A dose-response study to assess the renal hemodynamic, vascular, and hormonal effects of eprosartan, an angiotensin II AT_1-receptor antagonist, in sodium-replete healthy men

Study design: The effects of orally administered eprosartan on changes induced by angiotensin II in blood pressure, renal hemodynamics, and aldosterone secretion were evaluated in healthy men in this double-blind, randomized, single-dose, placebo-controlled crossover study, which was conducted in three parts. Part 1 (n = 12) assessed the onset and duration of the effect of eprosartan 350 mg or placebo; part 2 (n = 14) assessed the dose-response profile of placebo or 10, 30, 50, 70, 100 or 200 mg eprosartan; and part 3 (n = 5) assessed the duration of the effect of 50, 100, or 350 mg eprosartan.

Results: In part 1 of the study, 350 mg eprosartan caused complete inhibition of angiotensin II–induced pressor and renal blood flow hemodynamic effects (effects on effective renal plasma flow [ERPF]) and inhibited angiotensin II–induced stimulation of aldosterone secretion from 1 to 3 hours after administration. Eprosartan, 350 mg, inhibited the effects of exogenous angiotensin II by approximately 50% to 70% from 12 to 15 hours after dosing. Eprosartan had no angiotensin II agonistic activity and produced an increase in ERPF starting at 1 to 4 hours after dosing. In study part 2, at 3 hours after single doses of 10, 30, 50, 70, 100, and 200 mg, eprosartan inhibited angiotensin II–induced decreases in ERPF by 39.1%, 49.9%, 33.0%, 56.0%, 71.0%, and 85.7%, respectively, compared with placebo. In study part 3, 50, 100, and 350 mg eprosartan produced measurable inhibition of angiotensin II–induced decreases in ERPF from 12 to 15 hours after administration. In parts 2 and 3, the eprosartan angiotensin II antagonism on blood pressure response and aldosterone secretion mirrored the angiotensin II antagonism on ERPF. (Clin Pharmacol Ther 1997;63:471-81.)

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The renin-angiotensin system is a complex of enzymes, proteins, and peptides that are involved in blood pressure regulation and fluid and electrolyte balance.[1] Angiotensin II, the primary effector hormone of the renin-angiotensin system, produces arteriolar smooth muscle contraction and stimulation of aldosterone production in the adrenal cortex through the AT_1-receptor as two of its principal actions. The net effect of activation of the renin-angiotensin system is an elevation of blood pressure and sodium retention.

Regulation of the renin-angiotensin system occurs primarily in the kidney and provides a rapid and efficient mechanism for producing acute changes in blood pressure and fluid and electrolyte balance. Decreases in renal perfusion pressure, increases in renal β-adrenergic stimulation, and sodium depletion are the major stimuli of renal renin release, which results in increased angiotensin II production.

The renin-angiotensin system is often activated in diseases such as hypertension, congestive heart failure, and

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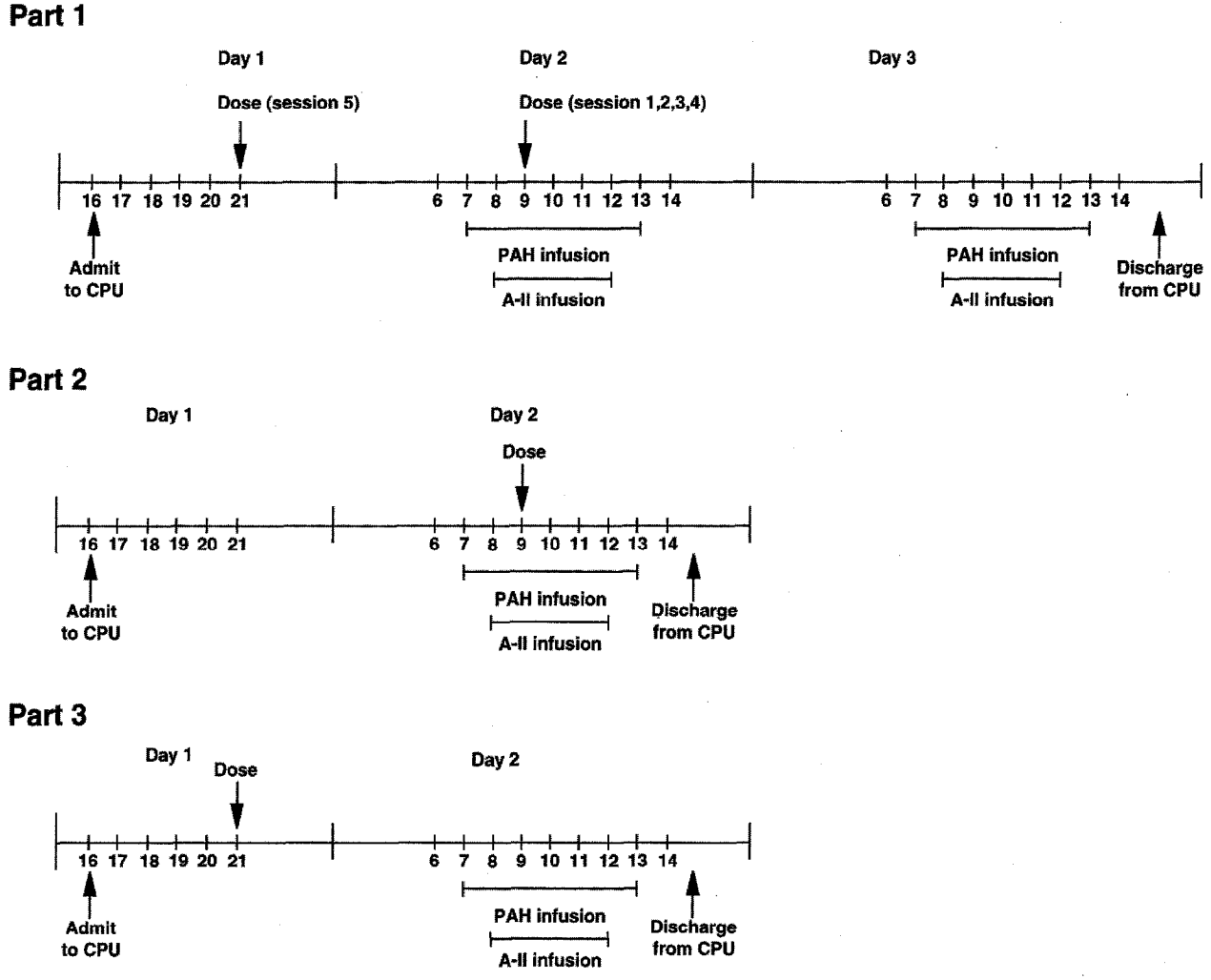

Fig. 1. Study schematic for parts 1, 2, and 3. Time of day is on a 24-hour clock. CPU, Clinical Pharmacology Unit; PAH, *para*-aminohippurate; A-II, angiotensin II.

chronic renal failure and plays a central role in the pathophysiology of these disorders. A number of nonpeptide angiotensin II antagonists have been described recently[2,3] that differ from the angiotensin converting enzyme inhibitors in that they directly inhibit angiotensin II at the AT_1-receptor level rather than blocking angiotensin II production. Eprosartan [Teveten] is a non-biphenyl, non-tetrazole angiotensin II receptor antagonist, chemically distinct from other angiotensin II receptor blockers, that has been shown in rats and dogs to be a potent and highly selective competitive antagonist of angiotensin II at the AT_1-receptor.[3-5] Eprosartan has recently been approved for the therapy of essential hypertension.

