The Effect of Captopril on Urinary Protein Excretion in Puromycin Aminonucleoside Nephrosis in Rats

HOWARD TRACHTMAN, BETH ZAVILOWITZ, BOYCE BENNETT, AND DAVID I. GOLDSMITH

Departments of Pediatrics (Division of Pediatric Nephrology) and Pathology, Albert Einstein College of Medicine, Bronx, New York 10461

ABSTRACT. We investigated the effect of captopril, an orally active angiotensin converting enzyme inhibitor, on urinary protein excretion in puromycin aminonucleoside nephrotic rats. The administration of captopril (10 mg/100 g body weight) decreased proteinuria on days 10–14 following the administration of puromycin aminonucleoside (73.0 *versus* 125.0 mg, $p < 0.01$), without affecting glomerular filtration rate. The beneficial effect of captopril was not abolished by the continuous intravenous infusion of angiotensin II (10 $\mu g/kg/h$ for 9 days) or subcutaneous injections of aprotinin (50,000 KIU/day for 3 days). Indomethacin, in moderate (5 mg/kg/day for 3 days) or high (10 mg/kg/day) doses, abolished the captopril attenuation in urinary protein excretion. The salutory effect of captopril was characteized by a reduction in the fractional excretion of protein without compromising the glomerular filtration rate. No difference in renal ultrastructure was noted in captopril-treated *versus* control animals. Captopril was ineffective in reducing urinary protein excretion in rats with adriamycin-induced glomerulopathy. We conclude that captopril acts to reduce proteinuria in renal disease states arising from depletion of the glomerular basement membrane polyanion. The mechanism of action is postulated to be an alteration in renal hemodynamics, namely increased blood flow and a decrease in the ultrafiltration coefficient, that are the consequence of increased intrarenal prostaglandin production. (*Pediatr Res* 19: 828–834, 1985)

Abbreviations

AI, angiotensin I
AII, angiotensin II
ACE, angiotensin converting enzyme
BW, body weight
FEpr, fractional excretion of protein
GBM, glomerular basement membrane
GFR, glomerular filtration rate
k_f, ultrafiltration coefficient
PAN, puromycin aminonucleoside
Q_A, renal blood flow

Many vasoactive substances are capable of altering quantitative urinary protein excretion in normal and disease states. The

short-term infusion of AII into normal animals causes an increase in the fractional and absolute excretion of protein, provided the GFR is preserved (1–3). In contrast, indomethacin administration to patients with the nephrotic syndrome results in a parallel decline in absolute urinary protein excretion and GFR (4, 5).

Captopril, an orally active ACE inhibitor, interacts with several important hormonal systems that control intrarenal hemodynamics (6). By virtue of its capacity to act as a converting enzyme inhibitor, this drug interferes with the conversion of AI to AII. Under the name kininase II, this same dipeptidase is responsible for the proteolytic inactivation of bradykinin, causing elevated blood kinin concentrations and increased urinary kinin excretion (7). Captopril also acts, either directly or indirectly, to stimulate intrarenal prostaglandin synthesis and release. Each of these alterations, singly or in combination, might affect renal hemodynamic factors or intrinsic permselectivity properties of the glomerular capillary that influence macromolecular clearance (8, 9). We, therefore sought to determine whether captopril could modify the degree of proteinuria observed in nephrotic rats and, if so, to elucidate its mechanism of action.

METHODS

Male Sprague-Dawley rats, weighing 125–275 g, were paired by weight and maintained in individual metabolic cages. They were fed *ad libitum* a diet of standard laboratory rat food. After a period of acclimatization to these conditions, 24-h urine samples were collected for 4 days to establish the baseline urinary protein and creatinine excretion rates. On day 5, both animals in each pair received a single intraperitoneal injection of PAN (Sigma Chemical Co., St. Louis, MO) 15 mg/100 g BW dissolved in 0.15 M saline. Immediately after the administration of PAN, animals randomly assigned to the experimental group were started on captopril. The daily dose was 10 mg/100 g BW dissolved in 0.15 M saline and administered by oral gavage. Control animals received an equal volume of saline vehicle by the same route. Five successive protocols were performed and are described below (Fig. 1).

Protocol I ($n = 9$ pairs): no additional drugs were administered. The duration of study was 10 days following PAN administration.

Protocol II ($n = 5$ pairs): all animals received a continuous infusion of AII (Sigma), 10 $\mu g/100$ g BW/h, via intraperitoneally implanted osmotic minipumps (Alzet, Inc., Palo Alto, CA). The infusion commenced on day 6 (1 day post-PAN administration) and was maintained for 9 days.

