Chronic Angiotensin II Infusion But Not Bradykinin Blockade Abolishes the Antiproteinuric Response to Angiotensin-Converting Enzyme Inhibition in Established Adriamycin Nephrosis

FRITS H. WAPSTRA,* GERJAN NAVIS,*† PAUL E. DE JONG,* and DICK DE ZEEUW*†

*Groningen Institute of Drug Studies (GIDS), Department of Medicine, *Division of Nephrology and †Department of Clinical Pharmacology, University Hospital and Groningen School of Medicine, The Netherlands.*

Abstract. Angiotensin-converting enzyme (ACE) inhibition reduces proteinuria in established adriamycin nephrosis. To investigate whether the reduction in proteinuria is due to decreased generation of angiotensin II (AngII) or to decreased degradation of bradykinin, four series of experiments in established adriamycin nephrosis were performed. In the first series, 2 mg/kg lisinopril reduced BP from 117 ± 4 to 67 ± 2 mmHg and proteinuria from 335 ± 66 to 57 ± 10 mg/24 h after 2 wk of treatment. Subsequent continuous intraperitoneal infusion of AngII (250 ng/kg per min) for 2 wk partially restored proteinuria to 180 ± 42 mg/24 h, whereas BP increased to 97 ± 3 mmHg. Subsequent withdrawal of AngII restored the antiproteinuric effects of lisinopril, whereas subsequent withdrawal of lisinopril restored proteinuria to pretreatment values. In the second series, AT1 receptor blockade induced a fall in BP and proteinuria similar to that by lisinopril. In the third series, lisinopril reduced BP from 121 ± 5 to 68 ± 2 mmHg and proteinuria from 355 ± 90 to 101 ± 10 mg/24 h. Subsequent intraperitoneal infusion of bradykinin antagonist (HOE 140; 1 mg/kg per 24 h) for 2 wk did not affect BP (72 ± 2 mmHg) or proteinuria (92 ± 15 mg/24 h). In the fourth series, bradykinin (3 mg/kg per 24 h) was infused for 2 wk to mimic decreased bradykinin breakdown. This did not affect proteinuria, but induced a fall in BP from 114 ± 3 to 93 ± 4 mmHg. The BP-lowering effect of exogenous bradykinin was completely reversed by 1 wk infusion of HOE 140 (93 ± 4 to 113 ± 4 mmHg), while proteinuria remained unchanged. In conclusion, the antiproteinuric effect of ACE inhibition appears to be independent of bradykinin in this model, supporting a main role for reduction of AngII in the antiproteinuric action of ACE inhibition.

Angiotensin-converting enzyme (ACE) inhibition reduces urinary protein excretion in experimental as well as human renal disease (1–3). We previously reported the antiproteinuric efficacy of lisinopril in established adriamycin nephrosis (3). Reduction of generation of angiotensin II (AngII) is assumed to be a main mechanism mediating the effects of ACE inhibitors. As infusion of pharmacologic doses of AngII induces proteinuria in normal animals (4,5), increased intrarenal AngII has been postulated as a mechanism contributing to the development of proteinuria (6). Reduction of AngII may thus be a mechanism contributing to the antiproteinuric effect of ACE inhibition.

ACE—or kininase II—however, is not specific for angiotensin as it hydrolyzes may other peptides as well. ACE degrades bradykinin and kallidin to inactive peptides. Accordingly, ACE inhibition not only can lead to decreased formation of AngII, but also to decreased bradykinin degradation (7–10). A role for decreased bradykinin generation in the effects of ACE inhibition is suggested by studies in human (11) and in experimental renal disease (12,13), but other studies provided conflicting results (14,15). The role of decreased bradykinin generation in the antiproteinuric effect of ACE inhibition, therefore, is debated.