This study was designed to evaluate the inhibitory effects of eprosartan on angiotensin II–induced changes in blood pressure, renal hemodynamics, and aldosterone secretion in sodium-replete healthy men. This was the first study to evaluate the pharmacologic effects of eprosartan in humans. Because the renal circulation is sensitive to angiotensin II, effective renal plasma flow (ERPF, as determined by the plasma clearance of *para*-aminohippurate) is more sensitive to the effects of angiotensin II than either blood pressure or aldosterone secretion.[6] ERPF was therefore evaluated as the primary pharmacodynamic end point of the study. The onset and duration of angiotensin II blockade and the dose-response relationship of angiotensin II blockade after administration of oral eprosartan were also characterized. Subjects were maintained on a high-salt diet (up to 200 mEq sodium and 100 mEq potassium per day) throughout the study to suppress endogenous angiotensin II formation.

METHODS

The study was conducted at the SmithKline Beecham Clinical Pharmacology Unit (Philadelphia, Pa.). The protocol and statement of informed consent were approved by the Institutional Review Board of the Presbyterian Medical Center of Philadelphia before the start of the study, and the study was conducted in accordance with the Declaration of Helsinki and Title 21 of the U.S. Code of Federal Regulations. Subjects gave written informed consent before they were enrolled in the study. Single oral eprosartan doses of up to 350 mg were evaluated in this study because doses up to 350 mg were safe and well tolerated in an initial safety dose-rising study in healthy subjects (data on file, SmithKline Beecham Pharmaceuticals). Eprosartan was safe and well tolerated at single oral doses up to 350 mg by the subjects in this study.

Study design

A schematic of the study design is presented in Fig. 1. The study was conducted in three parts (parts 1, 2, and 3). Each part of the study was a randomized, double-blind, placebo-controlled, four-period (sessions 1, 2, 3, and 4), period-balanced crossover design. Subjects participated in only one part of the study. Part 1 assessed the onset and duration of the inhibitory effect of eprosartan or placebo on angiotensin II–induced decreases in ERPF with subjects receiving single oral doses of 350 mg eprosartan or placebo during angiotensin II infusions. An additional open-label study session (session 5) was added to part 1 of the study to further investigate the activity of 350 mg eprosartan from 12 to 15 hours after dosing. Part 2 assessed the dose-response profile of eprosartan or placebo on the inhibition of angiotensin II–induced decreases in ERPF from 0 to 3 hours after dosing. During part 2, subjects received four of seven possible regimens: placebo or 10, 30, 50, 70, 100, or 200 mg eprosartan during angiotensin II infusions. Part 3 assessed the inhibitory effect of selected eprosartan doses or placebo on angiotensin II–induced decreases in ERPF from 12 to 15 hours after dosing; subjects received single oral doses of placebo or 50, 100, or 350 mg eprosartan at 12 hours before angiotensin II infusions.

During each part of the study, subjects received *para*-aminohippurate (PAH) infusions to measure ERPF, a sensitive pharmacodynamic marker for angiotensin II effect.[6,7] Subjects were not water loaded for their renal hemodynamic studies and were studied during fasting conditions. The duration of each PAH infusion was 6 hours, and the duration of each angiotensin II infusion was 4 hours. During the first 15 minutes of the infusion, angiotensin II was titrated up to 10 ng/kg/min, a dose that has been shown to have vasopressor, renal vasoconstrictive, and aldosterone secretory effects.[6] The times of administration of study medication in relation to PAH and angiotensin II infusions for each part of the study are given in Fig. 1.

Study population

Thirty-one healthy men between 19 and 40 years old who weighed at least 50 kg and were within 10% of ideal body weight were enrolled in part 1 ($n = 12$), part 2 ($n = 14$), or part 3 ($n = 5$). Subjects participated in only one part of the study. Each subject had a complete medical history, medication history, daily diet history, physical examination (including vital signs), 12-lead electrocardiogram (ECG), and clinical laboratory tests performed within 30 days of study enrollment. Eligible subjects had no clinically relevant abnormalities at screening, and urine drug screen results were negative. After the study was initiated, the protocol was amended to restrict participation to white men because black men were noted to have a heightened pressor response to angiotensin II infusion.

Dietary electrolyte intake

Based on a dietary history obtained at screening, subjects adhered to a 2500-calorie diet that contained approximately 200 mEq sodium and 100 mEq potassium. Subjects received up to 3 gm sodium chloride (1 gm tablets, Eli Lilly and Company, Indianapolis, Ind., or an equivalent amount of salt supplement as bouillon cubes, each containing approximately 1 gm of sodium per cube if they could not tolerate the salt tablets because of nausea) twice daily and 30 mEq potassium chloride (10 mEq K-Tab tablets, Abbott Laboratories, Abbott Park, Ill.) during the 5 days before and throughout the study. Each subject collected a 24-hour urine specimen on the day before each study session and returned the urine collection to the Clinical Pharmacology Unit on the morning of the day of admission for that session. These specimens were analyzed for sodium, potassium, creatinine, and volume to assess daily sodium and potassium excretion. Salt supplements were given on the basis of the urine collection results to achieve a sodium excretion of 200 mEq/day and a potassium excretion of 100 mEq/day.

Pharmacodynamic and safety assessments

Study sessions for parts 1, 2, and 3 of the study were conducted in a similar fashion. Subjects arrived at the Clinical Pharmacology Unit at approximately 4 PM on

day 1 of each study session, a standardized meal was served at approximately 5 PM, and the subject remained in the Clinical Pharmacology Unit overnight. Blood and urine specimens were collected before administration of the study medication to perform safety laboratory studies. A 12-lead ECG was obtained and a brief physical examination was performed. The subject was awakened at approximately 5:15 AM the following morning and urinated in the bathroom. The subject then remained supine for the remainder of the study. An intravenous cannula was placed in a vein in each forearm.