Protocol III ($n = 5$ pairs): all animals received aprotinin (Sigma) dissolved in a 5% dextrose solution, 50,000 kallikrein inhibitory units daily, administered subcutaneously in two divided doses (10). The animals were studied for 7 days after the administration of PAN; aprotinin was given on the final 3 days.
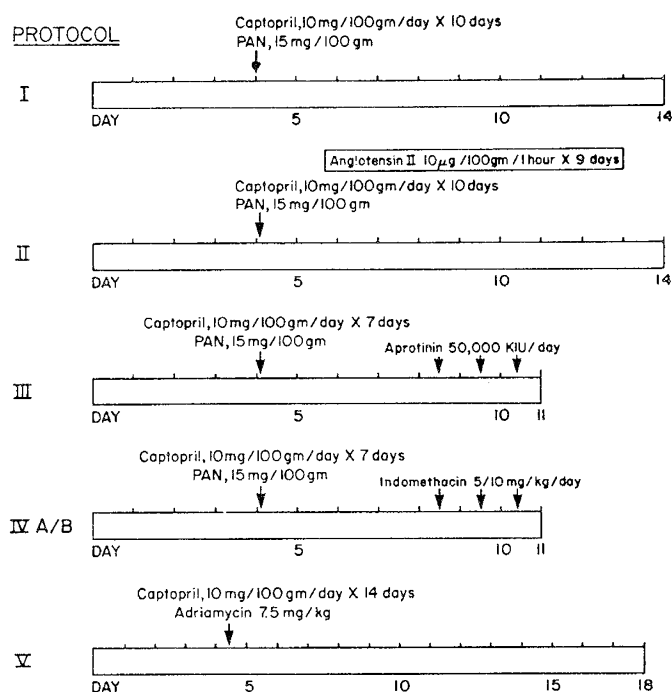
Fig. 1. Graphic illustration of the experimental design used in protocols I-V.

Protocol IVA (n = 5 pairs): all animals received indomethacin (Merck, Sharp & Dohme, West Point, PA), dissolved in TRIS-HCl buffer (pH 8), 5 mg/kg BW as a loading dose and then 5 mg/kg BW/day, administered intraperitoneally in two divided doses. The animals were studied for 7 days following the administration of PAN; indomethacin was given on the final 3 days.

Protocol IVB (n = 5 pairs): all animals received indomethacin dissolved in TRIS-HCl buffer (pH 8), 10 mg/kg BW as a loading dose and then 10 mg/kg BW/day administered intraperitoneally in two divided doses. The animals were studied for 7 days after the administration of PAN; indomethacin was given on the final 3 days.

Protocol V (n = 4 pairs): nephrosis was induced by the intravenous administration of a single dose of adriamycin (Adria Laboratories Inc., Columbus, OH) 7.5 mg/kg BW. Experimental animals received captopril, 10 mg/100 mg BW/day by gavage, while control animals were given an equal volume of saline vehicle. No additional drugs were administered to these animals. The animals were studied for 14 days after the intravenous infusion of adriamycin.

The animals were weighed and 24-h urine samples were collected daily for the duration of each protocol. Measurements of urinary protein and creatinine excretion were made on each sample. On the final day of study, the rats were sacrificed using pentobarbital anaesthesia, 50 mg/kg BW, given intraperitoneally. Serum samples were obtained for the determination of albumin and creatinine concentration. The kidneys were rapidly excised and portions placed in Dubosq-Brazil and cacodylate-buffered 2.5% glutaraldehyde solutions for fixation for light and electron microscopic examination, respectively. Thin sections were cut from paraffin-embedded material and stained with hematoxylin and eosin, periodic acid-Schiff, and Masson trichrome methods for light microscopy. Portions of the tissue for electron microscopy were postfixed in osmium tetroxide and stained with lead citrate and uranyl acetate. Ultrathin sections were cut from plastic embedded material and viewed with an electron microscope. Renal histological specimens were examined by a single pathologist (B.B.) who was blinded as to the protocol and experimental assignment of each animal.

Serum and urinary protein concentrations were determined spectrophotometrically (Guilford model) following sulfosalicylic

acid precipitation (11). Serum and urinary sodium and potassium concentrations were measured with a flame photometer with lithium as an internal standard. Serum and urinary creatinine concentrations were measured using the Jaffe reaction (12).

The endogenous creatinine clearance was assumed to be equivalent to the GFR. The FEpr and creatinine clearance were calculated using standard formulas.

Statistical comparisons between experimental and control groups within each protocol were made using Student's paired t test. Multiple comparisons between groups were made using the Duncan multiple range test and an anlysis of variance. Results were considered statistically significant if $p < 0.05$.

RESULTS

The mean weight gain of the experimental and control animals was similar in all of the study protocols except in protocol III, in which captopril-treated animals gained more weight than controls ($p < 0.01$) (table 1). All animals became visibly edematous and developed moderate to marked degrees of ascites within 7 days of receiving PAN. No measurements of serum total protein, albumin, or cholesterol were made. Daily urine output and fluid intake tended to rise in parallel with the appearance of sustained heavy proteinuria in all animals studied. Each animal manifested at least a 2-fold rise above its baseline daily urinary protein excretory rate following the administration of PAN.