The role of kinins in renal disease and specifically in the nephrotic syndrome has not been well characterized, but there is some evidence for reduced activity of the kallikrein-kinin cascade in proteinuric renal disease (16). A higher proteinuria has been found to be associated with lower urinary kallikrein excretion, with a return of kallikrein toward normal with resolution of proteinuria (17). The availability of specific angiotensin subtype 1 (AT1) receptor antagonists (18,19) and bradykinin antagonists (20,21) may allow us to elucidate the respective contributions of interference in the renin-angiotensin system and the kallikrein-kinin cascade in the antiproteinuric effect of ACE inhibitors.

In the present study, we sought to delineate between these potential mediating pathways of antiproteinuric action of ACE inhibitors. We investigated first whether the antiproteinuric

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Correspondence to Dr. Gerjan Navis, Department of Nephrology, University Hospital, Hanzeplein 1, 9713 GZ Groningen, The Netherlands. Phone: +31 5036 12621; Fax: +31 5036 19310; E-mail: g.j.navis@int.azg.nl

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effect of lisinopril could be abolished by chronic infusion of AngII or a bradykinin antagonist, respectively, in conscious rats with established adriamycin nephrosis. In addition, the antiproteinuric effects of an AngII antagonist were compared with those of lisinopril. Finally, we tested whether chronic infusion of exogenous bradykinin is able to induce proteinuria.

Materials and Methods

Experimental Design

Adriamycin nephrosis was induced in four series of 12 male Wistar rats (Harlan, Zeist, The Netherlands) with an average weight of 290 ± 19 g, by a single slow injection of adriamycin (2 mg/kg; Pharmachemie BV, Haarlem, Holland) in the penis vein under anesthesia. All animals received a low sodium diet (0.05% NaCl, 20% protein; Hope Farms, Inc., Woerden, The Netherlands) and received daily fresh tap water *ad libitum*. During the next 8 wk, 24-h urine samples were collected twice a week in metabolic cages with free access to food and water. Body weight and BP were measured twice a week; during the chronic infusion periods, BP was measured daily. The intervention studies started after stabilization of proteinuria, *i.e.*, 6 wk after disease induction. Previous studies have shown that in this model, BP and proteinuria remain stable between 6 and 12 wk after disease induction (3,22,23).

AngII Experiments

Group I: Chronic AngII infusion *versus* Vehicle in Lisinopril-Treated Rats. ACE inhibitor treatment (lisinopril 5 mg/kg per 24 h) was started 6 wk after adriamycin injection. Lisinopril was added to the drinking water, adjusted for water intake. During the second week of treatment, an intraperitoneal catheter was implanted under anesthesia. After 2 wk of ACE inhibition, the animals were divided into two groups, matched for proteinuria and BP. During the next 2 wk, these groups received a continuous intraperitoneal infusion of AngII (250 ng/kg per min; Sigma Chemie, Brussels, Belgium) or vehicle (glucose 5%). This dose was selected based on a pilot study to obtain the dose that titrates BP back to baseline during lisinopril 5 mg/kg per 24 h. At the end of this period, the infusions were stopped in all animals; lisinopril was continued for another week. Finally, lisinopril was withdrawn as well, and the recovery was studied for 1 wk.

Group II: AT1 Receptor Blockade *versus* Lisinopril. Six weeks after the adriamycin injection, two groups of six animals were matched for proteinuria and BP. These groups received either the AT1 receptor blocker L158, 809 (10 mg/kg per 24 h; Merck, Sharp & Dohme, Rahway, NJ) or lisinopril 5 mg/kg per 24 h for 2 wk. L158, 809 and lisinopril were administered in the drinking water, adjusted for water intake.

Bradykinin Experiments

Group III: Bradykinin Antagonist Infusion *versus* Vehicle in Lisinopril-Treated Rats. Twelve rats received lisinopril 5 mg/kg per 24 h as of 6 wk after adriamycin injection. During the second week of ACE inhibitor treatment, an intraperitoneal catheter was implanted. After 2 wk of ACE inhibition, two groups of six animals were matched for proteinuria and BP. These groups were subsequently infused with either the bradykinin antagonist HOE 140 (1 mg/kg per 24 h; Hoechst, Frankfurt, Germany) or vehicle (glucose 5%) for 2 wk. The dose of HOE 140 was selected based on data from the literature and from the manufacturer. Pharmacologic efficacy of this dose to block bradykinin effects was verified by the experiments in group IV.

