid compounds.

Calculated IC_{50} values are listed in table 1. Moexiprilat proved to be more active against purified ACE than the diacid of enalapril (table 1).

Because of the absence of esterases in the purified ACE preparation, the prodrug inhibitors were not converted to the active diacids *in vitro*. Accordingly, the prodrug ACE inhibitors moexipril and enalapril were found to be only weak inhibitors of purified ACE from rabbit lung (table 1). The

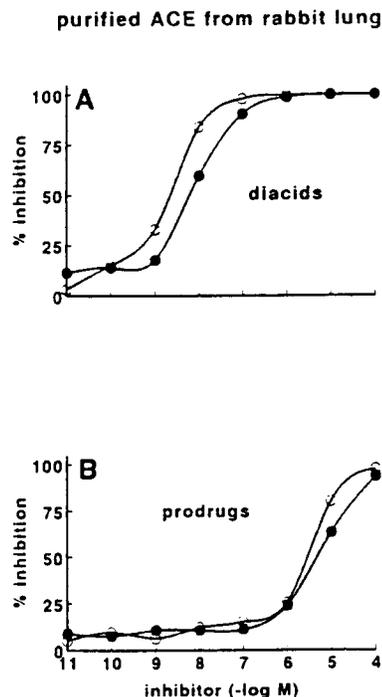


Fig. 3. A) *In vitro* inhibition of purified ACE from rabbit lung (active diacids); \circ moexiprilat, \bullet enalaprilat. B) *In vitro* inhibition of purified ACE from rabbit lung (prodrugs); \circ moexipril, \bullet enalapril. The inhibitors were added to the ACE assay incubation at final concentrations of 10^{-4} M– 10^{-11} M. Control ACE activity of purified ACE solution ranged from 101 to 105 nmol of His-Leu/ml/min.

TABLE 1

In vitro inhibition of purified ACE from rabbit lung and ACE in rat plasma (IC_{50} values)

ACE Inhibitor	Purified ACE			Rat Plasma		
	Prodrug (nM)	Active Drug (nM)	Ratio	Prodrug (nM)	Active Drug (nM)	Ratio
Moexipril	2700	2.1	1300	165	1.75	94.3
Enalapril	7700	5.4	1400	200	6	33.3

IC_{50} values were calculated from the curves shown in figures 3 and 4.

ratios between IC_{50} values for prodrugs and their respective diacids (table 1) reveal that the active diacids were 1300 times (moexiprilat) to 1400 times (enalaprilat) more active against purified ACE than the respective prodrugs.

***In vitro* inhibition of ACE activity in rat plasma.** The concentration-dependent inhibition *in vitro* of plasma ACE activity by the prodrug ACE inhibitors moexipril and enalapril and their respective active diacid forms is shown in figure 4.

The *in vitro* prodrug activities in plasma were markedly enhanced when compared with purified enzyme preparations because of a partial biotransformation to the active diacids by plasma esterases. The ratios between IC_{50} values for prodrugs and their respective diacids (table 1) reveal that the active diacids were 33 times (enalaprilat) to 94 times (moexiprilat) more active against plasma ACE than the respective prodrugs. Moexipril revealed an inhibitory potency close to that of enalapril, and its active diacid, moexiprilat, was found to have a higher ACE-inhibitory potency than enalaprilat.

Time course of changes in the plasma RAS of SHR after oral treatment with moexipril for 3 days. SHR were treated orally by gavage for 3 days with 10 mg/kg/day

moexipril, and plasma parameters of the RAS were determined 1, 2, 4, 8 and 24 h after the last dosage.

The concentration of HPLC-separated ANG II in plasma decreased to 9% and 28% of control values 1 and 2 h after dosage, respectively, but recovered to control levels 24 h after dosage. In parallel, the HPLC-separated ANG I concentration increased 8.6-fold 1 h after dosage but returned to basal conditions 24 h after dosage (fig. 5). Plasma ACE activity was completely blocked 1 h after drug administration and was still inhibited by 56% 24 h afterward.

Effect of 4-week oral treatment with different doses of the ACE inhibitor moexipril on blood pressure and plasma and tissue ACE activity in SHR. Oral treatment of SHR with moexipril at doses ranging between 0.1 and 30 mg/kg/day produced dose-dependent decreases in arterial blood pressure (fig. 6).