The results in protocol I are illustrated graphically in Figure 2, in which mean daily protein excretion is plotted *versus* day. There was no significant change in the mean daily urinary excretion during the first 3 days after the administration of PAN in either the control or experimental groups. However, on day 8 there was an abrupt onset of proteinuria in both groups. Thereafter, the mean daily protein excretion was significantly reduced on days 10–14 in the rats given captopril compared to their untreated counterparts ($p < 0.01$).

The results obtained in protocol II and III are illustrated in Figures 3 and 4. All of the animals that received the infusion of pharmacologic doses of AII demonstrated a rise in proteinuria 1 day earlier (day 7) than the animals studied in protocol I. This discrepancy is a consequence of the AII-induced enhancement in the fractional and absolute excretion of macromolecules (2). Thereafter, on days 9–13 experimental animals given captopril displayed a significant attenuation in protein excretion compared to control rats ($p < 0.05$). A similar reduction in proteinuria was observed on days 8–11 ($p < 0.01$) in experimental *versus* control rats given aprotinin. These observations imply that captopril attenuation of proteinuria in PAN-nephrotic rats is not mediated via a reduction in the circulating levels of AII or a potentiation of intrarenal bradykinin activity.

The results obtained in protocols IVA and IVB are illustrated

Table 1. *Initial and final body wt of control and experimental animals in protocols I-V*

Protocol	Initial wt		Final wt	
	C*	E*	C	E
I	169.1	185.0	236.6	246.7
	±12.9	±21.5	±25.3	±23.9
II	140.8	138.8	168.6	151.6
	±5.2	±4.1	±13.9	±14.9
III	177.8	181.8	223.6	257.4†
	±2.5	±4.8	±5.8	±9.0
IVA	156.9	159.6	196.6	192.0
	±9.7	±9.3	±8.3	±10.5
IVB	166.4	169.4	181.0	177.2
	±4.3	±7.5	±7.3	±10.9
V	255.0	253.3	280.0	304.3
	±10.6	±10.6	±9.8	±13.3

* C, control (vehicle); E, experimental (captopril).
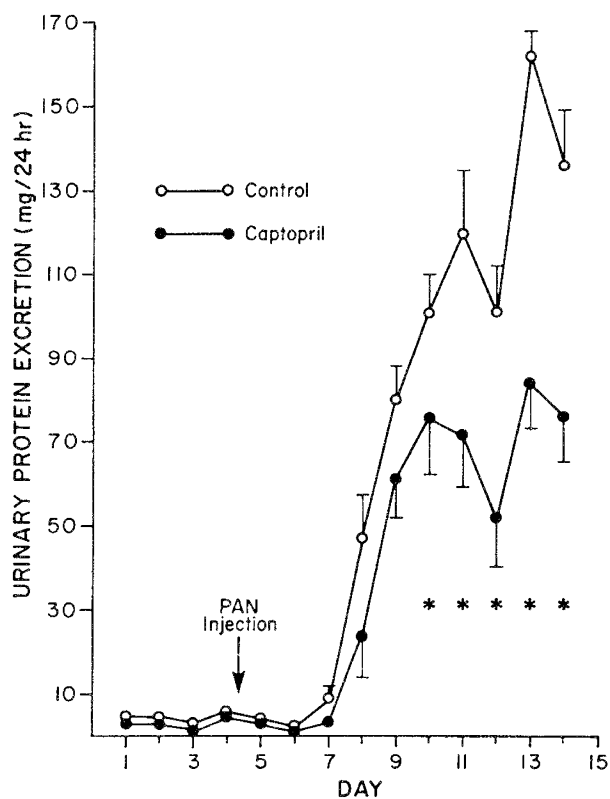Values reported as mean ± SEM; † $p < 0.01$.

Fig. 2. Urinary protein excretion plotted *versus* day in animals treated according to protocol I. Values are given as mean ± SEM; * $p < 0.01$.

Fig. 3. Urinary protein excretion plotted *versus* day in animals treated according to protocol II. Values are given as mean ± SEM; * $p < 0.05$.