On day 2 of part 1 (sessions 1 to 4) and part 2, study medication (10 or 50 mg oral eprosartan tablets [Teveten] or matching placebo, SmithKline Beecham Pharmaceuticals, King of Prussia, Pa.) was administered after an overnight fast at 9 AM with 200 ml water. ERPF was assessed from 0 to 3 hours (parts 1 and 2) and 24 to 27 hours (part 1 only) after administration. Intravenous PAH infusions were maintained for 6 hours during each study session from 7 AM to 1 PM on day 2 (parts 1 and 2) and day 3 (part 1 only). Intravenous angiotensin II infusions were maintained for 4 hours during each study session from 8 AM to noon on day 2 (parts 1 and 2) and day 3 (part 1 only).

On day 1 of part 1, session 5, and for all of part 3, study medication was administered at least 4 hours after the evening meal at 9 PM with 200 ml water. ERPF was assessed from 12 to 15 hours after dosing. PAH and angiotensin II infusions were maintained as described above on day 2 for these study sessions. Crossover treatment sessions were separated by at least 1 week for all parts of the study.

Subjects were monitored continuously by telemetry ECG, and supine blood pressure and heart rate were measured periodically by noninvasive sphygmomanometry equipment (Spacelabs, Redmond, Wash.) during PAH and angiotensin II infusions. During the infusion of angiotensin II, blood pressure and pulse rate were recorded at 5-minute intervals for the first 30 minutes of the infusion, then at intervals of 15 minutes during the remainder of the infusion and for 1 hour after the infusion was discontinued. Loading doses (LD) and maintenance infusion doses (MD) of PAH (aminohippurate sodium, 20% for injection; Merck Sharpe & Dohme, West Point, Pa.) were calculated on the basis of a subject's weight and estimated creatinine clearance[8,9]:

$$\text{LD (mg)} = 8 \text{ mg/kg} \cdot \text{subject's body weight (kg)}$$

$$\text{MD (mg/min)} = [15 - (CL_{CR} \cdot 0.125)]/2 + (CL_{CR} \cdot 0.125)$$

in which CL_{CR} (estimated creatinine clearance; ml/min/1.73 m^2) was derived from the subject's serum creatinine (S_{CR}), body weight, and body surface area by nomogram (BSA) by the equation:

$$CL_{CR} = \frac{(140 - \text{age}) \cdot \text{weight (kg)}}{72 \cdot \text{SCR (mg/dl)}} \cdot \frac{\text{BSA}}{1.73}$$

Angiotensin II (Hypertensin, Ciba Geigy Pharmaceuticals, Summit, N.J.) was administered by continuous intravenous infusion with use of an electronic syringe pump (AutoSyringe, model AS20GH-2, Baxter Healthcare, Deerfield, Ill.). The infusion was started at a rate of 0.3 ng/kg/min and then titrated as follows: 0.3 ng/kg/min for 5 minutes, 1 ng/kg/min for 5 minutes, 3 ng/kg/min for 5 minutes, and then 10 ng/kg/minute for the remainder of the infusion period. Sustained (>1 hour) elevations in supine systolic blood pressure of >35 mm Hg or diastolic blood pressure of >30 mm Hg, or reductions in pulse rate to <40 beats/min would necessitate discontinuation of the angiotensin II infusion and cause a subject to be excluded from further study participation.

Subjects were allowed to urinate spontaneously but were not permitted to stand to urinate during PAH and angiotensin II infusions. Subjects remained supine from approximately 1 hour before initiation of PAH infusions until completion of the PAH infusions. Water, soft drinks without caffeine, and fruit juices (other than grapefruit juice) were allowed ab libitum beginning 5 hours after administration of study medication. For parts 1 and 2, meals were served approximately 5, 11, and 28 hours after administration of study medication. For part 3, subjects continued to fast, except for sodium and potassium supplements and noncaffeinated beverages until 16 hours after the evening dose of study medication.

Blood specimens for PAH and serum aldosterone concentrations were collected during part 1 (sessions 1 to 4) as follows: –2 (aldosterone only), –1, 0 (predose), 1, 2, 3, 4, 23, 24, 25, 26, 27, and 28 hours after the morning dose of study medication. During part 2, samples were obtained at –2 (aldosterone only), –1, 0 (predose), 1, 2, and 3 hours after the morning dose of study medication. Sampling times for session 5 of part 1 and for part 3 were as follows: 10¾, 11, 12, 13, 14, 15, and 16 hours after the evening dose of study medication.

Hematology, clinical chemistry, and urinalysis studies were performed at screening, just before dosing, 24 hours after dosing during each study session, and 1 week after the last study session. A brief physical examination was performed and a 12-lead ECG was obtained at the end of each study session. Adverse events were recorded throughout the study.

Table I. Subject demographics

	Study part 1 (n = 12)	*Study part 2 (n = 14)*	*Study part 3 (n = 5)*	*Pooled (n = 31)*
Age (yr)				
Mean ± SD	25 ± 6	27 ± 5	24 ± 4	26 ± 5
Range	19-40	20-39	20-28	19-40
Body weight (kg)				
Mean ± SD	76.0 ± 7.7	76.0 ± 7.5	68.0 ± 1.8	74.9 ± 7.4
Range	64.8-89.0	66.6-88.8	67.4-71.6	64.8-89.0
Height (cm)				
Mean ± SD	180.0 ± 7.0	176.0 ± 4.8	173.0 ± 3.1	176.9 ± 6.0
Range	169.0-190.5	168.0-184.0	170.0-177.0	168.0-190.5

PAH assay method

The concentration of PAH in serum was quantified with use of a modified Bratton-Marshall method.[10] In this method, PAH was diazotized with sodium nitrite. Excess sodium nitrite was eliminated by addition of ammonium sulfamate. The resulting diazo compound was combined with *N*-1-naphthylethylenediamine hydrochloride to form a purple azo dye. The colored complex was measured photometrically at 550 nm with use of a Cobas Mira analyzer (Roche Diagnostic Systems, Montclair, N.J.).

Aldosterone assay method

Concentrations of aldosterone were determined by use of standard radioimmunoassay techniques (Coat-A-Count, Diagnostic Products Corp., Los Angeles, Calif.).

Pharmacodynamic analyses

ERPF was estimated by calculation of the plasma PAH clearance (CL_{PAH}), which served as the principal pharmacodynamic end point of the study. CL_{PAH} (in milliliters per minute) at steady state was calculated from the maintenance dose of PAH administered (in milligrams per minute) divided by the serum PAH concentration measured at the end of the infusion interval (in milligrams per milliliter).[8] The actual PAH maintenance dose administered was derived from the measured infusate concentration of PAH (in milligrams per milliliter) determined from an aliquot of the infusate for each subject multiplied by the PAH infusion rate (in milliliter per minute). CL_{PAH} data were analyzed by ANOVA with terms for sequence, subject (sequence), session, and regimen included in the model as appropriate. Point estimates and associated 90% confidence intervals (to assess angiotensin II agonist activity) or 95% confidence intervals (to assess angiotensin II inhibitory effects) were also constructed to compare CL_{PAH} differences between regimens.