Plasma ACE activity was inhibited dose-dependently after 3 days as well as after 2 and 4 weeks of treatment with moexipril (fig. 6). With the exception of the 10 mg/kg-dose, this effect appeared already to be maximal after 3 days of oral treatment with moexipril. ACE activity in tissue homogenates (lung, aorta, heart and kidney) was inhibited dose-dependently by moexipril with no significant difference between the 10-mg/kg and 30-mg/kg groups after 4 weeks of treatment. At a dose of 30 mg/kg/day, inhibition was highest in lung, followed by aorta, kidney and heart (fig. 7).

Effect of 4-week oral equidose treatment with moexipril and enalapril on systolic blood pressure and on parameters of the plasma RAS. Oral administration of moexipril and enalapril in SHR at a dose of 10 mg/kg/day lowered blood pressure to a similar extent over a treatment period of 4 weeks (fig. 8). The blood pressure reduction was most pronounced after 1 week of treatment. Plasma ACE activity was similarly inhibited in both treatment groups by 76% to 82% (moexipril) and 84% to 88% (enalapril) throughout treatment (fig. 8). Both inhibitors produced a significant inhibition of ACE activity in tissue homogenates after 4 weeks of treatment. Although ACE inhibition in the kidney was similar in both treatment groups (31% with moexipril, 35% with enalapril; fig. 9), moexipril more effectively blocked ACE activity in lung, aorta and heart (fig. 9) than enalapril: ACE activity in lung, aorta and heart was inhibited by 88%, 59% and 44%, respectively, after 4 weeks of treatment with moexipril, whereas the inhibiting effects of enalapril were only moderate or absent (47%, 8% and 0% in lung, aorta and heart, respectively). Both ACE inhibitors produced changes in the parameters of the plasma RAS characteristic for ACE inhibition. PRC and immunoreactive ANG I were increased and plasma angiotensinogen was reduced after 3 days as well as after 2 and 4 weeks of treatment (fig. 10). Enalapril consistently caused a greater increase in *ir*-ANG I and PRC than moexipril at all time-points (fig. 10).

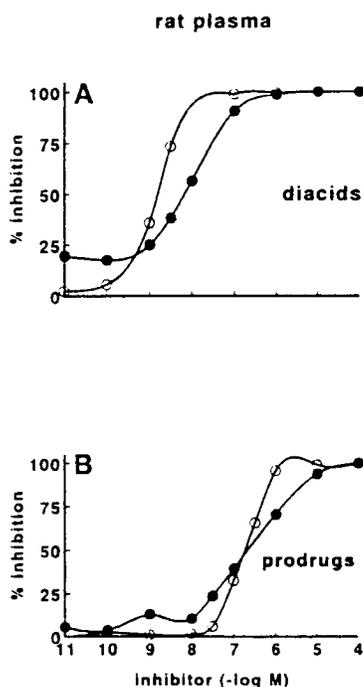


Fig. 4. A) *In vitro* inhibition of ACE in rat plasma (active diacids); ○ moexiprilat, ● enalaprilat. B) *In vitro* inhibition of ACE in rat plasma (prodrugs); ○ moexipril, ● enalapril. The inhibitors were added to the ACE assay incubation at final concentrations of 10⁻⁴ M–10⁻¹¹ M.