Fig. 4. Urinary protein excretion plotted *versus* day in animals treated according to protocol III. Values are given as mean ± SEM; * $p < 0.01$, † $p < 0.05$.
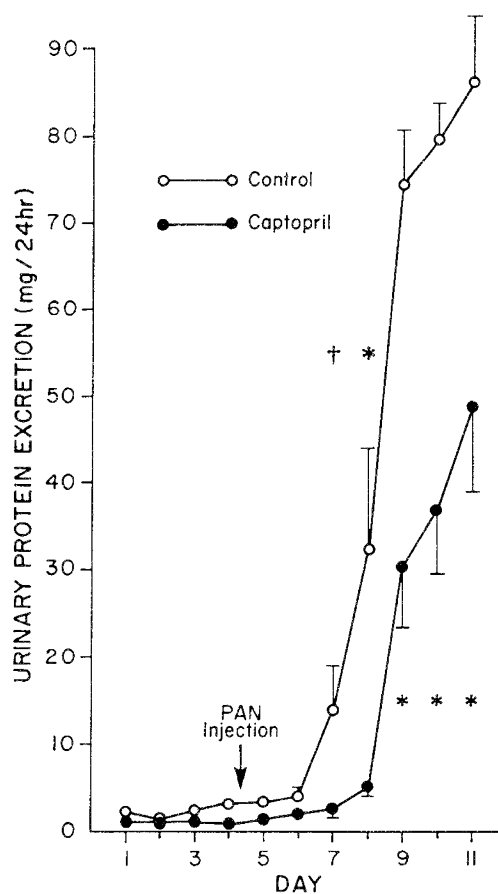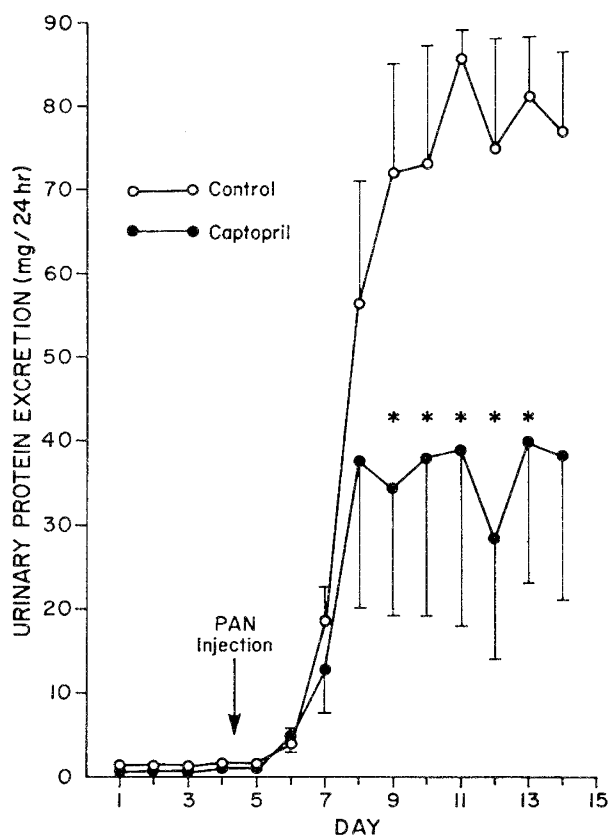
in Figure 5. In both studies, there was a rise in mean daily protein excretion on day 8, 4 days after PAN administration in experimental and control animals. However, on both the moderate and high dose indomethacin regimens, no significant differences in the daily excretion of protein were noted on days 9–11 between experimental and control animals. Thus, systemic treatment with a prostaglandin synthesis inhibitor abolished any difference in mean daily urinary protein excretion between the two treatment groups.

Animals given adriamycin to induce proteinuria demonstrated a gradual rise in urinary total protein excretion that was initially detected on day 5 after the intravenous injection and reaching a peak value by day 11. At no time during the 2-wk period of observation were any significant differences in mean daily protein excretion noted between captopril-treated and control animals (day 18: 144.9 ± 8.3 mg/24 h captopril *versus* 169.3 ± 7.2 control).

The data obtained on the final day of study in each protocol namely, mean protein excretion, GFR, and FE_{pr} are presented in Table 2. The mean urinary protein excretion on the final day of study in the experimental and control animals reflected the pattern that had been established during the preceding 3–5 days within each protocol. There was no significant difference in the mean value for GFR between the captopril-treated and control animals in any drug treament group. The serum creatinine concentrations in experimental and control rats paralleled the respective GFR determinations (Table 2). The administration of indomethacin, 10 mg/kg BW/day, resulted in a substantial reduction in the GFR in both groups of animals (0.21 ± 0.04 ml/min, captopril-treated *versus* 0.39 ± 0.07, control) compared to the values observed in the other protocols ($p < 0.05$). When the

daily protein excretion on the final day of the study was correlated with the individual values for GFR in the animals studies in protocol I, the r value was -0.62 and $+0.62$ in captopril-treated and control animals, respectively ($p < 0.05$) (Fig. 6). The magnitude and direction of the correlation coefficients changed in an analogous fashion in protocols II and III, although the changes were not statistically significant in either instance. In marked contrast, in protocol IVA the correlation coefficients were $+0.76$ and $+0.69$ in experimental and control animals, respectively, while in protocol IVB the values were $+0.67$ and $+0.71$ (Fig. 6). Using an analysis of variance, the differences in the correlation coefficients observed in protocols I and IV were statistically significant; no other comparisons were significantly different.