A measure of the inhibitory effect of eprosartan on angiotensin II–induced decreases in ERPF (ΔCL_{PAH}) was derived to express the change in CL_{PAH} associated with eprosartan plus angiotensin II relative to that associated with angiotensin II alone with use of the following equation:

$$\Delta CL_{PAH,t} = 100\% \cdot \frac{(CL_{PAH,t} - CL_{PAH,0})}{(CL_{PAH,-1} - CL_{PAH,0})}$$

in which t is equal to 1, 2, or 3 hours after dosing. The inhibitory effect of eprosartan on angiotensin II–induced changes in ERPF relative to placebo (part 3) was also calculated with the following equation:

$$\Delta CL^{pl}_{PAH,t} = 100\% \cdot \frac{(CL_{PAH,active,t} - CL_{PAH,placebo,t})}{(CL_{PAH,placebo,t})}$$

in which t is equal to 12, 13, 14, or 15 hours after dosing. Statistical analyses were performed with SAS, version 6.07, and SAS for Windows, version 6.08 (SAS Institute, Cary, N.C.).

RESULTS

Subject demographics and safety parameters

Thirty-one healthy men were enrolled in the study and received study medication. Demographics for these subjects are presented in Table I. Twenty-six white subjects completed the study ($n = 8$ in part 1, $n = 14$ in part 2, and $n = 4$ in part 3) and were included in the CL_{PAH} analyses. Two black subjects who received placebo withdrew during session 1 because of bradycardia. One black subject who received placebo during session 1 and one white subject who received eprosartan during session 1 and placebo during session 2 withdrew because of excessive blood pressure elevations during angiotensin II infusion. One white subject withdrew because of an episode of nonsustained ventricular tachycardia after placebo.

Nineteen subjects reported a total of 33 adverse experiences. Nine adverse experiences in eight subjects

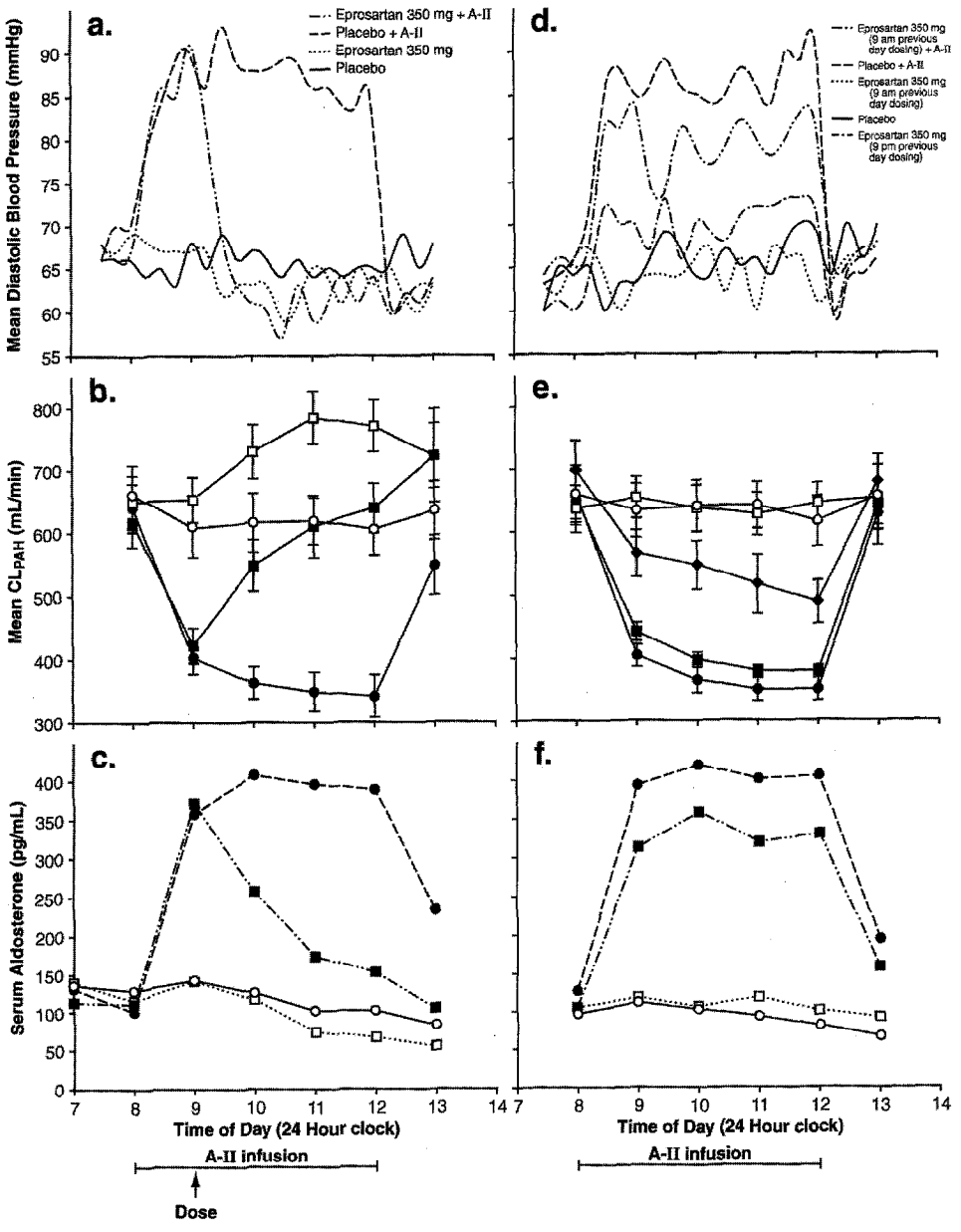

Fig. 2. **a,** Mean diastolic blood pressure (in millimeters of mercury) from –1 to 4 hours after administration of 350 mg eprosartan or placebo. **b** and **c,** Mean effective renal plasma flow (ERPF) (*para*-aminohippurate plasma clearance [CL_{PAH}], in milliliters per minute, ±SEM) and mean serum aldo-sterone (in picograms per milliliter) from –2 to 4 hours after administration of 350 mg eprosartan or placebo. **d,** Mean diastolic blood pressure (in millimeters of mercury) from 11 to 16 hours (9 PM administration on the previous day) and from 23 to 28 hours (9 AM administration on the previous day) after 350 mg eprosartan or placebo. **e** and **f,** Mean ERPF (CL_{PAH}, in milliliters per minute, ±SEM) and mean serum aldosterone (in picograms per milliliter) from 11 to 16 hours (9 PM administration on the previous day) and from 23 to 28 hours (9 AM administration on the previous day) after 350 mg eprosartan or placebo. *Solid squares,* 350 mg eprosartan + antiotensin II; *open squares,* 350 mg eprosartan; *solid circles,* placebo + angiotensin II; *open circles,* placebo; *solid diamonds,* 350 mg eprosartan (9 PM administration on the previous day) + angiotensin II (study part 1; $n = 8$ per regimen).

