Plasma concentration and urinary excretion of N-terminal proatrial natriuretic peptides in patients with kidney diseases

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Plasma concentration and urinary excretion of N-terminal proatrial natriuretic peptides in patients with kidney diseases.

Background. Biologically active N-terminal fragments such as proANP(1-30), proANP(31-67), and proANP(1-98) derive from the prohormone of α-human atrial natriuretic peptide [proANP(99-126) or α-ANP]. No systematic data are available for patients with different kidney diseases.

Methods. Specific immunoassays were developed to determine plasma and urine concentrations of these fragments in 121 patients with different degrees of kidney function and urinary protein excretion, respectively.

Results. In patients with kidney disease and normal renal function without proteinuria, circulating proANP(1-30) and proANP(31-67) increased 2.8-fold and 6.5-fold, respectively. Urinary excretion of proANP(31-67) increased by a factor of 7.7 in these patients, whereas proANP(1-30) was not affected. Patients with impaired renal function had a dramatic increase of urinary proANP(31-67) excretion even before serum creatinine levels started to rise. The progression of renal failure caused a significant rise of circulating proANP(1-30) (4.3-fold) and proANP(31-67) (3.0-fold) compared with patients with normal renal function. Urinary excretion of proANP peptides significantly increased, particularly when the serum creatinine level was >5.0 mg/dL [proANP(1-30) 26-fold, proANP(31-67) 8.4-fold]. Urinary excretion of proANP(1-30) increased up to 4.4-fold and urinary excretion of proANP(31-67) increased up to 2.4-fold in patients with proteinuria in excess of 3 g/24 h.

Conclusions. Plasma concentrations and urinary excretion of proANP(1-30) and proANP(31-67) are affected by kidney disease and function, but not by proteinuria per se. It is proposed that the diseased kidney increases early urinary excretion of proANP fragments to participate in the regulation of renal function as well as sodium and water excretion.

Atrial natriuretic peptide [proANP(99-126); α-ANP] was discovered as a cardiac hormone in which the 126 amino acid prohormone proANP(1-126) is stored in atrial granules [1–3]. α-ANP regulates natriuresis and diuresis through specific renal receptors by activation of the guanylate cyclase/cyclic guanosyl 3’5’ monophosphate (cGMP) system [2–4]. Recent studies have demonstrated that after proteolytic release of the C-terminal α-ANP, presumably during the secretion from cardiac cells, the N-terminal proANP(1-98) is further degraded in the heart and the circulation to render distinct peptide fragments, namely proANP(1-30), proANP(31-67), and proANP(79-98) [5, 6]. These fragments circulate in animal species and healthy humans exhibiting circadian variations inversely related to the levels of atrial blood pressure [7]. Furthermore, it has been demonstrated that N-terminal fragments have biological functions that are qualitatively similar to α-ANP [8–10]. No data are available regarding the activity of proANP(1-98). Unlike the atria, the kidney appears to secrete an atriapeptin (AP)-like protein that was detected in the cortical tubule fraction [11]. It was further demonstrated that normal rat kidney expresses ANP mRNA [12]. Urodilatin—proANP (95-126)—is the renal analogue of α-ANP, differentially processed in the kidney and detected in only the urine. Urodilatin being synthesized in the distal tubular region may be transported as a paracrine factor to the collecting duct, where it exerts its suppressing effect on the sodium reabsorption inducing diuresis and natriuresis [13, 14].

In particular, previous pharmacological studies have reported that proANP(31-67) promotes sodium excretion and affects vasodilation in the rat [10], dog [15], and human [16]. However, the mechanisms for the natriuretic actions of proANP(31-67) are not completely defined. It is suggested that the inhibition of the sodium-potassium-ATPase in both the kidney medulla and cortex is mediated by prostaglandin E2 [17]. A recently published experimental study in anesthetized dogs with a single intact kidney [18] could show that sustained intrarenal infusions of proANP(31-67) produced a 66% fall in renin secretion associated with significant increases in creatinine clearance, renal blood flow, urine flow, and sodium excretion. Thus, proANP(31-67) may represent an im-

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important hormonal mechanism involved in the regulation of body fluid balance.