The FE_{pr} was lower in captopril-treated rats compared to control counterparts in protocols I-III and V, although the difference was statistically significant only in protocol I (0.022 ± 0.004, captopril-treated versus 0.035 ± 0.005, control; $p < 0.03$). Of note was the similar degree of reduction in the FE_{pr} achieved by captopril treatment in protocols I-III, i.e. 35%. FE_{pr} was the same in experimental (0.008 ± 0.003) and control animals (0.009 ± 0.003) treated with indomethacin, 10 mg/kg BW/day; moreover, in animals treated with indomethacin, 5 mg/kg BW/day, the experimental group had a higher FE_{pr} (0.033 ± 0.01) compared to control animals (0.020 ± 0.004). Using the Duncan multiple range test, it was evident that the change in FE_{pr} associated with captopril administration was significantly different in animals treated according to protocols IVA and IVB from those handled according to protocols I, II, and III. Thus, indomethacin abolished the similar reduction in FE_{pr} achieved by captopril treatment in protocols I-III.

When samples of renal tissue were examined microscopically, all specimens showed fusion of epithelial cell foot processes and
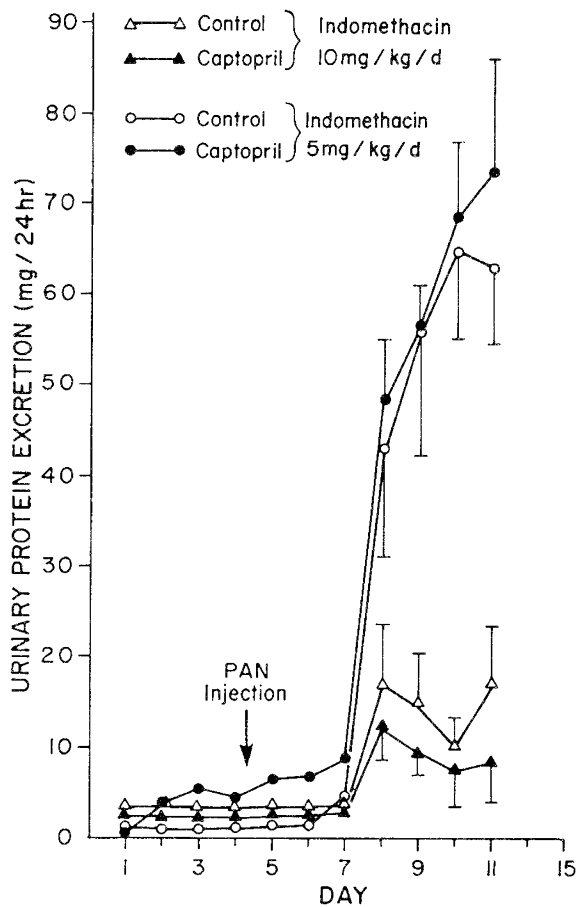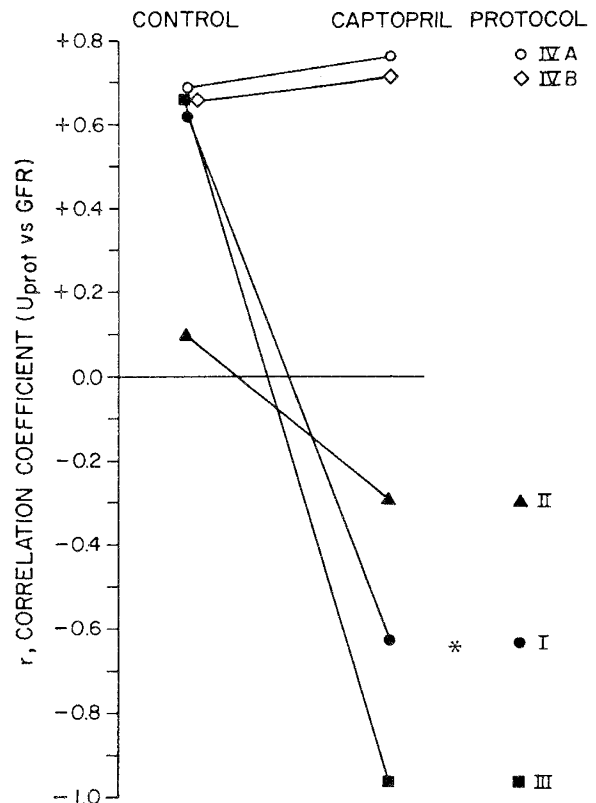

Fig. 5. Urinary protein excretion plotted versus day in animals treated according to protocols IVA and IVB. Circles indicate animals given indomethacin, 5 mg/kg/day while triangles denote animals given indomethacin, 10 mg/kg/day. The open figures are control animals (vehicle) while closed figures are experimental animals (captopril).

Fig. 6. Effect of captopril treatment on the correlation coefficient, r, relating urinary protein excretion (Uprot) versus GFR. *$p < 0.05$. ●———● protocol I; ▲———▲ protocol II; ■———■ protocol III; ○———○ protocol IVA; ◇———◇ protocol IVB.