Table II. Comparison of mean ERPF (in milliliters per minute; CL_{PAH}) from 0 to 4 hours after a single oral dose of placebo or 350 mg eprosartan in healthy sodium-replete men ($n = 8$, study part 1)

	Time after administration of study medication				
	0 Hours	*1 Hours*	*2 Hours*	*3 Hours*	*4 Hours*
350 mg eprosartan/no angiotensin II	654	731	785	772	724
Placebo/no angiotensin II	609	617	620	607	638
[Eprosartan] – [placebo]	45	113	165	165	87
90% CI	–28, 118	24, 203	75, 256	78, 253	–69, 242
350 mg eprosartan + angiotensin II	422	549	610	640	725
Placebo/no angiotensin II	609	617	620	607	638
[Eprosartan] – [placebo]	–187	–69	–10	33	88
95% CI	–275, –99	–177, 40	–120, 100	–73, 139	–101, 276
Placebo + angiotensin II	403	363	350	343	549
Placebo/no angiotensin II	609	617	620	607	638
[Placebo + angiotensin II] – [placebo/no angiotensin II]	–206	–255	–270	–264	–88
95% CI	–294, –118	–363, –146	–380, –160	–370, –158	–276, 100

ERPF, Effective renal plasma flow; CL_{PAH}, clearance of *para*-aminohippurate; CI, confidence interval.
Six-hour *para*-aminohippurate infusion from –2 hours before to 4 hours after administration; 4-hour angiotensin II infusion from –1 hour before to 3 hours after administration.

occurred after administration of placebo, and 24 adverse experiences in 13 subjects occurred after administration of eprosartan. All adverse experiences were mild to moderate in severity and resolved without sequelae. The most frequently reported adverse experience (four occurrences) was headache. Scattered abnormalities in clinical laboratory tests were noted; however, none were associated with clinical events or were considered to be related to eprosartan administration. There were no symptomatic changes in vital signs associated with eprosartan.

Renal hemodynamic, blood pressure and neurohormonal effects

Part 1: Onset and duration of effect of eprosartan 350 mg. For the placebo regimen, systolic and diastolic blood pressure increased by approximately 16 to 28 mm Hg and 17 to 30 mm Hg, respectively, in response to intravenous angiotensin II infusions of 10 ng/kg/min. ERPF decreased by 206 to 270 ml/min and aldosterone secretion was stimulated by approximately fourfold, as previously reported by others.[6] A single 350 mg oral dose of eprosartan caused complete (100%) inhibition of the angiotensin II–induced pressor and renal vasoconstrictive effects (i.e., ERPF) and inhibited the angiotensin II–induced stimulation of aldosterone secretion at 1 to 3 hours after dosing (Fig. 2, *a, b,* and *c;* Table II). Inhibition of the renal hemodynamic effects of angiotensin II was apparent at 12 hours (~70% inhibition) and 15 hours (~50% inhibition) after a single oral dose of 350 mg eprosartan but was attenuated by 24 hours after the dose (Fig. 2, *e;* Table III and IV). Inhibition of the pressor effects of angiotensin II was apparent at 12 to 15 hours (>50% inhibition) after a single oral dose of 350 mg eprosartan and was still present at 24 hours after this single dose (30% inhibition; Fig. 2, *d*). The effects of eprosartan on blood pressure were mirrored by partial inhibition of the aldosterone secretory effects of angiotensin II at 24 to 27 hours after a single oral dose of 350 mg eprosartan (Fig. 2, *f*).

In addition, the lack of agonist activity of eprosartan was assessed by investigation of the effects of eprosartan alone (without angiotensin II infusions) on blood pressure, ERPF, and aldosterone secretion. Eprosartan showed no angiotensin II agonist activity. A single oral dose of 350 mg eprosartan administered in the absence of angiotensin II resulted in a statistically significant increase in ERPF of up to 165 ml/min (approximately 27%) greater than placebo at 1 to 4 hours after dosing (Fig. 2, *b;* Table II). Eprosartan, 350 mg, had no vasopressor effect and did not stimulate aldosterone secretion (Fig. 2, *a* and *c,* respectively). Eprosartan, 350 mg, had no effect on ERPF at 23 to 28 hours after dosing compared with placebo (Fig. 2, *e;* Table IV).

Part 2: Dose response. Eprosartan caused dose-related inhibition of the angiotensin II–induced renal vasoconstriction. At 3 hours after single oral doses of 10, 30, 50, 70, 100, and 200 mg, eprosartan inhibited angiotensin II–induced decreases in ERPF by 39.1%, 49.9%, 33.0%, 56.0%, 71.0%, and 85.7%, respectively,

Table III. Comparison of mean ERPF (in milliliters per minute; CL_{PAH}) from 11 to 16 hours after a single oral dose of placebo or 350 mg eprosartan in normal sodium-replete men (n = 8, study part 1)

	Time after administration of study medication					
	11 Hours	*12 Hours*	*13 Hours*	*14 Hours*	*15 Hours*	*16 Hours*
350 mg eprosartan + angiotensin II	698	565	546	516	488	681
Placebo/no angiotensin II	661	634	642	643	617	659
[Eprosartan] – [placebo]	37	–69	–96	–127	–130	22
95% CI	–79, 153	–146, 7	–174, –18	–205, –49	–198, –61	–79, 123

Six-hour *para*-aminohippurate infusion from 10 to 16 hours after administration; 4-hour angiotensin II infusion from 11 to 15 hours after administration.