Clinical studies on circulating N-terminal proANP fragments have mainly focused on patients with cardiac disorders. It was found that their plasma concentrations correlated significantly with echocardiographic measurements of left ventricular structure and performance, the function of aortic and mitral valves, and mortality [19-22]. Our group has recently investigated the influence of periodic circulatory volume expansion on plasma concentrations of different proANP fragments in a large group of patients undergoing regular hemodialysis treatment [23]. It was found that circulating proANP fragments are influenced by a variety of factors such as end-stage renal disease, hemodialysis treatment per se, dialyzer membrane material, cardiac dysfunction, and/or hypertension.

N-terminal proANP fragments have been determined mainly in the circulation. To date, urinary excretion of proANP peptides has been investigated only in healthy humans [24] and dogs [25]. No systematic data are available about proANP fragments in nondialyzed patients with renal disease. Therefore, the present study was performed to evaluate proANP fragments in the plasma and urine of 121 patients with different kidney diseases, different degrees of impairment of renal function, and different degrees of urinary protein excretion as compared with healthy controls.

METHODS

Patients

We studied 121 patients, 63 women and 58 men, with various renal diseases. Patient ages ranged from 18 to 88 years, with a mean ± SD age of 54 ± 16 years. The etiology of kidney disease was membranous glomerulonephritis (N = 12), rapid proliferative glomerulonephritis (N = 10), IgA nephritis (N = 9), minimal change glomerulonephritis (N = 7), Henoch Schönlein glomerulonephritis (N = 2), focal segmental glomerulosclerosis (N = 9), diabetic nephropathy (N = 15), hypertensive nephropathy (N = 8), analgesic nephropathy (N = 8), polycystic kidney disease (N = 6), miscellaneous (N = 18), or shrunken kidney of unknown etiology (N = 17). In all patients, blood was drawn for blood chemistry, and urine was collected at 24 hours for analysis. Forty-three patients had a normal serum creatinine; in the remaining 78 patients, the serum creatinine level ranged between 1.3 and 6.7 mg/dL. In 29 patients, serum creatinine was between 1.3 and 2.5 mg/dL. In 39 patients, it was between 2.5 and 5.0 mg/dL, and in 10 patients, serum creatinine was >5.0 mg/dL. Urinary protein loss was <0.15 g/24 hours in 23 patients, between 0.15 and 1.0 g/24 hours in 41 patients, between 1.0 and 3.0 g/24 hours in 31 patients, and higher than 3.0 g/24 hours in 26 patients. Overall, 19 patients had normal renal function (normal serum creatinine and creatinine clearance) without a loss of urinary protein. These individuals had polycystic kidney disease or glomerulonephritis with complete remission after immunosuppressive therapy.

As a control group, 16 healthy volunteers from our hospital staff with a mean ± SD age of 36 ± 10 years (range 23 to 54) were studied. No drugs were taken by these volunteers, except hormonal contraceptives by some females.

Analytical methods

Blood samples were obtained by puncture of an antecubital vein after a supine rest of at least 10 minutes. All samples were collected in chilled tubes containing ethylenediaminetetraacetic acid (EDTA) and aprotinin, immediately placed on ice, and centrifuged within 10 minutes. Plasma was separated and stored at −70°C until analysis. Urine samples of a 24-hour collected urine were also stored at −70°C until analysis. Plasma and urine concentrations of proANP(1-30) and proANP(31-67) were determined by competitive and specific enzyme immunoassays [26]. The detection limits were 2.5 and 10 pmol/L. Cross-reactivity with proANP(1-98) was 68% and 108%, respectively, and no cross-reactivity was observed with proANP(79-98) or proANP(99-128).

Statistical analysis

Results are given as mean ± SD. Statistical analyses were performed using the Student t test of paired data when comparing differences between consecutive values in the same individuals. Differences between groups were compared by unpaired t test. Simple correlation analysis was performed by one-way analysis of variance (ANOVA). A P value less than 0.05 was considered significant.

