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ORIGINAL ARTICLE

Remission of nephrotic syndrome diminishes urinary plasmin content and abolishes activation of ENaC

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Abstract
Background Urinary plasmin activates the epithelial Na^+ channel (ENaC) in vitro and may possibly be a mechanism of sodium retention in nephrotic syndrome (NS). This study used a paired design to test the hypothesis that remission of NS is associated with a decreased content of urinary plasmin and reduced ability of patients' urine to activate ENaC.
Methods Samples were collected during active NS and at stable remission from 20 patients with idiopathic NS, aged 9.1±3.2 years. Plasminogen–plasmin concentration was measured with an enzyme-linked immunosorbent assay. Western immunoblotting for plasminogen–plasmin was performed in paired urine samples. The patch clamp technique was used to test the ability of urine to evoke an inward current on collecting duct cells and human lymphocytes.
Results The urinary plasminogen–plasmin/creatinine ratio was 226 [95 % confidence interval (CI) 130–503] μg/mmol in nephrotic urine versus 9.5 (95 % CI 8–12) μg/mmol at remission ($p<0.001$). Western immunoblotting confirmed the presence of active plasmin in urine collected during active NS, while samples collected at remission were negative. Nephrotic urine generated an inward amiloride- and α_2-anti-plasmin-sensitive current, whereas the observed increase in current in urine collected at remission was significantly lower (201±31 vs. 29±10 %; $p=0.005$).
Conclusions These findings support the hypothesis that aberrantly filtered plasminogen–plasmin may contribute to ENaC activation and mediate primary renal sodium retention during active childhood NS.

Keywords Edema · Sodium retention · Plasmin · ENaC · Aldosterone

Introduction

Childhood idiopathic nephrotic syndrome (NS) is characterized by the development of severe edema, but the molecular mechanism of renal sodium retention during NS is still incompletely understood. For many years, the traditional explanation for sodium retention was as a secondary event to increased activity of the renin–angiotensin–aldosterone system (RAAS) induced by reduced circulating blood volume [1]. This "underfill" hypothesis was eventually challenged because renin was observed to be suppressed in the majority of adult patients with NS, and inhibitors of RAAS did not attenuate sodium and fluid accumulation [2, 3]. Low levels of RAAS have also been reported in children with NS [4, 5], and it has been hypothesized that a renal disturbance in sodium handling may be the primary factor responsible for the avid sodium retention observed in NS patients [6, 7].

The pathophysiology of the intrarenal sodium retention during NS has primarily been investigated using animal models. It was shown that only the nephrotic kidney in a two-kidney model retained sodium [8] and that this retention could be prevented by the epithelial sodium channel (ENaC) blocker amiloride [9]. Svenningsen et al. recently reported that plasminogen and its active form, the serine

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protease plasmin, was present in urine from rats with puromycin aminoglycoside (PAN)-induced nephrosis [10]. In addition, plasmin in both rat and adult human nephrotic urine was found able to activate the epithelial sodium channel (ENaC) in vitro—an effect mimicked by pure plasmin [10, 11]. The efficacy of ENaC-mediated sodium reabsorption depends on the number of channels present at the apical surface and on the activation state of the channels. The latter is partially regulated by proteolytic cleavage of the extracellular domain of the γENaC subunit [12–14]. If urinary plasmin activity is related to ENaC activation and excessive sodium retention, then it can be predicted that remission of NS would attenuate or abolish urinary plasmin content and subsequently ENaC activation. In the study reported here, we used a paired observational design to test the hypothesis that plasmin is present in urine during active childhood NS with the ability to activate ENaC in vitro and this effect is resolved when remission is achieved. We therefore determined plasminogen–plasmin levels in plasma and urine and investigated the ability of the urinary samples to alter an inward current carried through ENaC in cortical collecting duct cells and human lymphocytes by single cell patch clamp. The results support a novel mechanism for sodium retention and edema formation during childhood NS with the potential for several new therapeutic targets.