Table IV. Comparison of mean ERPF (in milliliters per minute; CL_{PAH}) from 23 to 28 hours after a single oral dose of placebo or 350 mg eprosartan in healthy, sodium-replete men (n = 8, study part 1)

	Time after administration of study medication					
	23 Hours	*24 Hours*	*25 Hours*	*26 Hours*	*27 Hours*	*28 Hours*
350 mg eprosartan/no angiotensin II	634	651	635	625	643	653
Placebo/no angiotensin II	661	634	642	643	617	659
[Eprosartan] – [placebo]	–27	17	–7	–18	25	–6
90% CI	–123, 69	–46, 81	–72, 58	–83, 47	–32, 83	–90, 78
350 mg eprosartan + angiotensin II	652	439	396	378	378	643
Placebo/no angiotensin II	661	634	642	643	617	659
[Eprosartan] – [placebo]	–8.76	–195	–247	–265	–239	–16
95% CI	–125, 107	–271, –118	–324, –169	–343, –187	–308, –170	–117, 85
Placebo + angiotensin II	655	403	364	349	350	629
Placebo/no angiotensin II	661	634	642	643	617	659
[Placebo + angiotensin II] – [placebo/no angiotensin II]	–6	–231	–278	–294	–268	–30
95% CI	–122, 110	–307, –155	–356, –200	–372, –216	–336, –199	–131, 71

Six-hour *para*-aminohippurate infusion from 22 to 28 hours after administration; 4-hour angiotensin II infusion from 23 to 27 hours after administration.

relative to placebo (Fig. 3, *b*). Single oral doses of 10 to 200 mg eprosartan also inhibited the angiotensin II–induced pressor and aldosterone secretory effects from 1 to 3 hours after administration, but there was no clear dose-response relationship for these parameters (Fig. 3, *a* and *c*, respectively).

Part 3: Dose response from 12 to 15 hours after administration. A single oral dose of 350 mg eprosartan administered 12 hours before angiotensin II challenge inhibited the renal hemodynamic effects of angiotensin II by 36% (Fig. 4, *b*). Single oral doses of 50 and 100 mg eprosartan administered 12 hours before angiotensin II challenge resulted in less inhibition of angiotensin II effects than the 350 mg dose, and there was a suggestion of a dose-response relationship (Fig. 4, *b*). The effects of eprosartan on ERPF were mirrored by inhibition of angiotensin II–induced pressor effect and aldosterone secretion, although a dose-response relationship was less evident for these parameters (Fig. 4, *a* and *c*, respectively).

DISCUSSION

The renal circulation is sensitive to angiotensin II. Indeed, ERPF is more sensitive to the effects of angiotensin II than either blood pressure or aldosterone secretion.[6] ERPF was therefore evaluated as the primary pharmacodynamic end point of the study.

The effects of eprosartan on renal hemodynamics in the presence and absence of angiotensin II were examined in this study by measurement of plasma clearance of PAH, which was used as a marker of ERPF or renal vascular tone. By itself, eprosartan significantly increased ERPF at a dose of 350 mg. Single oral doses of 10 to 350 mg eprosartan effectively inhibited the renal vasoconstrictor effects of angiotensin II in a dose-related manner with complete (100%) inhibition of the effects of angiotensin II after a single oral dose of 350 mg. The duration of angiotensin II inhibitory activity for eprosartan in the renal vascular bed was noted for 12 to 15 hours after a single oral dose of 350 mg and the inhibitory

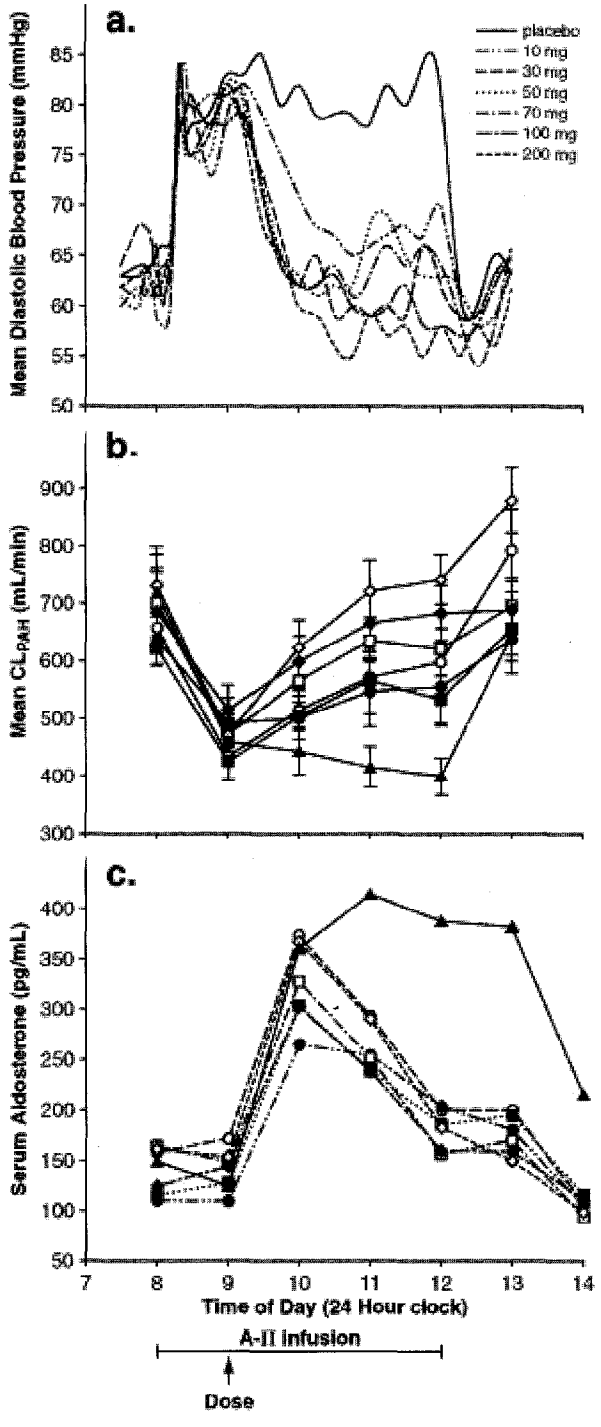

Fig. 3. a, Mean diastolic blood pressure (in millimeters of mercury) from –1 to 4 hours after placebo or eprosartan doses ranging from 10 to 200 mg. **b** and **c,** Mean ERPF (CL_{PAH}, in milliliters per minute, ± SEM) and mean serum aldosterone (in picograms per milliliter) from –1 to 4 hours after placebo or eprosartan doses ranging from 10 to 200 mg. *Solid triangles,* Placebo; *solid circles,* 10 mg; *open circles,* 30 mg; *solid squares,* 50 mg; *open squares,* 70 mg; *solid diamonds,* 100 mg; *open diamonds,* 200 mg (study part 2; $n = 8$ per regimen).