Group IV: Chronic Bradykinin Infusion *versus* Vehicle Followed by Bradykinin Antagonist or Vehicle Administration. This group of 12 rats served (*1*) to test whether exogenous bradykinin infusion can reduce proteinuria, and (*2*) to verify the pharmacologic efficacy of the bradykinin antagonist. Six weeks after adriamycin injection, an intraperitoneal catheter was implanted, and two groups of six animals were formed matched for proteinuria and BP. These groups were subsequently infused with either bradykinin (3 mg/kg per 24 h; Sigma Chemie, Brussels, Belgium) or vehicle (glucose 5%) for three wk. During the third week of bradykinin or vehicle infusion, the bradykinin antagonist HOE 140 (1 mg/kg per 24 h) or vehicle (glucose 5%) was added. The dose of bradykinin was selected based on a pilot experiment; doses >3 mg/kg per d failed to further reduce BP and proteinuria.

BP Measurements

Systolic BP was measured in conscious rats with an automated multichannel system (Apollo 179, IITC; Life Science, Woodland Hills, CA) (24). This system uses tail cuffs and photoelectric sensors to detect tail pulse. To this end, rats are placed in the test chamber in restrainers appropriate for their size and body weight. Although not the gold standard for BP measurement in rats, this method was adopted because it is noninvasive. Several measures were taken to enhance the reliability of the measurements. Animals were trained before the experiments to get accustomed to the BP measurement procedure. To rule out interindividual differences and environmental influences, all measurements were performed by the same observer in one single room with no other animals present. During each BP measurement session, five measurements were recorded for each animal. BP was taken as the mean of the last three recordings. Finally, during the infusion studies, BP was measured daily to provide a reliable estimate of the time course of the responses.

Biochemistry

Urinary protein was determined by the Pyrogallol-red-molybdate method (RA-1000; Technicon).

Implantation of Intraperitoneal Cathether

For the implantation of the intraperitoneal catheter, the rats were anesthetized with NO_2/O_2 halothane. A small abdominal midline incision was made through which a small silicon catheter was introduced into the peritoneal cavity. After fixation to the abdominal wall, the catheter was tunneled subcutaneously to the head, where it was coupled to a stainless steel L-shaped connector fixed to the skull. After surgery, the rats were allowed to recover for 5 d. For the remaining part of the study, the animals were housed individually in large metabolic cages allowing them to move freely despite the infusion cannula, with free access to food and water, and with the possibility to connect them all parallel to an infusion pump, by a swivel system.

Statistical Analyses

Statistical comparisons were performed by ANOVA. Unless specified otherwise, data are given as means ± SEM. A P value <0.05 was considered statistically significant.

Results

Group I: Chronic AngII Infusion versus *Vehicle in Lisinopril-Treated Rats*

Figure 1 shows the time course of proteinuria and BP in the first group. Six weeks after adriamycin injection, a stable