RESULTS

Plasma concentrations and urinary excretion of proANP(1-30) and proANP(31-67) in patients with renal disease but preserved renal function

In healthy volunteers (N = 16), the plasma concentrations of proANP(1-30) and proANP(31-67) varied between 0.23 and 0.41 nmol/L. The urine concentrations of proANP(1-30) and proANP(31-67) were 88 ± 39 and 181 ± 130 nmol/24 hours, respectively. In patients with renal disease but preserved renal function and without proteinuria (N = 19), plasma levels of proANP(1-30) were 2.8-fold (P < 0.001) and of proANP(31-67) were 6.5-fold (P < 0.0001) higher than in the control group (Fig. 1A). Urinary excretion of proANP(1-30) was not different between both groups, but urinary proANP(31-67) elimination was 7.7-fold (P < 0.0001) elevated as compared with the control group (Fig. 1B). In the control group, the mean plasma level of proANP(31-67) was
Figure 1. Plasma concentration (A) and urinary excretion (B) of two different N-terminal fragments of the proatrial natriuretic peptide [proANP(1-30) and proANP(31-67)] in patients with renal disease but preserved renal function and without proteinuria (■) as compared with healthy controls (■). The fragments were determined by radioimmunoassay as described in the Methods section. Data are mean ± SD (N = 19); **P < 0.0001 compared with healthy controls (N = 16).

Figure 2. Plasma concentration (A) and urinary excretion (B) of two different N-terminal fragments of the proatrial natriuretic peptide [proANP(1-30) and proANP(31-67)] in patients with renal disease and normal serum creatinine (■, N = 43), serum creatinine between 1.3 and 2.5 mg/dL (□, N = 29), serum creatinine between 2.5 and 5.0 mg/dL (▲, N = 39), and of those patients with a serum creatinine >5.0 mg/dL (◆, N = 10). Data are mean ± SD; *P < 0.004 and **P < 0.0001 compared with patients with normal serum creatinine.

Plasma concentration and urinary excretion of proANP(1-30) and proANP(31-67) in patients with renal disease and impaired renal function

Plasma concentrations and urinary excretion of proANP(1-30) and proANP(31-67) were determined in patients with renal disease and different degrees of renal failure according to the serum creatinine level (range 0.7 to 6.8 mg/dL). Figure 2 demonstrates that patients with a serum creatinine in the normal range had the lowest plasma concentrations and urinary excretion of proANP(1-30) and proANP(31-67). Progression of renal failure was associated with a consecutive rise of plasma proANP(1-30) and proANP(31-67) concentrations. However, urinary proANP(1-30) and proANP(31-67) excretion significantly increased as soon as serum creatinine levels exceeded 2.5 mg/dL and were distinctly higher when serum creatinine levels were above 5.0 mg/dL (Fig. 2B). Circulating proANP(1-30) maximally increased 4.3-fold, and proANP(31-67) rose 3.0-fold. Urinary proANP(1-30) excretion maximally increased up to 26-fold, whereas urinary proANP(31-67) elimination rose 8.4-fold in the group of patients with serum creatinine levels over 5.0 mg/dL.

Plasma concentration and urinary excretion of proANP(1-30) and proANP(31-67) in patients with renal disease and different degrees of proteinuria

Plasma concentration and urinary excretion of proANP(1-30) and proANP(31-67) were determined in patients with renal disease according to their daily urinary protein excretion rates (range 0 to 20.3 g/24 hours). There was no significant difference for circulating proANP(1-30) or proANP(31-67) whether the patients had no proteinuria or were severely proteinuric (Fig. 3A). A significantly (P < 0.05) higher urinary excretion of proANP(1-30) was found in patients when proteinuria exceeded 1.0 g/24 h (Fig. 3B). Urinary excretion of proANP(31-67) increased according to the rise of proteinuria, but the
difference was not statistically significant until the urinary protein loss was >5.0 g/24 h. (Fig. 3B). However, patients with proteinuria between 1.0 and 3.0 g/24 hours and those with a urinary protein loss >5.0 g/24 hours had significantly higher serum creatinine levels (2.9 ± 1.9 and 2.6 ± 1.6 mg/dL) than patients with proteinuria <1.0 g/24 hours and <0.15 g/24 hours (2.3 ± 1.5 and 1.7 ± 0.9 mg/dL). Urinary excretion of proANP(1-30) increased by a maximum of 4.4-fold. Urinary excretion of proANP(31-67) was elevated by a maximum of 2.4-fold in the group of patients with proteinuria >3.0 g/24 hours.