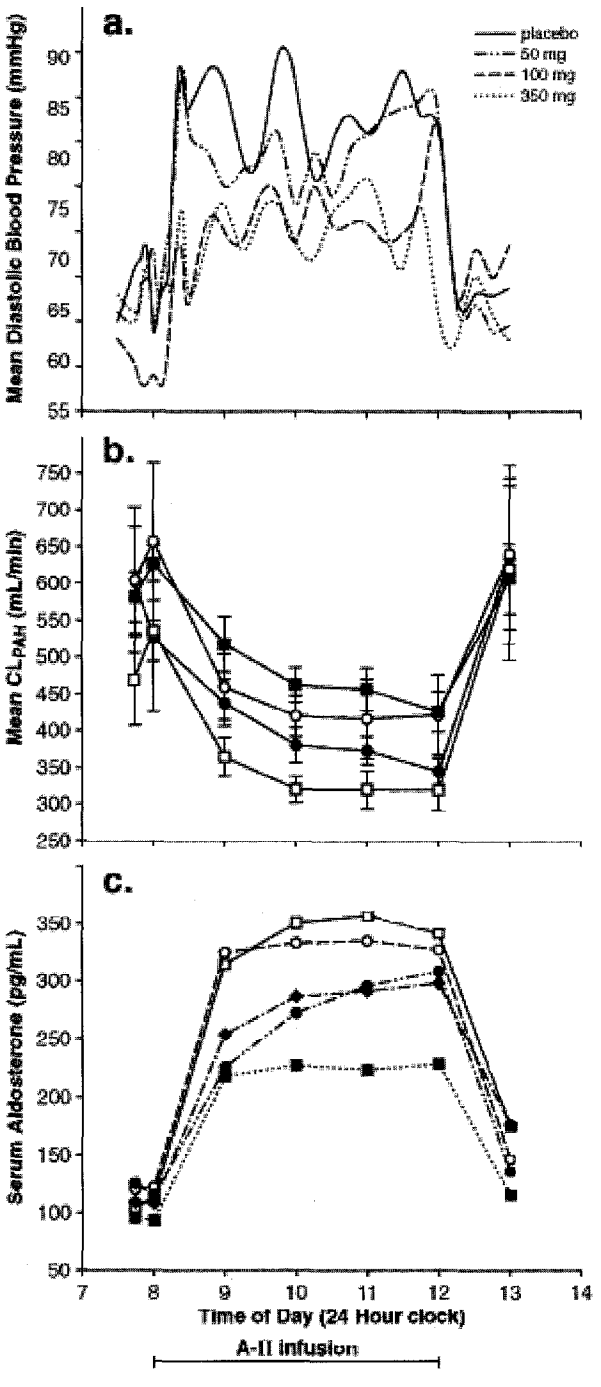

Fig. 4. a, Mean diastolic blood pressure (in millimeters of mercury) from 11 to 16 hours after placebo or 50, 100, or 350 mg eprosartan (9 PM administration on the previous day). **b** and **c,** Mean ERPF (CL_{PAH}, in milliliters per minute, ±SEM) and mean serum aldosterone (picograms per milliliter from 11 to 16 hours after placebo or 50, 100, or 350 mg eprosartan (9 AM administration on the previous day for part 1, $n = 8$; (9 PM administration on the previous day for part 3, $n = 5$). *Open squares,* Placebo; *solid circles,* 50 mg; *open circles,* 100 mg; *solid squares,* 350 mg (part 3); *solid diamonds,* 350 mg (part 1, session 5).

effect of eprosartan on angiotensin II pressor effect was still present at 24 hours after dosing. Changes in ERPF after eprosartan were mirrored by commensurate inhibition of angiotensin II–induced changes in blood pressure and serum aldosterone. Eprosartan blunted both the pressor response and aldo-sterone secretory response to exogenous angiotensin II. The effects of eprosartan observed in this study are consistent with the pharmacology of eprosartan as a direct antagonist of the angiotensin II AT_1-receptor. By comparison, in a similar study of salt-replete normotensive subjects, a single oral dose of 50 mg losartan had no vasodilatory effect on ERPF and inhibited the renal vasoconstrictive effects of angiotensin II at 10 ng/kg/min by approximately 50%.[11] In the losartan study, angiotensin II infusions were started 3 hours after losartan administration to achieve peak levels of E-3174, the primary pharmacologically active metabolite of losartan. The data from the current study would suggest that a single oral dose of 350 mg eprosartan may provide more complete blockade of the renal hemodynamic effects of angiotensin II than does a single oral dose of 50 mg losartan. These doses of eprosartan and losartan are comparable because daily doses of 400 to 600 mg eprosartan and 50 mg losartan, respectively, are recommended starting doses of these medications in the treatment of essential hypertension.

In the current study, changes in ERPF induced by eprosartan in the presence or absence of exogenous angiotensin II provide important insights into the pharmacodynamic effects of eprosartan. Brunner et al.[12] suggested that the antihypertensive dose range of the angiotensin converting enzyme inhibitors captopril and enalapril could have been defined with inhibition of angiotensin I pressor effects in healthy volunteers as a marker of pharmacologic activity of these drugs. These techniques were used in the current study to target effective antihypertensive doses of eprosartan for clinical trials in patients with essential hypertension.

The effect of angiotensin II on both the peripheral and renal vasculature is influenced by the level of sodium intake and sodium balance.[6,13,14] When sodium intake is restricted and the renin-angiotensin system is activated, both saralasin (a peptide angiotensin II antagonist) and angiotensin converting enzyme inhibitors induce a dose-related increase in renal blood flow and an increase in sodium excretion. This study was conducted under conditions of a high sodium diet to dampen the activity of the renin-angiotensin system, to minimize systemic and tissue levels of angiotensin II, and to potentiate the systemic and renal vascular responses to exogenous infused angiotensin II.[6] In previous studies under these conditions, neither saralasin nor captopril increased ERPF.[7,15-17]

In the absence of exogenously administered angiotensin II, a single oral dose of eprosartan caused a clinically significant increase in ERPF in salt-replete individuals. The maximum increase in ERPF occurred 2 to 3 hours after eprosartan administration, with an increase in ERPF of approximately 165 ml/min, or 27% greater than placebo. The increase in ERPF in salt-replete individuals suggests that the intrarenal renin-angiotensin system may have a basal renal vasoconstrictive effect in the absence of circulating angiotensin II.[18]