Methods

Patients and definitions

Patients from eight pediatric departments in Denmark in the period from January 2008 to January 2010 were eligible for entry into our study. All included subjects were aged 1–14 years with debut or relapse of idiopathic NS. Exclusion criteria were evidence of secondary NS and specific glomerulonephritis. The study was approved by the Regional Committee on Biomedical Research Ethics. Patients were not included before written and oral informed consent was obtained from both parents.

NS was defined as proteinuria >40 mg/m^2/h or an albumin/creatinine ratio of >200 mg/mmol, plasma albumin level of <25 g/L, and edema. Remission was defined by urinary albumin dipstick negative for 3 consecutive days; relapse was defined as 3 consecutive days with ≥2+ on urinary albumin dipstick. Patients were classified as steroid resistant when remission was not achieved with prednisone therapy at 60 mg/m^2/day for 4 weeks.

Blood and urine samples

Blood and urine samples were collected before treatment at debut/relapse of NS and when stable remission was achieved, between 30 and 120 days after inclusion. Data on weight, height, age, gender, and medical treatment were noted. Blood samples for measurements of plasma creatinine, albumin, and sodium were analyzed at the Department of Clinical Biochemistry using standard procedures. The estimated glomerular filtration rate (eGFR) was calculated using the revised Schwartz formula (k=0.413) [15]. For determination of plasma plasminogen, venous blood samples were collected in EDTA-vacutainers and immediately placed on ice. The samples were centrifuged (1,500 g, 4 °C, 15 min) and the plasma frozen at −80 °C until analysis. The urine at debut/relapse was collected for a 6-h period after the first morning void was discarded. Treatment with oral diuretics and steroids (60 mg/m^2/24 h) was first initiated after blood sampling and after the 6-h urine collection was terminated. At remission, a 24-h urine collection was performed. Measurements of urinary creatinine, albumin, and sodium were analyzed at the Department of Clinical Biochemistry using standard procedures. Urine for the experimental procedures was frozen at −80 °C until analysis. To avoid proteolysis during storage, one tablet Complete Mini® (1 tablet/40 mL; Roche Diagnostics, Mannheim, Germany) was dissolved in the urine samples.

Experimental procedures

Western blotting

Urine samples were centrifuged at 10,000 rpm for 30 s, and experiments were done using the supernatant. Urine samples were pretreated using Aprotinin (USB, Cleveland, OH) coupled to CNBr-activated Sepharose 4B (Amersham Bioscience, Hillerod, Denmark) as previously described [10] using 0.4 mL of urine. The beads were pelleted and washed thoroughly. Bound proteins were eluted using 40 μL phosphate buffered saline, pH 2, and 4 μL 1 mol/L Trizma was added per sample. The aprotinin plasminogen–plasmin purification eluate was mixed with NuPAGE® Sample Reducing Agent (10×) (Invitrogen, Carlsbad, CA) and NuPAGE® LDS Sample Buffer (4×) (Invitrogen) and then heat denatured. Samples were run on a Ready Gel 7.5 % Tris–HCl (BioRad Laboratories, Hercules, CA) and subsequently blotted onto a Immobilon™ polyvinylidene difluoride membrane (Millipore, Immobilon-P transfer membrane, pore size 0.45 μm; Millipore Corp., Bedford, MA). The polyclonal goat anti-human plasminogen antibody 6189-100 (Abcam, Oxford, UK) was used as the primary antibody and horse radish peroxidase-conjugated anti-goat (DakoCytomation, Glostrup, Denmark) was used as secondary antibody. Blots were developed using the AmershamTM ECL™ system (Amersham Bioscience).

Plasminogen–plasmin concentration in plasma and urine

Measurement of plasminogen–plasmin in plasma and urine was performed using a commercially available enzyme-

linked immunosorbent assay (ELISA) (Immunology Consultants Laboratory, Newberg, OR) according to the manufacturer's instructions.