Plasma concentration and urinary excretion of proANP fragments in patients with normal serum creatinine: Nonproteinuric versus proteinuric patients

Figure 4 shows plasma levels and urinary excretion of proANP(1-30) and proANP(31-67) in patients with a normal serum creatinine value and urinary protein excretion <0.15 g/24 hours (N = 19) as compared with patients with a normal serum creatinine value but proteinuria >1.0 g/24 hours (N = 17). Mean ± SD urinary protein loss was 0.1 ± 0.1 g/24 hours in the nonproteinuric group and 5.3 ± 5.2 g/24 hours in the proteinuric group (P < 0.0001). Circulating proANP(1-30) and proANP(31-67) did not differ between the two groups (Fig. 4A). No significant difference was also found for urinary excretion of proANP(1-30) and proANP(31-67) between the two groups (Fig. 4B).

Correlations

proANP(1-30) and proANP(31-67) in plasma and urine, and their correlation to serum creatinine levels and proteinuria. Overall, there was a good correlation between plasma concentrations of proANP(1-30) and proANP(31-67) (correlation coefficient R = 0.9, P < 0.0001), as well as between their respective urinary excretion rates (R = 0.8, P < 0.0001). No correlation was found between circulating proANP(1-30) and urinary excretion of proANP(1-30), but circulating proANP(31-67) significantly correlated with proANP(31-67) urine excretion (R = 0.5, P < 0.0001). The serum creatinine levels of the patients correlated well with both plasma concentrations and urinary elimination of proANP(1-30) and proANP(31-67) (R between 0.4 and 0.6, P < 0.0001). However, urinary protein
loss did not correlate with either plasma levels nor with urinary excretion of proANP(1-30) or proANP(31-67).

Plasma concentrations and urinary excretion of pro-ANP(1-30) and proANP(31-67) and their correlation to urinary sodium excretion, systolic and diastolic blood pressure. Urinary sodium excretion did not correlate with proANP(1-30) or proANP(31-67) in either plasma or urine. Systolic blood pressure slightly correlated with plasma proANP(31-67) concentration (R = 0.3, P < 0.01) and urinary excretion of proANP(1-30) (R = 0.3, P < 0.03), whereas diastolic blood pressure did not correlate with either of the propeptides in plasma or urine. The correlation between diastolic blood pressure and urinary sodium excretion was slightly significant (R = 0.3, P < 0.05), but not for the systolic blood pressure.

DISCUSSION

Some fragments that derive from the N-terminus of proANP, namely proANP(1-30) and proANP(31-67), have been shown to exert biological functions qualitatively similar to the C-terminal α-ANP [8–10]. The major stimulus for prohormone synthesis and ANP release is atrial stretch caused by volume overload [2–4]. It was shown that proANP(31-67) levels determined by radioimmunoassay were a better guide to small changes in salt and water metabolism than levels of α-ANP. In persons with chronic heart failure, serum levels of proANP(31-67) were reportedly the most sensitive indicator of the degree of disease, allowing differentiation of individuals in the New York Heart Association class I from normal healthy individuals [19–22]. Patients with end-stage renal disease undergoing regular hemodialysis treatment have markedly increased plasma concentrations of proANP fragments [23]. Whereas hemodialysis never corrects the defect in the metabolism of these peptides [23], successful kidney transplantation decreases the circulating levels of α-ANP, proANP(31-67), and proANP(1-98) within the first 24 hours after surgery, and the levels return to normal within 7 to 10 days [27]. However, until now there have been no systematic data available that focus on proANP fragments in patients with different degrees of renal disease before hemodialysis is instituted. Furthermore, as our assays are suitable for different biological specimen, urinary excretion was included in our studies.