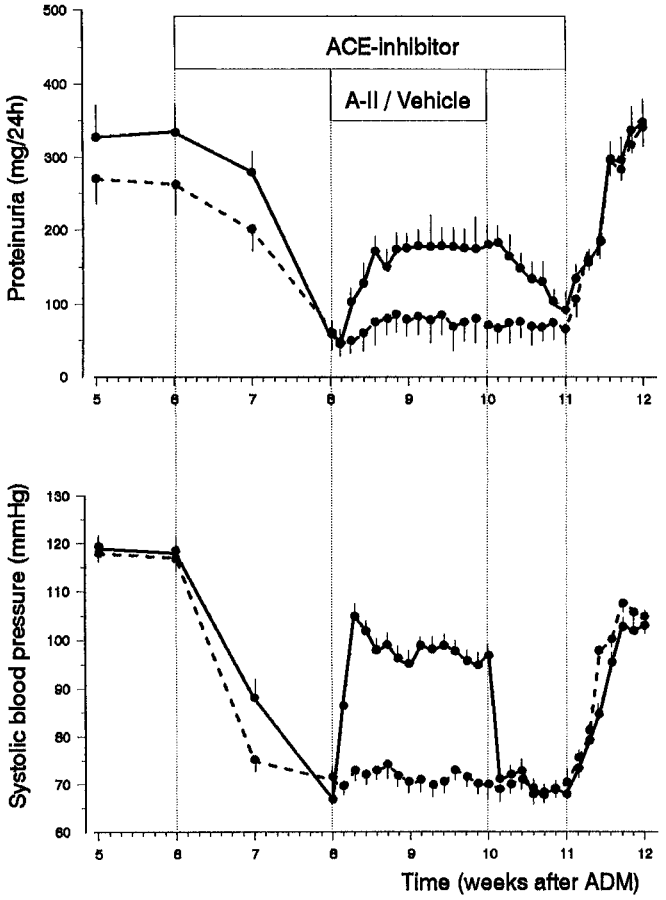

Figure 1. Group I: Chronic angiotensin II (AngII) infusion *versus* vehicle in lisinopril-treated rats. Time course of proteinuria and systolic BP during angiotensin-converting enzyme (ACE) inhibition. From week 8 through 10, either AngII (A-II; continuous lines) or vehicle (broken lines) was infused. Results are given as means ± SEM.

proteinuria of 355 ± 66 mg/24 and 263 ± 57 mg/24 h and a systolic BP of 118 ± 4 mmHg and 117 ± 4 mmHg was reached in the groups that were to be allocated to AngII and vehicle, respectively. After 2 wk of lisinopril in these groups, proteinuria had decreased to 57 ± 10 mg/24 h and 67 ± 8 mg/24 h ($P < 0.01$) and systolic BP to 67 ± 2 mmHg and 71 ± 3mmHg ($P < 0.01$), respectively. Intraperitoneal infusion of AngII for 2 wk led to an immediate and sustained rise in systolic BP (97 ± 3 mmHg at week 10; $P < 0.01$) and a gradual rise in proteinuria (180 ± 42 mmHg at week 10; $P < 0.01$). Vehicle had no effect on proteinuria (61 ± 10 mg/24 h at week 10) or on BP (70 ± 1 mmHg at week 10). After withdrawal of AngII, a gradual restoration of the antiproteinuric effect (90 ± 16 mg/24 h at week 11; $P < 0.05$) was observed with an immediate fall in BP (68 ± 3 mmHg at week 11; $P < 0.01$). After withdrawal of ACE inhibition, proteinuria increased to pretreatment values (347 ± 62 mg/24 h at week 12). Systolic BP increased as well, but did not reach pretreatment values (103 ± 3 mmHg at week 12). In the vehicle group, withdrawal of ACE inhibition restored proteinuria (342 ± 57 mg/24 h at week 12) and systolic pressure (105 ± 5 mmHg at week 12) in a manner similar to the AngII group. There was no significant difference in body weight between the groups (week 12: vehicle 360 ± 15 g; AngII 356 ± 18 g). Food intake was not affected by start of lisinopril or by the AngII infusion, and was similar for the groups.

Group II: AT1 Receptor Blockade versus *Lisinopril*

Figure 2 shows the time course of proteinuria and BP during the 2 wk of treatment with the AT1 receptor blocker and lisinopril. Six weeks after adriamycin injection, a stable proteinuria of 336 ± 41 mg/24 h and 330 ± 57 mg/24 and a systolic BP of 119 ± 1 and 119 ± 1 mmHg was reached in the two groups, respectively. After 2 wk of treatment with the AT1