Aldosterone determination in urine

Urine samples were centrifuged at 10,000 rpm for 30 s, and assays were run on the supernatant. Urine aldosterone concentration was measured using the Coat-A-Count® Aldosterone kit (Siemens Healthcare Diagnostics, Los Angeles, CA) following the manufacturer's instructions.

Whole-cell patch clamp of cortical collecting duct cells and human lymphocytes

Patch clamp experiments were performed to analyze the capability of urine to activate the inward amiloride-sensitive current in a collecting duct cell line (M-1) obtained from Boras, Sweden. To ensure that the observed alteration in current was not a specific quality related to the M-1 cell line, we repeated the patch clamp experiments on human lymphocytes. All procedures have previously been described in detail [10, 16]. In brief, lymphocytes were isolated from blood samples collected from healthy volunteers through sedimentation over Dextran T500, washed, resuspended in RPMI-1640, and seeded onto coverslips. M-1 cells were grown to confluence in 25-cm^2 flasks (Nunc, Roskilde, Denmark) followed by trypsination and seeding onto coverslips in DMEM:F12 (Life Technologies, Taastrup, Denmark) supplemented with 5 μM dexamethasone (Sigma, Broendby, Denmark). M-1 cells were incubated at 37 °C/5 % CO_2, and single cell patch clamp experiments were performed 24–48 h after cell seeding. Lymphocytes were incubated at 37 °C/5 % CO_2, and single cell patch clamp experiments were performed 2–6 h after cell seeding. Experiments were performed at room temperature in the tight-seal whole-cell configuration of the patch clamp technique using heat-polished patch pipettes with a resistance that varied from 5 to 7 MΩ. Seal resistance was in the range of 2–15 GΩ. High-resolution membrane currents were recorded with an EPC-9 patch clamp amplifier (HEKA Electronik, Lambrecht/Pfalz, Germany) controlled by PULSE v8.11 software on a Power Macintosh G3 computer. The current was monitored by the response to a voltage step of −160 mV for 200 ms from a holding potential of −60 mV (this pulse was repeated every 3 s throughout the entire experiment). After 30–60 s, the cell was gently flushed with urine and the current monitored. To block ENaC, amiloride was added to the bath solution (2 μmol/L), and the patch clamp experiments were repeated as described above with nephrotic urine. To test for plasmin activity, α_2-anti-antiplasmin was added to the urine (1 μmol/L) and the patch clamp experiments repeated as described above.

Statistical analysis

Statistical analysis was performed using Stata statistical software for Windows (release 10; Stata Corp., College Station, TX). A p value of <0.05 was considered to be statistically significant. Normally distributed data were expressed as means and standard deviations (SD). Comparisons between such data was performed using paired t tests. To obtain normal distributions, we performed logarithmic transformation on urinary plasminogen–plasmin/creatinine ratio data and Na^+/creatinine ratio data, and these data are presented as the geometric means with 95 % confidence interval (CI). Data not normally distributed were expressed as medians and ranges, and comparisons between such data were performed using Wilcoxon signed ranks tests. Correlations were analyzed by linear regression analysis, and the model was checked by diagnostic plots of the residuals. Based on urine (U) and plasma (P) measurements, the urinary fractional excretion (FE) of x was determined as: $(FE_x) = (U_x/P_{creatinine})/(P_x/U_{creatinine})$.

Results

Demographical characteristics of subjects

A total of 20 patients (mean age 9.1 years, male:female ratio 2:1) were included in the study (Table 1). Response to steroid treatment was seen in 85 % of patients. Renal biopsy was done in three steroid-resistant patients, each showing minimal change disease. Remission was achieved with calcineurin inhibitors in combination with steroids. Two patients with relapse received angiotensin converting enzyme inhibitors (ACE) at inclusion. At remission, four patients were treated with ACE. None of the patients were treated with diuretics at inclusion or at remission.