Table 2. Renal function and urinary protein excretion on the final day of study in control and experimental animals, protocols I-V

Protocol	Uprot* (mg/24 h)		GFR (ml/min)		FEpr	
	C	E	C	E	C	E
I ($n = 9$)	136.0 ± 13.1	75.5 ± 11.0†	0.63 ± 0.09	0.75 ± 0.11	0.035 ± 0.005	0.022 ± 0.004†
II ($n = 5$)	71.9 ± 14.3	38.1 ± 16.8	0.55 ± 0.08	0.47 ± 0.04	0.013 ± 0.003	0.008 ± 0.004
III ($n = 5$)	86.5 ± 8.5	48.9 ± 9.8	0.55 ± 0.11	0.76 ± 0.24	0.029 ± 0.007	0.019 ± 0.003
IVA ($n = 5$)	62.7 ± 8.1	73.2 ± 13.0	0.43 ± 0.06	0.56 ± 0.20	0.020 ± 0.004	0.033 ± 0.01
IVB ($n = 5$)	16.8 ± 6.4	8.4 ± 4.6	0.39 ± 0.07	0.21 ± 0.04	0.009 ± 0.003	0.008 ± 0.003
V ($n = 4$)	169.3 ± 7.2	144.9 ± 8.3	1.26 ± 0.12	1.23 ± 0.16	0.035 ± 0.003	0.028 ± 0.005

* Uprot, urinary protein excretion; C, control (vehicle); E, experimental (captopril).
Values reported as mean $\pm$ SEM; † p 0.01 C versus E.

increased cytoplasmic vacuolization. No differences were noted in the appearance of captopril-treated or control animals in any experimental protocol. Thus, PAN uniformly induced a nephrotic state in all of the experimental animals.

DISCUSSION

We have demonstrated that captopril, an orally active ACE inhibitor, is capable of effecting a 50% reduction in mean daily protein excretion in rats rendered nephrotic by the administration of PAN. This action was primarily a quantitative one and did not correct the underlying glomerular lesion since the degree of nephrosis was similar in all experimental and control animals. The inability of pharmacological doses of angiotensin II and aprotinin to diminish the efficacy of captopril in reducing proteinuria indicates that the drug acts via a pathway that is independent of AII or kinin production. In contrast, the administration of indomethacin abolished the captopril effect and implicates intrarenal prostaglandin synthesis and release as the primary mediator of the drug action.

The dose of captopril used in these experiments was consistent with that used in previous investigations studying its efficacy as a chronic antihypertensive agent (12, 14, 15). At the dosage used in the present work, captopril is free of major toxic side effects. Moreover, although no blood pressure measurements are available, in the strain of rats used captopril does not exert any significant effect on systemic blood pressure (13–15). The doses of AII (2, 10, 16, 17), aprotinin (10, 18), and indomethacin (19, 20) used in this study are compatible with previously described usage, in the first instance, in both normal and nephrotic rats, and for the latter two drugs, in normal rats. In the case of aprotinin and indomethacin, they are associated with a substantial reduction in urinary kinin and prostaglandin excretion in normal rats (10, 18, 19). Although the differential actions of AII, aprotinin, and indomethacin on renal hemodynamics are not well characterized in nephrotic animals, their administration was appropriately controlled in each experimental protocol. Thus, one must conclude that captopril treatment was responsible for the differences in urinary protein excretion observed in experimental *versus* control animals in protocol I–III and that indomethacin did, in fact, abolish the difference. However, it should be noted that although comparable data are lacking regarding prostaglandins and AII, urinary kallikrein excretion is decreased in nephrotic rats (21), so that captopril treatment may have affected blood pressure differently than expected in normal rats.

One other study in the literture has explored the effect of captopril on proteinuria in the spontaneously hypertensive strain of rats with PAN nephrosis (22). The investigators concluded that captopril is unable to attenuate the proteinuria provoked by this nephrotoxic reagent. However, the experimental design utilized in this study was clearly different than ours since the daily urinary protein excretion was only measured until the initial, abrupt rise in PAN-induced proteinuria. We concur with the finding that captopril does not prevent the dramatic, early increase in protein excretion in response to PAN. However, if the animals are observed for an additional 7-day period, then a beneficial effect of captopril on PAN-induced proteinuria is clearly demonstrable.

Table 3. *Hemodynamic factors underlying the effects of vasoactive substances on urinary protein excretion*

	FEpr*	Q_A	k_f	ΔP	SNGFR
Vasoconstrictor substances, *e.g.* AII	↑	↓	↓	↑	↔
Vasodilator substances, *e.g.* PGE$_1$	↓	↑	↓	↔	↔

* FEpr, fractional excretion of protein; Q_A, renal blood flow; k_f, ultrafiltration coefficient; ΔP hydrostatic pressure gradient; SNGFR, single nephron glomerular filtration rate.