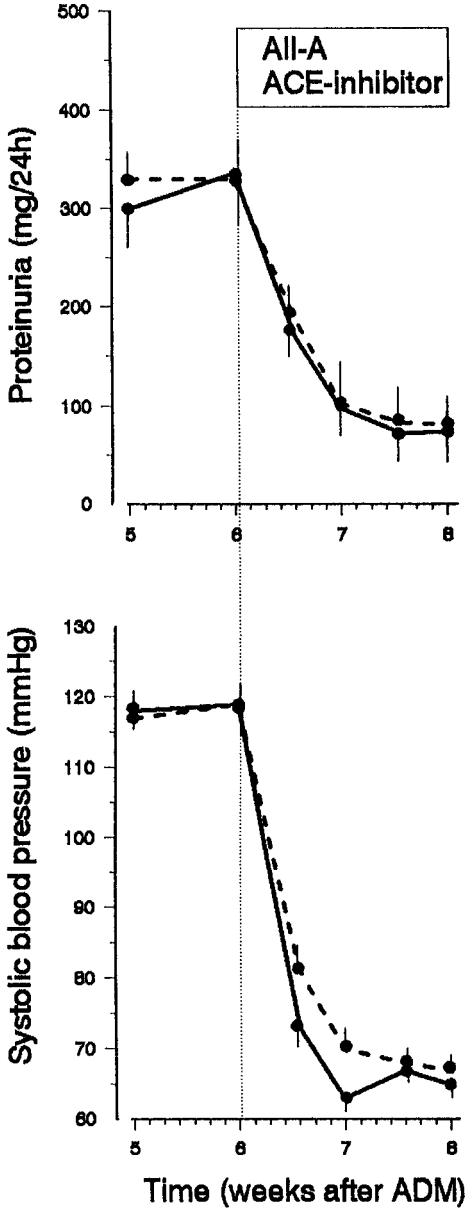

Figure 2. Group II: AT1 receptor blockade *versus* lisinopril. Time course of proteinuria and systolic BP during treatment with AT1 receptor blocker (AII-A; continuous lines) or ACE inhibition (broken lines). Results are given as means ± SEM.

receptor blocker, proteinuria had decreased to 73 ± 19 mg/24 h ($P < 0.01$) and systolic BP to 65 ± 2 mmHg ($P < 0.01$), whereas lisinopril resulted in a reduction of proteinuria to 82 ± 29 mg/24 h ($P < 0.01$) and a fall in BP to 67 ± 2 mmHg ($P < 0.01$). There was no difference in antiproteinuric and BP response between the lisinopril and the AT1 receptor blocker group. There was no significant difference in body weight between the groups (AT1 receptor blockade group: 343 ± 12 g; lisinopril group: 330 ± 15 g). Food intake was similar in the groups and not affected by treatment.

Group III: Bradykinin Antagonist Infusion versus *Vehicle in Lisinopril-Treated Rats*

Figure 3 shows the time course of proteinuria and systolic BP in the third group. Two weeks of lisinopril treatment induced a reduction in proteinuria from 324 ± 97 mg/24 h and 355 ± 90 mg/24 h to 101 ± 10 mg/24 h ($P < 0.01$) and 103 ± 17 mg/24 h ($P < 0.01$) with a fall in systolic BP from 122 ± 5 mmHg and 121 ± 5 mmHg to 68 ± 2 mmHg ($P < 0.01$) and 70 ± 2 mmHg ($P < 0.01$) in the groups allocated to bradykinin antagonist and vehicle, respectively. Subsequent infusion of the bradykinin antagonist HOE 140 for 2 wk did not alter proteinuria (92 ± 15 mg/24 h) or BP (72 ± 2 mmHg). Vehicle had no effect on proteinuria and BP either. There was no significant difference in body weight between the groups (week 12: vehicle 356 ± 14 g; HOE 140: 348 ± 8 g). Food intake was not affected by treatment and was similar for the groups.