The mean concentration of urinary sodium was 61± 54 mmol/L during active NS, which was significantly lower than that observed at remission (103±41 mmol/L; p<0.02,

Table 1 Patients' characteristics

Characteristics	Values[a]
Study cohort	20
Age (years)	9.1 (2.1–13.7)
Gender	
Female	7
Male	13
Clinical presentation	
Debut	14
Relapse	6
Response to steroids	
Sensitive	17
Resistant	3

[a]All values are presented as the number of patients, with the exception of Age, which is presented as the mean with the range in parenthesis

paired *t* test). FE_{Na}^{+} was 0.26±0.33 % at NS, compared to 0.45±0.16 % at remission (p=0.05, paired *t* test). Plasma albumin was significantly lower during active NS than at remission, while the urinary albumin/creatinine ratio was a 100-fold higher in nephrotic urine than in that collected at remission. All included patients had normal renal function, as estimated by eGFR (Table 2).

Plasminogen–plasmin in urine and plasma

A linear regression model was used to analyze the relationship between urinary albumin and plasminogen–plasmin concentration. A significant association between plasminogen–plasmin and albumin excretion in urine was found (p=0.04, r^2=0.23). Median plasma plasminogen–plasmin concentration was significantly decreased during active disease compared to remission [118 (79–137) vs. 148 (99–195) μg/mL, respectively; p<0.001, Wilcoxon signed ranks test]. The urinary plasminogen–plasmin/creatinine ratio was 255.8 (95 % CI 130.3–502.7) μg/mmol during active NS compared to 9.5 (95 % CI 7.7–11.7) μg/mmol at remission (p<0.001, paired *t* test) (Fig. 1). The median urinary $FE_{PLG/plasmin}$ was calculated as 0.024 during active NS, which was significantly higher than the $FE_{PLG/plasmin}$ of 0.0003 at remission (p<0.05, Wilcoxon signed ranks test). No relationship was found between absolute urinary plasminogen–plasmin concentration and urinary sodium concentration or when the fractional urinary excretion of plasminogen–plasmin and sodium was compared using a linear regression model (n=20, y=9.5–0.25x, r^2=0.07, p=0.3).

Table 2 Urine and plasma data from 20 patients investigated during active nephrotic syndrome and during remission

Urine and plasma parameters	Debut/relapse	Remission	*p* value
$Plasma_{Albumin}$ (g/L)	18 (12–39)	44 (37–50)	<0.001
$Plasma_{Creatinine}$ (μmol/L)	41 (26–80)	41 (26–66)	0.82
eGFR (ml/min/1.73 m^2)	124±38	119±21	0.55
$Urine_{Albumin}$ (g/L)	5.8 (0.5–38)	0.036[a]	<0.001
$Urine_{Albumin/creatinine\ ratio}$ (mg/mmol)[b]	594 (161–2436)	5 (3–9)	<0.001
$Urine_{Na+/creatinine\ ratio}$	4.2 (1.8–10.1)	14.1 (11.3–17.7)	<0.001

eGFR, Estimated glomerular filtration rate (Schwartz formula)

Data is presented as the median with the range given in parenthesis, or as the mean ± standard deviation (SD), where appropriate

[a] 0.036 is the lower detection limit of urinary albumin and corresponds to a negative urinary dipstick

[b] At remission, the urinary albumin concentration was defined to be 0.036 g/L

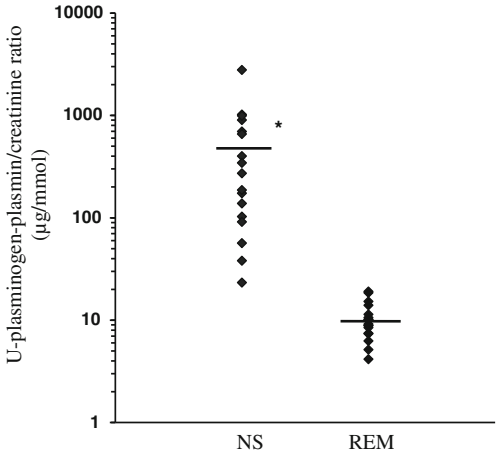

Fig. 1 Urinary plasminogen–plasmin/creatinine concentration ratios during active nephrotic syndrome (*NS*) and at remission (*REM*). There was a significantly higher ratio during active disease compared to remission (*p<0.001)