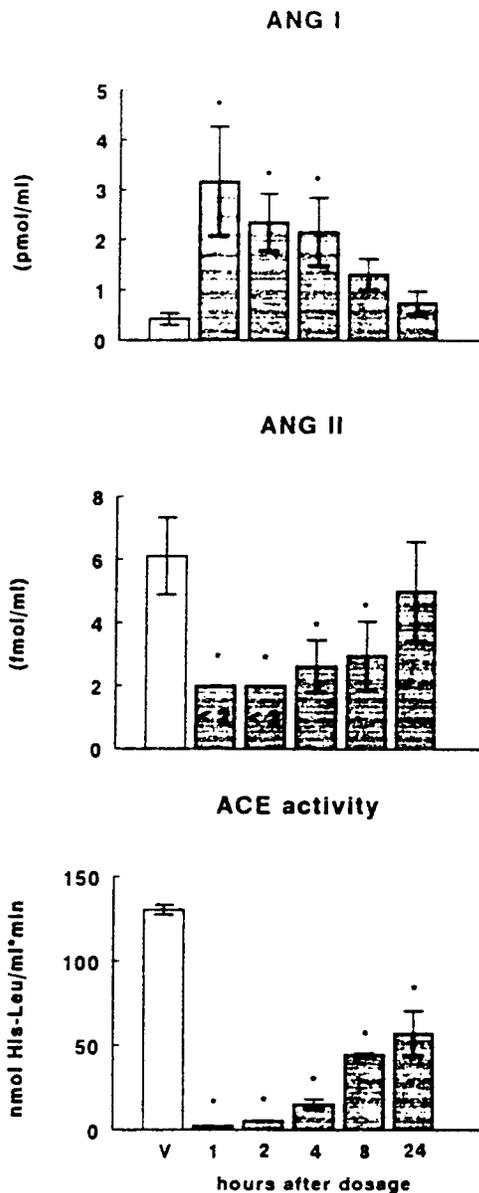


Fig. 5. Time course of changes in the plasma RAS (ANG I, ANG II, ACE activity) in SHR after oral gavage treatment with 10 mg/kg/day moexipril for 3 days. Plasma parameters of the RAS were determined 1, 2, 4, 8 and 24 h after the last dosage. Angiotensin peptides were extracted on phenylsilica columns and separated by HPLC before quantitation by radioimmunoassay. $n = 10$ for all time-points. V: vehicle treatment ($n = 10$); * $P < .05$ vs. vehicle.

Effect of 4-week oral equidose treatment with moexipril and enalapril on MAP, ANG I pressor responses and bradykinin depressor responses in conscious SHR. Oral equidose treatment with moexipril and enalapril at 10 mg/kg/day significantly decreased MAP after 4 weeks of treatment. At this time-point, MAP was 140 ± 6.5 mmHg (moexipril), 139 ± 3.5 mmHg (enalapril) and 174 ± 6.1 mmHg (vehicle) (fig. 11). The pressor responses to 100 ng ANG I i.v. were reduced by 85% in the moexipril-treated rats and by 88% in the enalapril-treated rats when compared with vehicle-treated control SHR (fig. 11), and ANG II (100 ng) i.v. produced similar pressor effects in all groups (fig. 11). The

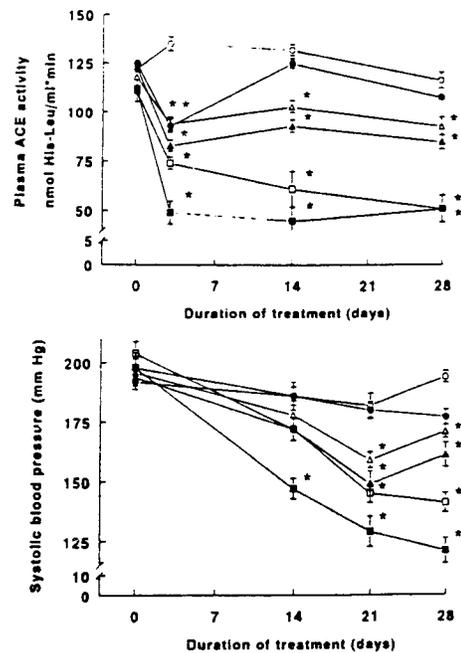


Fig. 6. Plasma ACE activity and systolic blood pressure of SHR treated for 4 weeks with different doses of moexipril in the overnight drinking water. \circ vehicle (tap water), \bullet 0.1 (mg/kg/day), \triangle 1 (mg/kg/day), \blacktriangle 3 (mg/kg/day), \square 10 (mg/kg/day), \blacksquare 30 (mg/kg/day); $n = 8$ to 10 per group, * $p < 0.05$ vs. vehicle.

depressor responses to i.v. bradykinin (10–1000 ng) were potentiated to a similar extent in both treatment groups by a factor of 2.9 to 3.9 (moexipril) and 3.1 to 4.6 (enalapril) (fig. 11).

Discussion

In the present study, the new ACE inhibitor moexipril was characterized pharmacodynamically in comparison with enalapril by investigation of its ACE-inhibitory potency *in vitro* and *in vivo* and by its time- and dose-dependent effects on blood pressure and on plasma and tissue components of the RAS after oral administration.

The active diacid of the prodrug ACE inhibitor moexipril proved to be a potent inhibitor of purified ACE from rabbit lung with a higher *in vitro* inhibitory potency than the diacid form of enalapril. Compared with their respective prodrugs, the active diacids were about 3 log orders more potent in inhibiting purified ACE from rabbit lung because of the lack of esterase activity present in this preparation.