The attenuation in proteinuria induced by captopril was not causally related to a direct effect on GFR. In protocols I–III, the captopril-treated animals had significantly lower levels of protein excretion than control rats despite comparable or even slightly higher values for GFR, unlike the case in protocol IVB in which high dose indomethacin drastically reduced the GFR and urinary protein excretion in tandem. However, this finding does not exclude the possibility that the captopril-induced changes in protein excretion were mediated via hormonal effects on hemodynamic factors (Table 3) (8). For example, Brenner and co-workers (2) have demonstrated that the augmentation of proteinuria induced by vasoconstrictor substances such as AII is a consequence of reductions in renal blood flow, Q_A and ultrafiltration coefficient, K_f and a balanced elevation in the hydrostatic pressure gradient, ΔP, without a change in GFR. In contrast, vasodilator substances such as kinins and prostaglandin E$_1$ may cause a reduction in the fractional excretion of protein with a near constancy of single nephron GFR as a result of an offsetting increase in Q_A and a fall in K_f (23).

The inability of an angiotensin infusion or aprotinin administration to abolish the captopril effect indicates that the drug is not acting via its inhibition of ACE to alter these glomerular hemodynamic factors. We postulate that the action of captopril in reducing protein excretion is, in all likelihood, mediated by increased prostaglandin synthesis and release, with consequent alterations in renal hemodynamics. Evidence in favor of this proposal is available from experiments using preparations of isolated glomeruli. *In vitro* cultures containing captopril and teprotide, directly and selectively, stimulated the glomerular synthesis and release of the vasodilatory prostanoids, prostaglandin E$_2$, and prostacyclin in the absence of AII or bradykinin (24). The hemodynamic changes induced by these prostaglandins are probably an increase in Q_A and a concomitant fall in k_f. We do recognize that this functional explanation for the captopril-mediated reduction in urinary protein excretion is based on hemodynamic findings in normal rats and must be verified in the circumstance of nephrotic animals.

There has been much clinical investigation into the mechanism of action regarding the antihypertensive effect of captopril (25–28). The consensus view is that the action of captopril in reducing blood pressure cannot be entirely explained by its inhibition of ACE. There is mounting evidence to suggest that captopril-induced changes in prostaglandin production can better account for the drug's antihypertensive action in essential hypertension (26, 28). Thus, the mechanism that we propose to explain the captopril attenuation in proteinuria in nephrotic rats is consonant with the pharmacological properties of the drug and the vascular response to its administration in a different setting. Moreover, the postulated hemodynamic changes involve known intrarenal actions of prostaglandins on Q_A and k_f (23).

Indirect evidence in favor of this hemodynamic mechanism of action for captopril may be gained by examining the drug effect in rats given adriamycin to induce nephrosis. Adriamycin causes proteinuria by altering the normal pore size distribution without affecting the GBM charge density (29, 30). This is in contrast to the well-studied effect of PAN which provokes proteinuria by depleting the glomerular capillary of its electronegative sialic acid residues (31, 32). Deen and Satvat (33) have demonstrated, using a mathematical model of macromolecular clearance, that the fracitonal excretion of protein is much more sensitive to alterations in Cm, the glomerular capillary charge density, than to increases in the effective pore radius. If the action of PAN is to cause a 30% reduction in cm to 100 mEq/liter, a change similar in magnitude to that observed in human minimal change disease (34), then alterations in Q_A will still exert a significant effect on macromolecular clearance. These theoretical considerations might explain the failure of captopril to exert an effect in animals given adriamycin in comparison to the PAN nephrotic rats. It is also conceivable that captopril acts to replenish the GBM charge density in PAN-treated animals by donating

its free sulfhydryl group. Such an effect would be consistent with previous work demonstrating inhibition by captopril of vascular permeability changes induced by histamine (35). It would explain the absence of any attenuation in proteinuria in rats given adriamycin. Future experiments including quantitative clearances of macromolecules of varying pKa and histochemical staining of the GBM with catatonic probes, will be needed to clarify whether captopril causes decreased proteinuria solely as a consequence of prostaglandin-mediated hemodynamic alterations or via a direct action on the GBM charge density.

It is important to note that under the experimental circumstances in which captopril was successful in decreasing proteinuria, it changed the correlation between daily protein excretion and GFR from a positive to a negative one. This relationship is important since it suggests that a similar inverse correlation will exist between urinary protein excretion and Q_A, since the latter is the major determinant of GFR. The administration of indomethacin abolished captopril's efficacy and restored a positive correlation between protein excretion and GFR. This agrees with prior investigations that described a direct GFR-dependent effect of indomethacin in reducing protein (4). These findings imply that when it is effective, the action of captopril was proportionately greater in animals with near normal levels of GFR. PAN, which is widely used to induce a model of nephrosis said to mimic human minimal change disease, is a nephrotoxic reagent that can cause progressive renal parenchymal injury (36–38). Since it is not possible to control the extent of renal damage induced by the PAN, it is conceivable that the treated animals represented a heterogeneous sample. The animals with less extensive PAN-induced injury to the kidney may have derived maximal benefit from captopril and demonstrated significant reduction in proteinuria, while the remaining animals with widespread glomerular sclerosis manifested minimal response to the drug. It will be necessary to perform further experiments in which animals are studied at vary times after the administration of PAN and in which the proteinuria is correlated with the histological findings to validate this speculation.