Western immunoblotting analysis of urine plasmin–plasminogen

Western immunoblotting was performed on paired urine samples in 18 of 20 patients during active NS and at remission. An affinity–purification protocol was used to extract plasmin from urine samples. Proteins in eluate were separated by sodiium dodecyl sulfate-polyacrylamide gel electrophoreisand subsequently immunoblotted for plasmin and plasminogen. Using the aprotinin plasminogen–plasmin purification eluate, we detected protein bands with a migratory pattern at approximately 90 kDa and 75 kDa, compatible with plasminogen and/or glycosylated full-length active plasmin. The 75-kDa product migrated similarly to pure human plasmin (Fig. 2). In the nephrotic phase, similar products were identified at 90–100 kDa and 75 kDa, respectively, in 15 of 18 paired samples. Most samples displayed both bands, with some variation between the intensity of the 75-kDa versus 90-kDa bands. Western immunoblotting of urine from the remission phase was negative at the 75-kDa location of active plasmin. The sample from one patient at remission displayed a band at approximately 65 kDa.

Aldosterone concentration in urine

The urinary aldosterone/creatinine ratio was 9,250 (95 % CI 4,870–17,560) ng/g in the nephrotic phase and was not statistically different from the ratio at remission, 5,580 (95 % CI 3,290–9,470) ng/g (p=0.22). Excluding the patients treated with ACEIs did not change the reported differences between the groups [8,810 (95 % CI 4,650–16,700) ng/g at debut vs. 4,760 (95 % CI 1,470–8,210) ng/g at remission; p=0.13]. The values measured in paired samples of urine from each patient are shown in Fig. 3. Five patients

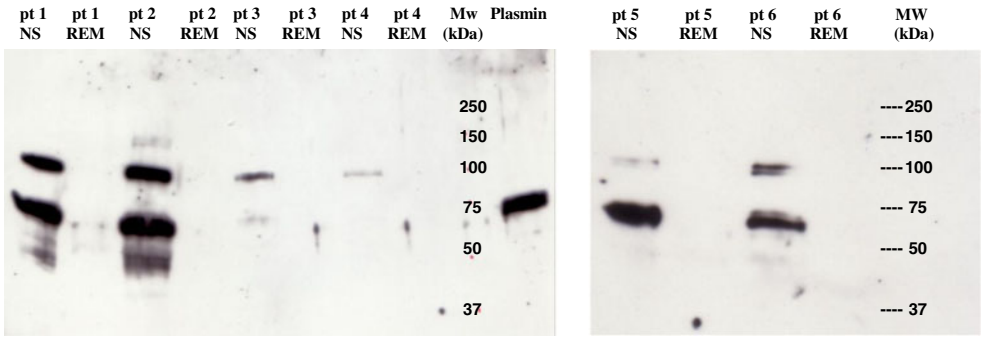

Fig. 2 Results of western immunoblotting of paired samples of patient (*pt*) urine during active nephrotic syndrome (NS) and at remission (REM) with an antibody directed against human plasmin(ogen). Pure plasmin was used as the positive control (*Plasmin*). Prior to gel electrophoresis, urine was purified by aprotinin-coated beads. In urine samples collected from patients with active NS, a migratory pattern consisting of three distinct proteins, at variable intensities in each sample, was detected: an approx. 90-kDa moiety compatible with plasminogen or fully glycosylated plasmin; a 75- to 80-kDa protein that migrated similarly as pure plasmin ("plasmin band") and thus compatible with active plasmin. No reaction was observed in matched samples at the 75-kDa position during remission. Gels are representative of 18 paired samples analyzed as described

showed markedly increased urinary aldosterone concentrations during active NS compared to remission, while the majority of patients (n=15) showed no change or a decreased urinary aldosterone concentration between the active NS and remission phases.