Moexiprilat showed a higher *in vitro* inhibitory potency against rat plasma ACE than enalaprilat. In contrast to purified ACE from rabbit lung, plasma contains sufficient esterases to hydrolyze ACE inhibitor prodrugs to their active diacids. Thus the prodrugs caused *in vitro* inhibition of plasma ACE because of partial hydrolysis to their respective active forms. The degree of bioactivation of prodrug ACE inhibitors in rat plasma can be estimated from the prodrug-to-active-diacid ratio of IC_{50} values. These data indicate that moexipril appeared to be less activated to moexiprilat by rat plasma, as shown by the high ratio of 94.3, than enalapril (ratio of 33.1). The results obtained for the *in vitro* inhibition

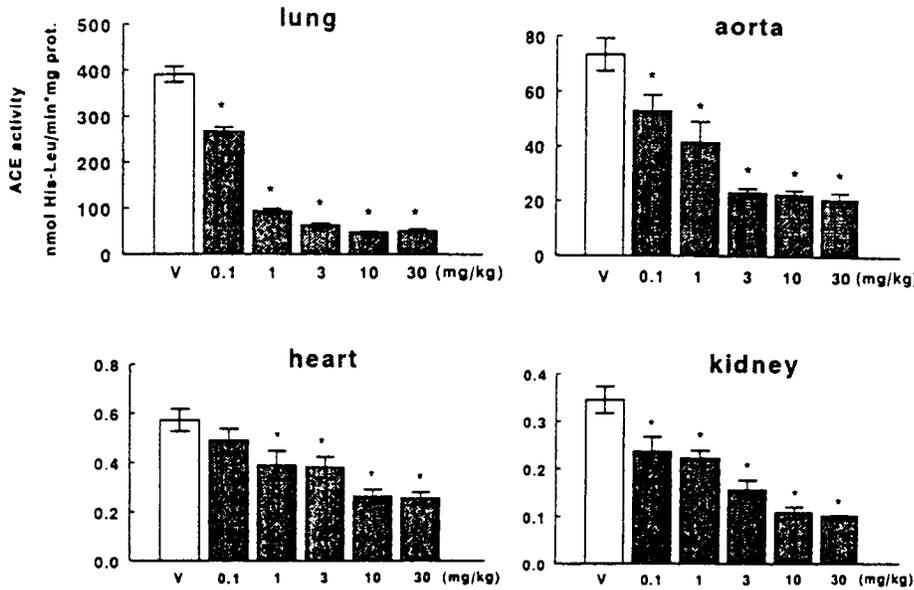


Fig. 7. ACE activity in various tissues of SHR after 4 weeks of treatment with different doses of moexipril in the overnight drinking water ($n = 8$ per group). V: vehicle (distilled water; $n = 10$), * $P < .05$ vs. vehicle.