These findings offer the possibility of alternate modes of therapy for steroid-dependent or steroid-resistant forms of childhood minimal change nephrotic syndrome. Our results may also be relevant in early diabetic nephropathy which, like human minimal change disease, is characterized by depletion of GBM charge density (34, 39). Each of these glomerulopathies is marked, in its incipient stages, by a normal or elevated GFR so that the response to captopril might be anticipated to be maximal (40). Other agents, such as the calcium channel antagonists, may also be effective in reducing glomerular proteinuria by the same mechanism (41, 42). It remains to be proven whether the use of drugs such as captopril, which can reduce but not abolish urinary protein excretion, is able to arrest the progressive downhill course observed in many glomerular disorders. However, clinical data in patients with focal segmental glomerulosclerosis, indicating an accelerated rate of renal functional deterioration with increasing amounts of proteinuria, underscore the importance of therapeutic maneuvers that reduce proteinuria (43).

In summary, we have demonstrated that the administration of captopril to PAN-nephrotic rats causes a marked attenuation in the degree of proteinuria normally observed in these animals. The effect was preserved despite pharmacological doses of AII or aprotinin but was abolished by the administration of indomethacin. We conclude that the action of captopril in reducing is probably mediated by alterations in renal hemodynamics, namely an increase in blood flow and a fall in the ultrafiltration coefficient, that are the consequence of increased intrarenal prostaglandin production.

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Plasma Protease Inhibitors in α_1-Antitrypsin-Deficient Children

TOMAS SVEGER

Departments of Pediatrics and of Clinical Chemistry, Malmö General Hospital, University of Lund, Malmö, Sweden

ABSTRACT. Seven plasma protease inhibitors were analyzed in 8-yr-old children—75 PiZ (severely deficient), one PiZ—(with a null gene), 32 PiSZ (moderately deficient), and 35 normals. In PiZ and PiSZ α_1-antitrypsin-deficient children, significantly higher concentrations of α_2-macroglobulin ($p < 0.001$) and antithrombin III ($p < 0.001$) were found and significantly lower concentrations of α_2-antiplasmin ($p < 0.001$), C$\bar{\text{I}}$-inactivator ($p < 0.01$) and plasminogen (PiZ $p < 0.02$, PiSZ $p < 0.01$). The inter-α-trypsin inhibitor concentrations differed in PiSZ children only ($p < 0.01$). At this age the high α_2-macroglobulin levels may be of importance to protect the lung tissue against free proteolytic activity, while importance of the other differences are unknown or reflect changes of purely theoretical interest. (*Pediatr Res* 19: 834–835, 1985)

Abbreviations

α_1AT, α_1-antitrypsin
α_1ATD, α_1-antitrypsin deficiency
α_1AC, α_1-antichymotrypsin
α_2AP, α_2-antiplasmin
α_2M, α_2-macroglobulin
ATIII, antithrombin III
C$\bar{\text{I}}$IA, C$\bar{\text{I}}$ inactivator
I-α-I, inter-α-trypsin inhibitor
PG, plasminogen

In α_1ATD, one important protease inhibitor is significantly reduced—in the PiZ state to 15% the PiSZ state to 40% and in the PIZ-state to 7.5% of the normal plasma level (1). Plasminogen and seven protease inhibitors—α_1AT, α_2M, α_1AC, α_2AP, C$\bar{\text{I}}$IA, ATIII, I-α-I—were studied in 8-yr-old children with α_1ATD followed prospectively since birth (2).

MATERIALS AND METHODS

EDTA-plasma (vacutainer) was drawn from 75 PiZ, one PiZ–, 32 PiSZ, and 35 age-matched control children. The age of both α_1ATD and control children was 8 yr 3 months ± 3 months. All were apparently healthy and had normal blood cell counts and sedimentation rates. None had clinical signs of liver disease. One-third of the PiZ, but none of the PiSZ children, had slightly increased plasma aminotransferase concentrations (2).

Plasma was stored at −20° C until analyzed. Protein concentrations were determined by electroimmunoassay using antibodies available at the laboratory of clinical chemistry (3). The concentrations are given as percentages of Seronorm (batch 103), except that of ATIII for which a standard pool of blood-donor plasma (EDTA) is used. One hundred percent corresponds to 1.5 g α_1AT/liter, 2.5 g α_2M/liter; the Seronorm protein concentrations of the other inhibitors are not properly known.

RESULTS

The protease inhibitor concentrations of the 8-yr-old α_1ATD and control children are given in Table 1. α_1ATD children (both PiZ and SZ) had significantly higher concentrations of α_2M (*p*