Effect of urine from nephrotic patients on whole-cell current in collecting duct cells (M1 line) and human lymphocytes

Urine from six patients at debut/relapse and remission was tested using the patch clamp technique. These six patients represented patients with both a high and low urinary concentration of plasminogen–plasmin ranging from 561 to 22,700 ng/mL. Whole-cell current traces were recorded before and after exposure to urine samples from each patient at debut and remission. Compared to baseline, exposure to nephrotic urine samples rapidly and significantly stimulated inward currents (Fig. 4a, e). Superfusion of single cells with urine from the same patient at remission failed to significantly stimulate inward current (Fig. 4b, e). The average increase in current due to the addition of NS urine to the M-1 cells was 201±31 %; in comparison, the average increase was 29±10 % when urine from patients in the remission phase was added (p=0.005) (Fig. 4e). The stimulatory effect of urine from patients with active NS on the inward current in M-1 cells was significantly inhibited by the ENaC blocker amiloride (201±31 to 19±10 %; p<0.001) (Fig. 4c, e). A similar reduction in current was observed when α_2-anti-plasmin was added to the nephrotic urine (201±31 to 29±8 %; p= 0.002) (Fig. 4d, e). Nephrotic urine also generated an inward current in human lymphocytes (Fig. 4f). On average, the current increased by 232±95 % when lymphocytes were flushed with nephrotic urine compared to 32±19 % when urine collected from patients at remission was added (p= 0.03). The effect of nephrotic urine was inhibited with amiloride (Fig. 4f).

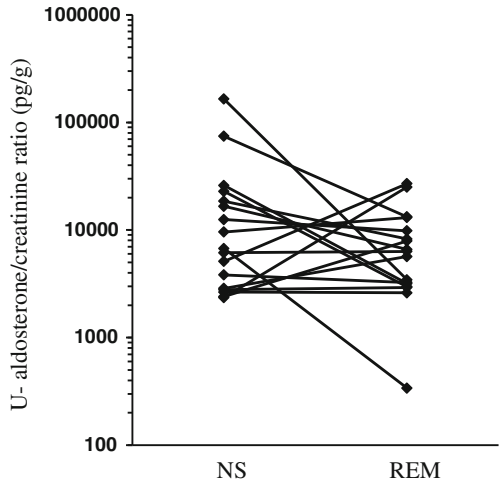

Fig. 3 Log-transformed urine aldosterone/creatinine ratios in paired samples obtained during active nephrotic syndrome (NS) and at remission (REM) from each patient. There were no significant differences

Discussion

Severe edema is the clinical hallmark of childhood NS, and its management is often challenging because of resistance even to large doses of diuretics. The results of our study show quantitative and reversible loss of plasminogen from plasma to urine and the presence of active plasmin in the urine, with the ability of urine to activate an inward, amiloride- and antiplasmin-sensitive current in collecting duct cells and lymphocytes in vitro during active NS. All of these effects waned during remission, supporting a central role of urinary plasmin in the activation of ENaC and sodium retention in patients with NS.

In agreement with other reports from adult patients [17], plasminogen–plasmin was excreted in large quantities