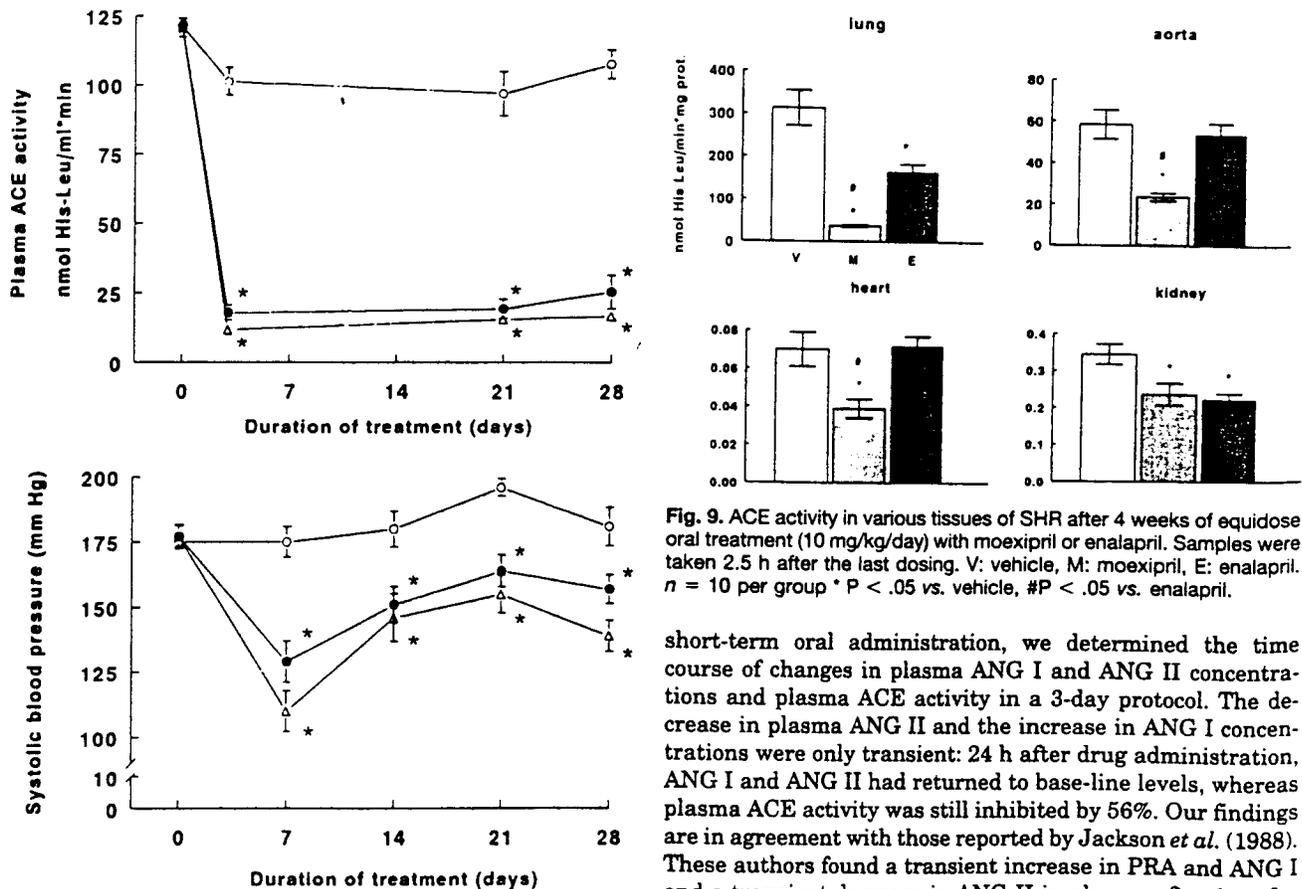


Fig. 8. Plasma ACE activity and systolic blood pressure of SHR 2.5 h after 10 mg/kg/day moexipril or enalapril by gavage during a 4-week treatment protocol. \circ vehicle (distilled water), \bullet moexipril, \triangle enalapril; $n = 10$ per group, * $P < .05$ vs. vehicle.

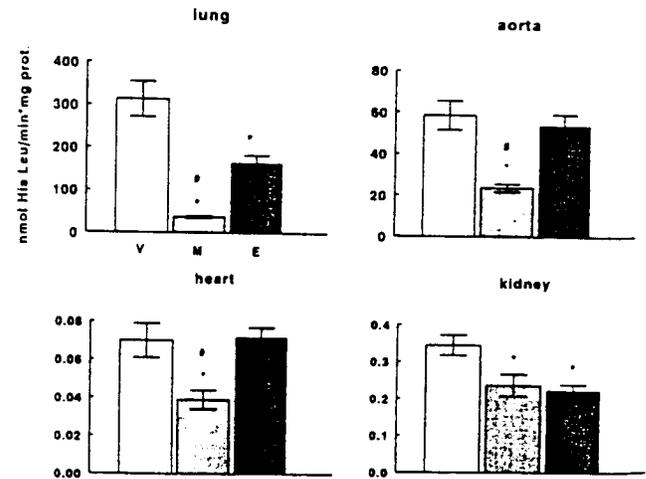


Fig. 9. ACE activity in various tissues of SHR after 4 weeks of equidose oral treatment (10 mg/kg/day) with moexipril or enalapril. Samples were taken 2.5 h after the last dosing. V: vehicle, M: moexipril, E: enalapril. $n = 10$ per group * $P < .05$ vs. vehicle, # $P < .05$ vs. enalapril.

short-term oral administration, we determined the time course of changes in plasma ANG I and ANG II concentrations and plasma ACE activity in a 3-day protocol. The decrease in plasma ANG II and the increase in ANG I concentrations were only transient: 24 h after drug administration, ANG I and ANG II had returned to base-line levels, whereas plasma ACE activity was still inhibited by 56%. Our findings are in agreement with those reported by Jackson *et al.* (1988). These authors found a transient increase in PRA and ANG I and a transient decrease in ANG II in plasma after 1 mg/kg perindopril in normotensive rats. However, in contrast to our results, basal ACE activity was restored 24 h after drug administration in their study.