Regarding the specificity of the immunoassays used, both also recognize proANP(1-98) and possibly other peptides derived from this precursor as long they contain the respective epitopes from the regions (1-30) and (31-67). However, since the levels measured are not well correlated, we conclude that the majority of the immunoreactive peptides are recognized in only one of the assays. Species that contain both epitopes and are measured in both assays represent only a small fraction. A detailed quantitation of the relative amounts would be only possible if the major circulating peptides are unequivocally identified.

We investigated 121 patients with renal disease and different degrees of renal failure as well as different degrees of urinary protein loss. In addition to the plasma concentrations of proANP(1-30) and proANP(31-67), we also determined their respective urinary excretion expressed as nmol/24 h. Since ANP is reportedly synthesized in the kidneys [11, 12] and some of its forms may have important autocrine or paracrine regulatory function [11], the urinary excretion of proANP fragments seemed to be of interest.

The present study reveals that patients with renal disease but normal kidney function and no proteinuria had significantly higher plasma concentrations of proANP(1-30) and proANP(31-67) than healthy controls (Fig. 1A). Their urinary excretion of proANP(31-67) was considerably elevated when compared with healthy subjects (Fig. 1B). Interestingly, the mean plasma concentration of proANP(31-67) was only 1.7-fold higher than the mean plasma concentration of proANP(1-30), as compared with a 13.8-fold higher urinary excretion of proANP(31-67) than that of proANP(1-30). It seems that in the diseased kidney, although renal function is normal, there are factors that determine proANP synthesis, specifically the renal clearance and degradation of the peptides.

None of the patients with normal renal function in our study had evidence for volume overload. Mean sodium excretion was 188 ± 128 mmol/24 hours and was higher compared with healthy controls (119 ± 41 mmol/24 hours). Their mean arterial blood pressure was within normal ranges (systolic blood pressure 127 ± 15 mm Hg, diastolic blood pressure 80 ± 9 mm Hg), but 6 out of 19 patients received antihypertensive medication (5 patients took 1 drug and 1 patient took 3 drugs). Schmid et al investigated hypertensive patients with polycystic kidney disease who were on a low-sodium and high-sodium diet [28]; their natriuresis-blood pressure curve showed an upward shift (resetting) and a positive slope (sodium sensitivity) associated with an exaggerated response of ANP to sodium loading. The resetting of the natriuresis-blood pressure relationship and the increased blood pressure sensitivity to sodium was observed irrespective of whether the glomerular filtration rate was normal or reduced [28]. Another five patients were actually treated with immunosuppressive agents. One patient received cyclophosphamide. Another patient was treated with cyclosporine, and three patients received low-dose prednisone. Finally, the age of this patient population was significantly higher than in healthy controls (50 ± 17 vs. 36 ± 10 years, P < 0.001). Thus, we cannot exclude that factors such as hypertension, immunosuppressive treatment, or the patients’ age could be responsible for...
the observed elevated plasma concentrations and urinary excretion of proANP peptides in patients with renal disease but normal kidney function.

Ritter, Needleman, and Greenwald demonstrated the de novo synthesis of an atriopeptin (AP)-like protein (AP126ir) in neonatal rat kidney cultures [11]. Unlike the atria, kidney cells appear to secrete AP solely by constitutive means. In primary adult rat kidney cultures, most of the AP126ir was detected in the cortical tubule fraction, demonstrating that these cells could secrete AP126ir. It was hypothesized that the renal AP may be as important as an autocrine or paracrine regulator of renal function [11]. Also, Greenwald et al demonstrated that normal rat kidney expresses ANP mRNA [12]. This study further substantiates the synthesis of ANP in the mammalian kidney. Unlike the mammalian heart, the kidney may contain two distinct ANP gene transcripts [12]. Our results support the hypothesis that proANP is synthesized in the kidney and indicate that a renal ANP secretion serves as an adaptive mechanism to preserve renal function in kidney disease.