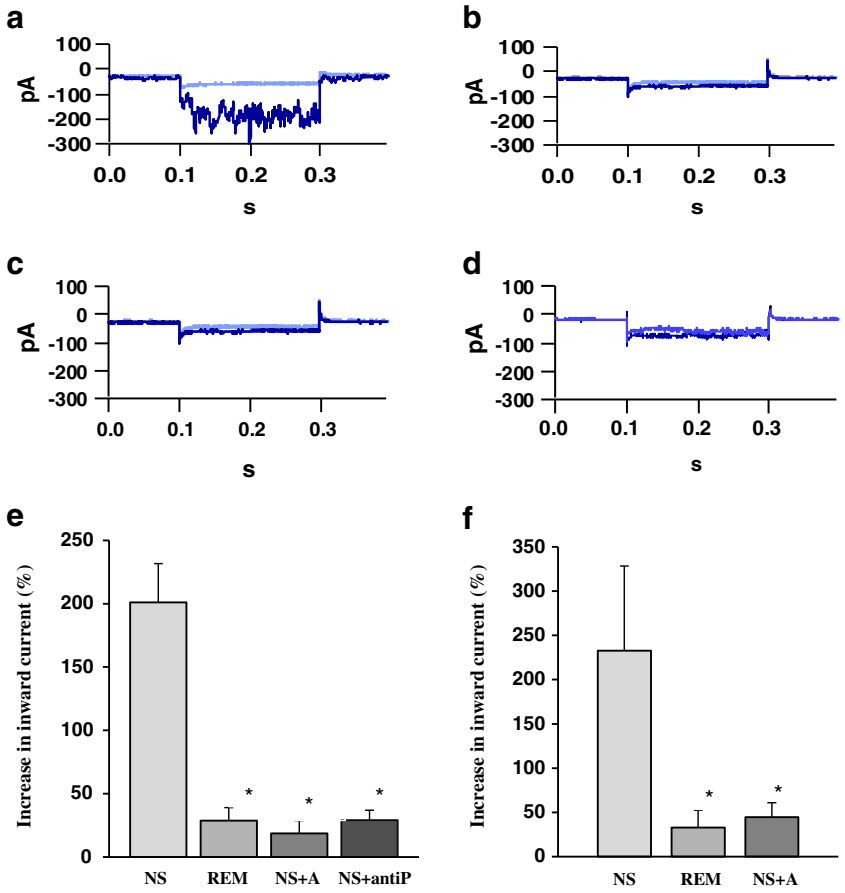

Fig. 4 Data from whole-cell patch clamp experiments with M-1 murine cortical collecting duct cells (**a**–**e**) and freshly isolated human lymphocytes (**f**). Paired urine samples collected from patients during active nephrotic syndrome (NS) and at remission (REM) were tested. **a**, **b** Whole-cell current trace (*dark lines*) after exposure of M-1 cells to nephrotic urine (**a**) and urine collected from the same patient at remission (**b**). **c** Currents from M-1 cells exposed to nephrotic urine with the addition of the epithelial Na^+ channel (ENaC) blocking drug amiloride. **d** Currents from M-1 cells exposed to nephrotic urine with the addition of alpha-2 antiplasmin. **e** *Columns* Average increase in current (%) after M-1 cells were exposed to urine samples from 6 patients collected during NS and REM. NS urine and REM urine increased the whole-cell current by 201±31 vs. 29±10 %, respectively ($p<0.001$). The stimulatory effect of nephrotic urine was inhibited by the addition of amiloride (*NS + A*) and α_2-anti-plamin (*NS + antiP*). **f** Average inward current from human lymphocytes flushed with NS urine from five patients increased significantly compared to the current evoked by urine from the same patients in REM phase and when nephrotic urine with amiloride (*NS + A*) were tested ($p=0.03$). Data are presented as the mean ± standard error of the mean. *Asterisk* indicates statistical significant difference from nephrotic phase urine at $p<0.05$

during active NS compared to remission (Fig. 1). Because the urinary albumin concentration correlated with the urinary plasminogen–plasmin content and because the plasma plasminogen concentration was lower during active NS, these observations are in accordance with an aberrant filtration of plasminogen across the defective glomerular filtration barrier during NS. The excessive filtration of plasminogen during NS might be a consequence of the similarity in molecular weight of albumin and plasminogen. Our western immunoblotting assay showed that plasmin was the predominant species detected in urine collected during the nephrotic phase (Fig. 2). The conversion of plasminogen to active plasmin in the tubular lumen may be mediated by urokinase plasminogen activator (uPA) when it binds to the cognate receptor which is present in both human and rat renal tissue [10, 18], along with the recently cloned plasminogen receptor [19]. There was a clear association between the disappearance of active plasmin, as illustrated by western blotting, a change to low or non-detectable levels of urine plasminogen–plasmin by ELISA, and conversion of patient urine to a state of inability to activate ENaC in vitro. Furthermore, we showed that the inward current induced by nephrotic urine was inhibited by amiloride, similar to findings with urine from adult patients with NS [10].