The initial fall of plasma ANG II concentrations to almost undetectable levels after oral administration of moexipril is consistent with the notion that ANG II reduction is the principal mechanism of the antihypertensive action of ACE inhibitors in SHR, although other mechanisms, such as po-

of ACE in rat plasma by enalapril agree with data from earlier studies (Unger *et al.*, 1986; Gohlke *et al.*, 1989).

To investigate the effect of moexipril on the RAS after

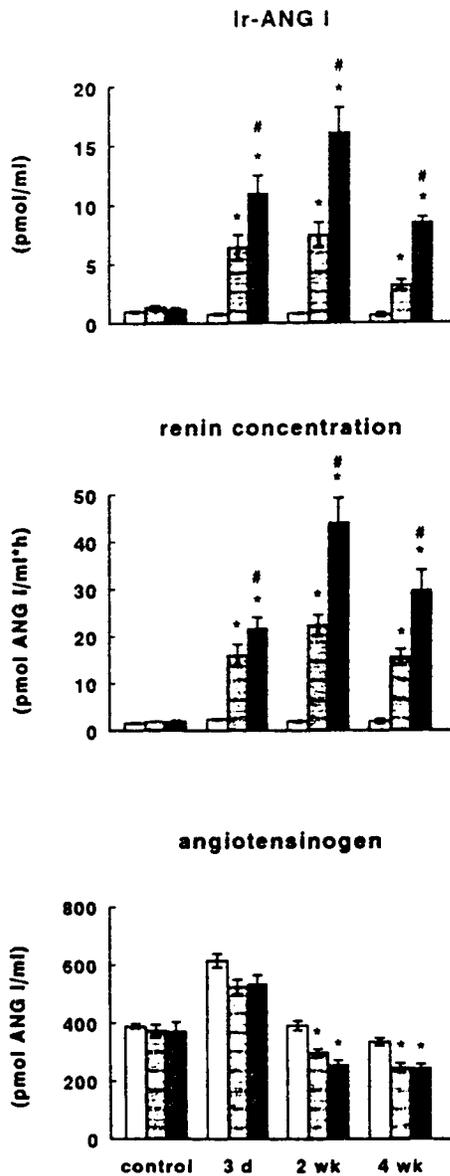


Fig. 10. Plasma parameters of the RAS in SHR during 4 weeks of oral treatment (10 mg/kg/day) with moexipril (grey bars, $n = 10$), enalapril (black bars, $n = 10$) or vehicle (distilled water; open bars, $n = 10$), * $P < .05$ vs. vehicle, # $P < .05$ vs. enalapril; d: days; wk: weeks; Ir-ANG I: immunoreactive angiotensin I.

tentiation of direct, prostaglandin- or endothelial-derived relaxing factor (EDRF)-mediated effects of bradykinin, are also relevant in other forms of hypertension (Bao *et al.*, 1992). The concentrations of ANG II in plasma reported here are in accordance with other studies in normotensive Sprague-Dawley rats and in normotensive volunteers investigating the time course of plasma ANG II concentration after ACE inhibition (Jackson *et al.*, 1988, Mooser *et al.*, 1990). Our results confirm a complete blockade of ANG II formation in plasma at peak inhibition of ACE activity, as proposed by Nussberger *et al.* (1985). In contrast, Campbell *et al.* (1994) have recently reported plasma ANG II concentrations of 30% to 40% of control (26 pg/ml) after treatment with various doses of perindopril in normotensive Sprague-Dawley rats.