In 1988, Winters et al demonstrated that chronic renal failure was associated with significantly increased circulating concentrations of proANP(1-30) and proANP(31-67) compared with healthy controls [5]. However, the authors have investigated 15 patients with high-grade renal impairment only [5]. Our group recently published a study on proANP peptides in a larger group of patients undergoing regular hemodialysis treatment [23]. We demonstrated that end-stage renal failure is associated with increasing plasma concentrations of proANP(1-30) and proANP(31-67) [23]. Our present study shows that with the progression of renal failure the maximal relative increase was slightly higher for circulating proANP(1-30) than for proANP(31-67) (4.3- vs. 3.0-fold). However, the highest plasma levels measured were much lower than predialytic plasma concentrations of patients with end-stage renal failure undergoing regular hemodialysis treatment. The increase of urinary excretion of proANP(1-30) and proANP(31-67) was not significant until serum creatinine exceeded 2.5 mg/dL (Fig. 2B). In patients with high-grade renal failure (serum creatinine >5.0 mg/dL), urinary excretion of proANP fragments was markedly elevated, proANP(1-30) 26-fold, but proANP(31-67) only 8.4-fold (Fig. 2B). In end-stage renal failure, the increased concentrations of circulating proANP fragments in part may reflect reduced renal clearance. However, we have demonstrated that simultaneously with the rise of proANP fragments in the circulation, the respective urinary excretion also increased. Overall, we found a highly positive correlation between the plasma concentrations of proANP(1-30) and proANP(31-67), as well as between their urinary excretions. Plasma concentrations and urinary excretion of proANP peptides were also correlated to the degree of renal failure expressed by the respective serum creatinine level. According to our findings, we assume that with the rise of circulating proANP fragments caused by progressive renal failure, the diseased kidney may increase the clearance rate of these peptides and probably also synthesizes proANP peptides in an attempt to increase diuresis.

In the present study, we further investigated whether the amount of urinary protein loss determines plasma concentrations and urinary excretion of proANP peptides. Proteinuria ranged between 0 and 20.3 g/24 hours, but the amount of urinary protein loss did not significantly affect the levels of circulating proANP fragments (Fig. 3A). Urinary excretion of proANP(1-30) significantly increased when proteinuria was higher than 1.0 g/24 hours, and urinary excretion of proANP(31-67) was significantly elevated when proteinuria exceeded 3.0 g/24 hours (Fig. 3B). This can be explained by differences in renal function. In patients with normal serum creatinine, the amount of urinary protein loss did not significantly relate to the plasma concentration or urinary excretion of proANP(1-30) or proANP(31-67) (Fig. 4). Overall, urinary protein loss did not correlate with any of the proANP fragments in plasma or urine.

Although N-terminal fragments of the pro-ANP are thought to have biological function such as natriuresis and diuresis, the sodium excretion (mmol/day) of the patients did not correlate with either proANP(1-30) or proANP(31-67) excretion or with their respective plasma concentrations. Systolic blood pressure was 137 ± 20 mm Hg (mean ± SD) and correlated slightly with plasma levels of proANP(31-67) and urinary excretion of proANP(1-30). Diastolic blood pressure was 81 ± 9 mm Hg (mean ± SD) and did not correlate with any of the proANP peptides. Diastolic blood pressure, however, showed a weak but significantly positive correlation to urinary sodium excretion.

In conclusion, plasma concentrations and urinary excretion of proANP(1-30) and proANP(31-67) are affected by renal failure, but not by proteinuria per se. Other factors such as hypertension, immunosuppressive agents, and/or age of the patients also could affect plasma and urinary proANP peptides. However, it is presumed that the diseased kidney increases urinary excretion of proANP peptides to participate in the regulation of renal function and possibly to synthesize atrial natriuretic prohormone. It is hypothesized that the early and dramatic increase of circulating and excreted fragments of proANP in renal pathology and deteriorating renal function might exert a mechanism of counter-regulation. These peptides could actually compensate for vascular and renal effects of messengers like catecholamines, angiotensin, and endothelin, which all increase because of renal pathology. This hypothesis seems reasonable, since it has recently been shown that the administration of proANP(31-67) helps to counter-regulate deteriorating re-
nual function and tubular damage in rats with acute renal failure [29]. Thus, our findings on fragments of proANP might actually disclose just one arm of a complex system working to protect vascular and renal functions under conditions of different renal pathology.

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