Group IV: Chronic Bradykinin Infusion versus *Vehicle followed by Bradykinin Antagonist or Vehicle Administration*

Figure 4 shows the time course of proteinuria and systolic BP in the fourth group. During the 2 wk of bradykinin infusion, no reduction in proteinuria was observed; the time course of proteinuria was similar to that in the vehicle group. Systolic BP fell from 114 ± 3 mmHg to 93 ± 4 mmHg ($P < 0.01$) during bradykinin, whereas vehicle did not affect BP. Subsequent infusion with the bradykinin antagonist for 1 wk did not influence proteinuria, either in the bradykinin group or in the vehicle group. BP, on the other hand, immediately increased during bradykinin antagonist infusion to pre-bradykinin levels (113 ± 4 mmHg; $P < 0.01$) in the bradykinin-infused animals, but did not change in the vehicle-infused animals. Body weight was similar in the groups (vehicle: 350 ± 10 g; HOE 140: 355± 8 g); food intake was similar as well, and not affected by treatment.

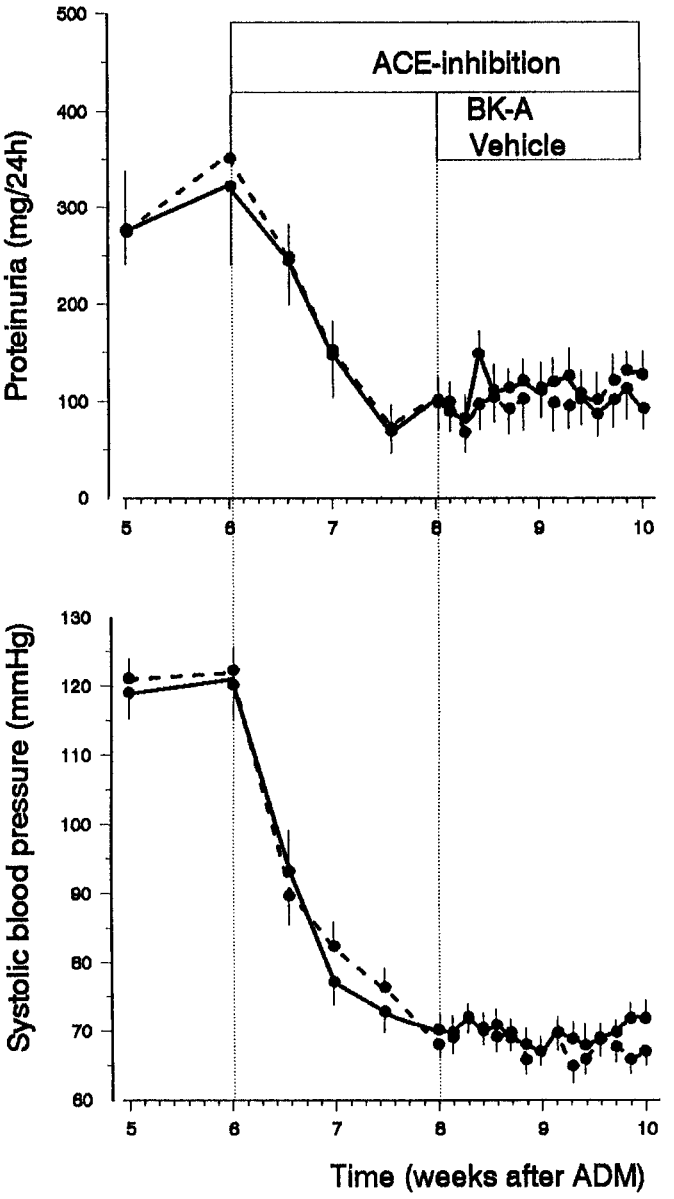

Figure 3. Group III: Bradykinin antagonist infusion *versus* vehicle in lisinopril-treated rats. Time course of proteinuria and systolic BP in lisinopril-treated rats. From week 8 through 10, either the bradykinin antagonist (BK-A; continuous lines) or vehicle (broken lines) was infused. Results are given as means ± SEM.

Discussion

In the present study in established adriamycin nephrosis, we found that chronic AngII infusion immediately and largely abolished the BP-lowering effect of lisinopril, but only partially and gradually attenuated the antiproteinuric effect of lisinopril. The AT1 receptor blocker had an effect on proteinuria and BP similar to lisinopril. Chronic infusion of a bradykinin antagonist did not influence the effects of lisinopril on BP or proteinuria. Infusion of exogenous bradykinin did not affect proteinuria in spite of a reduction in BP.