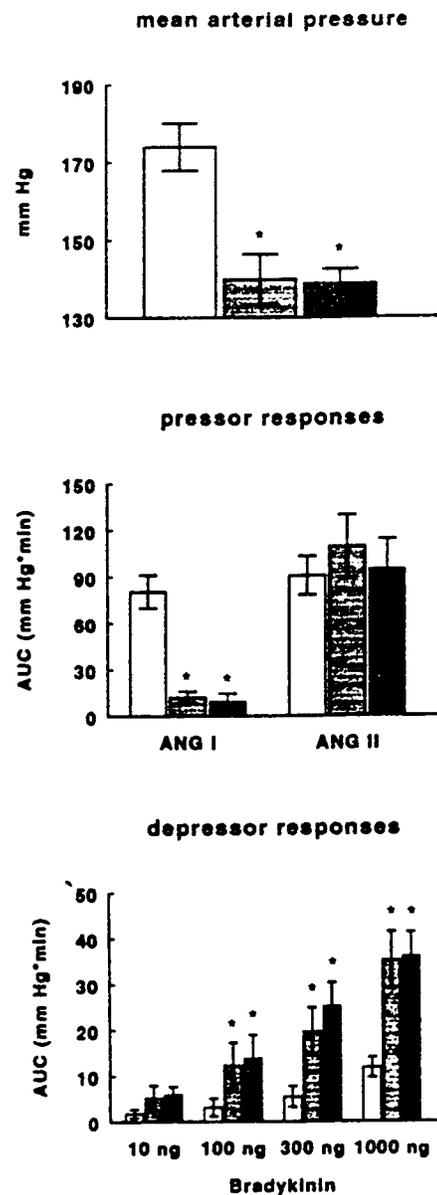


Fig. 11. MAP and blood pressure responses to i.v. ANG I (100 ng), ANG II (100 ng) and bradykinin after 4 weeks of oral treatment (10 mg/kg/day) with ACE inhibitors in SHR. Grey bars: moexipril; black bars: enalapril; open bars: vehicle (distilled water), $n = 10$ per group, * $P < .05$ vs. vehicle. AUC: area under the curve.

The authors suggested that an alternative pathway of conversion of ANG I to ANG II or direct conversion of angiotensinogen to ANG II by a serine protease may be responsible for the lack of complete inhibition of ANG II generation (Okunishi *et al.*, 1987; Urata *et al.*, 1990). According to Campbell *et al.* (1993), the alternative pathway of ANG II generation may contribute 10% of the plasma ANG II formation in intact rats. However, these conclusions were drawn from studies in normotensive animals and remain to be confirmed in hypertensive strains.

On the other hand, the recovery of plasma ANG II concentration within 24 h after drug administration can also be explained by the action of residual ACE activity on an in-

creased amount of circulating ANG I (Nussberger *et al.*, 1986).

The rise in PRC and plasma ANG I is a consistent feature of ACE inhibition because of withdrawal of the ANG II-mediated negative feedback on renin release (Hackenthal *et al.*, 1990). After treatment with moexipril, the increases in PRC and plasma ANG I were less pronounced than those after treatment with enalapril, acutely after 3 days as well as under chronic treatment after 4 weeks. This result was probably due to a more pronounced reduction of circulating ANG II after enalapril, although for technical reasons we did not measure HPLC-controlled ANG II levels in this part of the study.

Our results demonstrate that the ACE inhibitor moexipril has a marked antihypertensive potency in SHR after chronic treatment. The threshold dose for antihypertensive action was between 0.1 mg/kg/day and 1 mg/kg/day. Blood pressure was permanently lowered at a dose of 1 mg/kg/day and reached normotensive levels at a dose of 30 mg/kg/day. The blood pressure lowering effect of moexipril was paralleled by a dose-dependent inhibition of ACE activity in plasma and tissues. Moexipril and enalapril proved to be equipotent in lowering blood pressure and inhibiting plasma ACE activity in SHR during chronic oral treatment. With respect to blood pressure, this was demonstrated by measurement of systolic blood pressure *via* tail plethysmography during treatment as well as by direct measurement of MAP *via* an arterial catheter at the end of treatment. Similar inhibition of ACE activity with both drugs was demonstrated by direct measurement of plasma ACE activity at various time-points of treatment as well as by *in vivo* inhibition of pressor responses to ANG I and potentiation of depressor responses to bradykinin at the end of treatment. It should be pointed out that both drugs were used in formulations with comparable molecular weights, *e.g.*, moexipril-HCl (MW 535); enalapril-maleate (MW 502). Thus, although moexipril is more potent *in vitro* against purified ACE from rabbit lung or rat plasma ACE when compared with enalapril, both substances are equipotent *in vivo* when administered orally as prodrugs.

These observations can be explained on the basis of our *in vitro* experiments. Moexipril is 4 times more potent against ACE in rat plasma than enalapril, but enalapril is converted 3 times faster to its active diacid compound than moexipril. Furthermore, the bioavailability of enalapril (40%) is higher than that of moexipril (17%–26%), possibly because the absorption of moexipril is limited to specific sites of the gastrointestinal tract (Grass and Morehead 1989).