Therapies that are directed against the renin-angiotensin-aldosterone system have been shown to have a renal protective effect in patients with diabetic nephropathy and with other glomerular diseases. In patients with type I, insulin-dependent diabetes mellitus and diabetic nephropathy, captopril therapy reduces proteinuria and protects against deterioration in renal function and is significantly more effective than blood pressure control alone.[19] Benazapril has been shown to retard the progression of renal disease in a variety of glomerular diseases but not in patients with interstitial nephritis or adult polycystic kidney disease.[20] The interpretation of the benazapril data has been complicated by the observation that blood pressure control was better in the benazapril-treated patients than in the control group. The salutary effects of angiotensin converting enzyme inhibitors are presumably the result of reduction of glomerular capillary pressure, although this has never been proven in humans.[21,22] Brenner[23] suggested that a decrease in the filtration fraction (glomerular filtration rate/renal blood flow ratio) and reductions in proteinuria could be considered as surrogate markers for a decrease in the glomerular capillary pressure. Therapies that result in an increase in renal blood flow and little or no change in glomerular filtration rate would result in a decrease in filtration fraction and glomerular capillary pressures. Such therapies would have the potential to reduce proteinuria and retard the progression of glomerular sclerosis and chronic renal failure, especially in patients with type I diabetes mellitus. As mentioned above, captopril therapy has been shown to reduce proteinuria and protect against deterioration of renal function in these patients. Eprosartan, through direct antagonism of angiotensin II at the AT_1-receptor, would be expected to produce similar salutary effects by increasing renal blood flow and thereby decreasing filtration fraction, glomerular capillary pressure, and proteinuria. The long-term clinical implications of the renal vasodilatory effect observed with eprosartan in this study warrant further evaluation in patients with essential hypertension and chronic renal insufficiency.

In conclusion, the findings of this first study of eprosartan pharmacology in humans confirm the angiotensin II antagonistic activity of eprosartan. Consistent with this mechanism of action, a single oral dose of 350 mg eprosartan completely (100%) antagonized the vasopressor, renal hemodynamic, and aldosterone secretory effects of exogenous angiotensin II infusions in healthy subjects. A dose-response relationship has also been shown for these effects. In the absence of exogenously administered angiotensin II, a single oral dose of 350 mg eprosartan caused a clinically significant increase in ERPF in salt-replete individuals, suggesting that the intrarenal renin-angiotensin system may have a basal renal vasoconstrictive effect in the absence of circulating angiotensin II. The clinical implications of the renal vasodilatory effect observed with eprosartan in this study warrants further evaluation in patients with essential hypertension and chronic renal insufficiency.

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References

1. Oparil S, Haber E. The renin-angiotensin system. N Engl J Med 1974;291:389-401.
2. Duncia JV, Chiu AT, Carini DJ, Gregory GB, Johnson AL, Price WA, et al. The discovery of potent non-peptide angiotensin II receptor antagonists: a new class of potent antihypertensives. J Med Chem 1990;33:1312-29.
3. Weinstock J, Keenan RM, Samanen J, Hempel J, Finkelstein JA, Franz RG, et al. 1-(Carboxybenzyl)imidazole-5-acrylic acids: potent and selective angiotensin II receptor antagonists. J Med Chem 1991;34:1514-7.
4. Edwards RM, Aiyar N, Ohlstein EH, Weidley EF, Griffin E, Ezekiel M, et al. Pharmacological characterization of the nonpeptide angiotensin II receptor antagonist, SK&F 108566. J Pharmacol Exp Ther 1992;260:175-81.
5. Brooks DP, Fredrickson TA, Weinstock J, Ruffolo RR, Edwards RM, Gellai M. Antihypertensive activity of the non-peptide angiotensin II receptor antagonist, SK&F 108566, in rats and dogs. Arch Pharmacol 1992;345:673-8.
6. Hollenberg NK, Chenitz WR, Adams DF, Williams GH. Reciprocal influence of salt intake on adrenal glomerulosa and renal vascular responses to angiotensin II in normal men. J Clin Invest 1974;54:34-42.
7. Hollenberg NK, Williams GH, Burger B, Ishikawa I, Adams DF. Blockade and stimulation of renal, adrenal, and vascular angiotensin II receptors with 1-Sar, 8-Ala angiotensin II in normal man. J Clin Invest 1976;57:39-46.
8. Earle DP, Berliner RW. A simplified clinical procedure for measurement of glomerular filtration rate and renal plasma flow. Proc Soc Exp Biol Med 1946;62:262-4.
9. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41.
10. Bratton AC, Marshall EK. A new coupling component for sulfanilamide determination. J Biol Chem 1939;128:537-50.
11. Gandhi SK, Ryder DH, Brown NJ. Losartan blocks aldosterone and renal vascular responses to angiotensin II in humans. Hypertension 1996;28:961-6.
12. Brunner HR, Waeber B, Nussberger J. Does pharmacological profiling of a new drug in normotensive volunteers provide a useful guideline to antihypertensive therapy? Hypertension 1983;5(suppl III):101-7.
13. Williams GH, Hollenberg NK, Brown C, Mersey JH. Adrenal responses to pharmacological interruption of the renin-angiotensin system in sodium restricted normal man. J Clin Endocrinol Metab 1978;47:725-31.
14. Oelkers W, Brown JJ, Fraser R, Lever AF, Morton JJ, Robertson JI, et al. Sensitization of the adrenal cortex to angiotensin II in sodium-deplete man. Circ Res 1974; 40:69-77.
15. Hollenberg NK, Meggs LG, Williams GH, Katz J, Garnic JD, Harrington DP. Sodium intake and renal responses to captopril in normal man and in essential hypertension. Kidney Int 1981;20:240-5.
16. Hollenberg NK, Swartz SL, Passan DR, Williams GH. Increased glomerular filtration rate following converting enzyme inhibition in essential hypertension. N Engl J Med 1979;301:9-12.
17. Williams GH, Hollenberg NK. Accentuated vascular and endocrine response to SQ 20881 in hypertension. N Engl J Med 1977;297:184-8.
18. Hollenberg NK, Fisher NDI. Renal circulation and blockade of the renin-angiotensin system: is angiotensin-converting enzyme inhibition the last word? Hypertension 1995;26:602-9.
19. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. N Engl J Med 1993;329:1456-62.
20. Maschio G, Alberti D, Janin G, Locatelli F, Mann JFE, Motolese M, et al. Effect of the angiotensin-converting-enzyme inhibitor benazapril on the progression of chronic renal insufficiency. The Angiotensin-Converting-Enzyme Inhibition in Progressive Renal Insufficiency Study Group [see comments]. N Engl J Med 1996;334:939-45.
21. Anderson S, Meyer TM, Rennke HG, Brenner BM. Control of glomerular hypertension limits glomerular injury in rats with reduced renal mass. J Clin Invest 1985; 76:612-9.
22. Zatz R, Dunn R, Meyer TM, Anderson S, Rennke HG, Brenner BM. Prevention of diabetic glomerulopathy by pharmacological amelioration of glomerular capillary hypertension. J Clin Invest 1986;77:1925-30.
23. Brenner BM. The experimental basis for designing strategies for arresting progression of renal disease. Presented at the American Society of Hypertension, New York, May 11-14, 1994.