We previously demonstrated that ACE inhibition effectively reduces proteinuria in the rat model of established adriamycin nephrosis (3). To investigate whether the reduction of proteinuria by ACE inhibition was due to decreased AngII activity, we attempted to restore pretreatment conditions by chronic infusion of exogenous AngII. This did not quite restore BP to pre-lisinopril levels. However, BP during exogenous AngII was similar to BP after withdrawal of lisinopril, suggesting that the pharmacologic effect of lisinopril on BP was effectively and almost completely abolished by AngII. The antiproteinuric

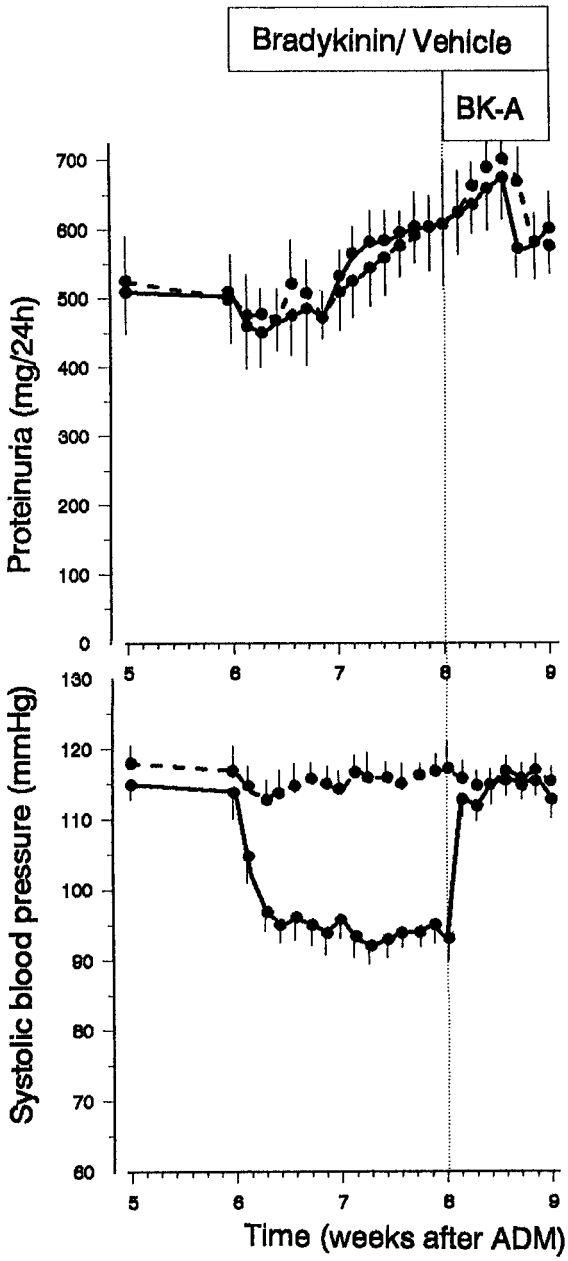

Figure 4. Group IV: Chronic bradykinin infusion *versus* vehicle followed by bradykinin antagonist or vehicle administration. Time course of proteinuria and systolic BP during either infusion of exogenous bradykinin (continuous lines) or vehicle (broken lines), combined with infusion of the bradykinin antagonist (BK-A) from week 8 through week 9. Results are given as means ± SEM.