After chronic oral treatment, moexipril caused a higher *ex vivo* inhibition of ACE in lung, aorta and heart tissue homogenates than enalapril, whereas in kidney homogenates, both drugs produced a similar ACE inhibition. Therefore, there does appear to be a correlation between antihypertensive potency and the ACE-inhibitory potency in plasma and specifically in the kidney. However, the relatively high degree of ACE inhibition in the kidney by enalapril can also be ascribed to an accumulation of the drug within its organ of elimination. The more lipophilic drug, moexipril, is preferentially eliminated by the liver (Unger and Gohlke, 1994) and thus probably does not accumulate in the kidney to the same extent as enalapril. Theoretically, the differences in tissue ACE inhibition between moexipril and enalapril could also be

explained by different binding characteristics (Ranadive *et al.*, 1992) or different bioactivation in tissues.

Results similar to those reported here were obtained in a previous study from our laboratory comparing ramipril and enalapril (Unger *et al.*, 1985). Administration of the more lipophilic ACE inhibitor ramipril yielded greater ACE inhibition in lung, aorta and heart of SHRSP than enalapril, whereas in the kidney, both ACE inhibitors produced equal ACE inhibition. In another study, Cushman *et al.*, (1989) examined the inhibition of tissue ACE as a function of time after administration of equivalent doses of seven ACE inhibitors including enalapril. Enalapril produced only a small and transient (1-h) inhibition of ACE in heart tissue, whereas in other tissues, such as lung, aorta and kidney and in plasma, ACE inhibition was more pronounced and more persistent.

The tissue-specific differences in ACE inhibition in the present study are unlikely to be related to pharmacokinetic properties of the two compounds. Tissue collection as well as measurement of blood pressure and ACE activity were performed 2.5 h after drug administration. At that time-point, both ACE inhibitors exerted maximal inhibitory activity. This was demonstrated in a study by Cohen and Kurz (1982) wherein maximal ACE inhibition in serum and tissues occurred 1 to 3 h after oral administration of enalapril. Peak blood levels of moexipril have been reported 1.5 to 2.8 h after treatment (Grass and Morehead, 1989), which is consistent with the results reported in the present study showing a complete inhibition of plasma ACE activity and a reduction in plasma ANG II levels for at least 2 h after drug application.

Another important factor influencing the degree of tissue ACE inhibition has been described recently by Kinoshita *et al.* (1993). These authors found that the dissociation of the enzyme-inhibitor complex during tissue processing is a crucial factor that usually leads to an underestimation of the actual inhibition of ACE in tissues. Differences between moexipril and enalapril in their binding characteristics to ACE may thus contribute to the observed differences in tissue ACE inhibition.

Although, except for the kidney, we observed tissue-specific differences in ACE inhibition between moexipril and enalapril, these were obviously not correlated with the antihypertensive action of the drugs. Therefore, the contribution of ACE inhibition in tissues to the antihypertensive action of ACE inhibitors seems to be limited. This view is supported by a study of Chevillard *et al.* (1988) comparing tissue ACE inhibition in serum and tissues of Sprague-Dawley rats after the administration oftrandolapril and enalapril. Almost complete ACE inhibition in serum, heart ventricle, renal inner cortex, lung, aorta, adrenal cortex and medulla was achieved with doses between 3 and 300 µg/kg oftrandolapril without any effect on blood pressure, and the latter was reduced only after 3 mg/kgtrandolapril and 30 mg/kgenalapril, respectively.

However, local ACE inhibition at cardiac and vascular sites can produce beneficial actions on organ function and metabolism and can affect structural parameters independently of a reduction of blood pressure and plasma ACE activity, as demonstrated in recent studies in SHR and SHRSP after long-term, low-dose treatment with ramipril (Unger *et al.*, 1992; Gohlke *et al.*, 1993, 1994). Therefore,

ACE inhibitors with high affinity to ACE located at cardiac and vascular sites, such as ramipril and moexipril, may produce beneficial cardiovascular actions even at low doses that do not affect blood pressure.

In summary, moexiprilat, the active diacid of the new ACE inhibitor moexipril, proved to be a potent inhibitor of ACE with a higher *in vitro* inhibitory potency than enalapril. *In vivo*, its prodrug moexipril dose-dependently inhibited ACE activity in different tissues with a higher inhibitory potency than enalapril. However, moexipril and enalapril produced a similar reduction in blood pressure and inhibition of the plasma RAS.

Our data reveal that the antihypertensive potency of ACE inhibitors cannot always be predicted from their *in vitro* characteristics and that ACE inhibition in tissue homogenates does not correlate with the antihypertensive action of ACE inhibitors but may rather be linked to organ-specific functional and metabolic changes.

Acknowledgments

We thank Mrs. R. Rössle and Mrs. S. Högenauer for their excellent technical assistance.

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