Proteolytic activation of ENaC by cell-attached proteases was discovered by Vallet et al. [13], and Svenningsen et al [10] identified plasmin as the predominant soluble protease activity in nephrotic urine, simultaneously with Passero et al., using urine from rats with metabolic syndrome [11]. In our study, the increase in current induced by nephrotic urine was similar in two different reporter cells that express

endogenous ENaC and was abolished by α_2-anti-antiplasmin, which also implicates plasmin as the culprit in children's nephrotic urine.

We observed sodium retention during active NS, but there was no linear relationship between urinary plasminogen–plasmin concentration and sodium retention. This is not to be expected, as even low concentrations of plasmin can activate ENaC through a cascade involving cell-attached prostasin [20]. Additionally, the sodium intake was not standardized, and the activity of RAAS could also contribute to sodium retention. In our study, no overall significant difference in aldosterone level was detected in the urine of patients with overt NS and those at remission. Several other studies support the notion that children with NS may present with levels of RAAS that indicate both "underfill" and "overfill" situations [4, 6, 21, 22], and it has been reported that the volume status may change in the same patient [23]. The variability observed in aldosterone concentration likely reflects different stages of extracellular fluid derangement. In our study, the majority of patients (15/20) had lower or unchanged urinary aldosterone concentrations during active NS with edema, suggesting that sodium retention is not directly dependent on aldosterone in these patients. Our findings support the hypothesis that an intrarenal mechanism for excessive sodium retention may be active independent of the volume status of patients [6, 24]. The remaining patients (5/20) had markedly increased concentrations of aldosterone during disease, indicating a possible "underfill" situation. In these patients, RAAS may contribute to the excessive sodium retention.

Loop diuretics are used as the first drug of choice in the treatment of nephrotic edemas since they are considered the most potent diuretics currently available [25]. However, such diuretics often prove ineffective in controlling edema during NS [26]. The reported renal resistance to loop diuretics could be explained by decreased glomerular filtration, but the majority of children with idiopathic NS have normal eGFR [27] as also observed in our study. Furthermore, increased urinary protein binding of furosemide during NS has been suggested to blunt its ability to inhibit the Na^+-K^+-$2Cl^-$ co-transporter, but the displacement of loop diuretics from urinary proteins does not enhance sodium excretion [28]. A more likely contributing factor to the resistance to loop diuretics may be an enhanced sodium retention mediated by a more distal part of the nephron, such as through maximally activated ENaC in the collecting ducts. Ichikawa et al. reported that sodium retention only occurred in this distal part of the nephron during rat PAN nephrosis [8]; this is the part of the nephron where ENaC is expressed [29]. The suggested pathogenic link between plasminogen–plasmin in nephrotic urine and ENaC activation may provide new therapeutic perspectives. Amiloride is known to be a potent inhibitor of ENaC and also inhibits uPA in vitro [30]. Amiloride is thus potentially able to inhibit the conversion of plasminogen to plasmin. Furthermore, Deschenes et al. reported that in their in vitro experiment with isolated cortical collecting ducts from rats with PAN-induced NS, amiloride was able to revert the decrease in sodium excretion in the collecting ducts [9]. Deschênes et al. reported two human case studies involving 13 pediatric patients in which the treatment regimens consisted of amiloride alone and amiloride in combination with furosemide. Both treatment protocols were more effective compared to the controls, and the addition of amiloride was superior to furosemide alone in treating the NS [31]. Together with the present set of data, these findings predict an unexploited therapeutic potential of amiloride in these patients.

In conclusion, the results of our longitudinal paired observational study support a central role of filtered plasminogen–plasmin in the activation of ENaC and the avid primary renal sodium retention that occurs during active childhood NS. In perspective, amiloride should be systematically tested in a randomized trial for its ability to attenuate childhood nephrotic edema.

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