effect of lisinopril, however, was only partially counteracted, both when compared to pretreatment proteinuria and posttreatment proteinuria. This could mean that the antiproteinuric effect of lisinopril is only partially mediated by reduction of AngII generation. However, we also found that the antiproteinuric effect of blockade of endogenous AngII by the AT1 receptor blocker was similar to that of ACE inhibition. This is in line with findings by Remuzzi *et al.* (25) and suggests that ACE inhibition has no antiproteinuric effects other than those accounted for by decreased AngII levels, and decreased interaction of AngII with the AT1 receptor subtype. Yet, in line with findings in humans, in whom short-term AngII infusion during ACE inhibition failed to restore proteinuria (26), the reversal of the antiproteinuric effect of ACE inhibition by AngII was incomplete. This might be due to the fact that systemic delivery of AngII may not be able to restore AngII levels precisely at the sites of the pathophysiologic action of endogeously generated AngII. Because not only systemic, but also intrarenal levels of AngII may be relevant to the antiproteinuric effect of ACE inhibition, we performed a long-term infusion to raise systemic and intrarenal AngII. Zou *et al.* showed that prolonged AngII infusion ($\geq$10 d) is required to raise intrarenal AngII levels as opposed to a rapid rise in plasma AngII levels (27). Such a dissociated time course of AngII levels might well correspond to the time course of the effects of AngII on BP and proteinuria in our study, but we do not avail of AngII levels to substantiate this assumption. It could be argued that our dose of AngII was too low to induce renal effects; however, elevated renal AngII (27) and clear effects on renal hemodynamics and sodium renal handling have been found with even lower doses of chronically infused AngII (28).

An alternative explanation for the only partial restoration of proteinuria by AngII infusion could be that the antiproteinuric effect of ACE inhibition is due to factors other than AngII, such as interference in the renal kallikrein-kinin system. If increased bradykinin levels would have been involved in the antiproteinuric effect of ACE inhibition, then one would have expected the bradykinin antagonist to have blunted or abolished the antiproteinuric effect of lisinopril. However, 2 wk of infusion of HOE 140 had no effect on proteinuria or BP, thus refuting the assumption of a substantial role of bradykinin in the antiproteinuric effect of lisinopril in this model. This is at variance with some findings in other rat models of renal damage, but in accord with others. For instance, Hutchison *et al.* found that HOE 140 prevented the enalapril-induced decrease in BP and albuminuria in passive Heymann nephritis (12). In puromycin aminonucleoside nephrosis, the antiproteinuric effects of enalapril were blunted by HOE 140 in the acute phase of the disease model, whereas AngII receptor blockade did not affect proteinuria (29). In rats with 5/6 nephrectomy, HOE partially antagonized the effects of ramipril on BP, proteinuria, and glomerulosclerosis in one study (13). On the other hand, Nabokov *et al.* found that HOE 140 did not antagonize the protection against renal structural damage by ramipril in 5/6 nephrectomy (15). These discrepancies may be due to differences in the pathophysiologic characteristics of the disease models. Differences in study design might be relevant as well. It should be noted that in the above studies, the bradykinin antagonist was administered at the start of the ACE inhibitor treatment, whereas in our study it was attempted to reverse the established antiproteinuric action of the ACE inhibitor.

To further explore the possible role for bradykinin in the antiproteinuric effect of lisinopril, we infused exogenous bradykinin, mimicking the potentiated bradykinin activity during

ACE inhibition. In this experiment, bradykinin was clearly capable of reducing the BP, although not as effectively as the ACE inhibitor. However, this reduction in BP was not associated with an antiproteinuric effect. Subsequent infusion of the bradykinin antagonist almost immediately abolished the BP effect, demonstrating the pharmacologic efficacy of HOE 140, without influencing proteinuria, however. These experiments suggest that decreased bradykinin breakdown is not involved in the antiproteinuric mechanism of ACE inhibition in this model.

Our studies were conducted during low sodium intake, as this ensures a clear-cut antiproteinuric response to ACE inhibition (3). This implicates an activated state of the renin-angiotensin system, which should be taken into account in the interpretation of our results, because the mechanism of action of ACE inhibition may not be entirely similar during low and high sodium intake. For instance, in patients with essential hypertension, it was reported that renin-angiotensin system blockade accounts for the majority of the effects of ACE inhibition during low sodium, whereas effects on the prostaglandin system contribute during high sodium intake only (30).

In conclusion, the antiproteinuric effect of ACE inhibition in this model appears to be independent of bradykinin effects, supporting a main role for reduction of AngII activity in the antiproteinuric effects of ACE inhibition in this model.

Acknowledgments

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