Sodium retention by uPA-plasmin-ENaC in nephrotic syndrome

The authors reply

There is a great interest in exploring the mechanisms of oedema in nephrotic syndrome (NS) as evidenced by 2 parallel studies published in *Acta Physiologica* (1, 2). This has generated an editorial comment (3) which we respond to by this rebuttal. The interest in the nephrotic edema reflects not only the significant negative impact this has on the quality of life, but also the difficulties associated with identifying optimal treatment due to the lack of pathophysiological insight. Conventional diuretics, i.e. loop diuretics and thiazides, show reduced efficacy in this condition emphasizing the need for a diuretic intervention based on causal understanding. Contrary to the classical view of altered fluid distribution second to a low plasma oncotic pressure, accumulating evidence suggest that oedema formation in NS is due to aberrant renal sodium retention (4). It has been shown that pharmacologic blockade of the epithelial sodium channel (ENaC) channel using amiloride can prevent sodium retention and relieve oedema NS in both rats, mice and humans (5-8). As aldosterone is typically in normal range or suppressed in NS, possibly after a variable and short initial increase, the question is what activates ENaC in NS?

Proteolytic activation of ENaC by filtered proteases has emerged as a new concept and several serine proteases have been shown to cleave and activate ENaC (9). A number of studies have shown that NS is associated with increased urinary excretion of active serine proteases and the previous study by

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Bohnert et al. (8) was the first to document, in a mouse NS model, that the broad-spectrum serine protease inhibitor aprotinin attenuates sodium retention. This seminal observation indicates that serine protease activity contributes to nephrotic sodium retention in mouse NS (8); however, the importance of the individual protease(s) remains to be clarified. Plasmin generated by urokinase-type plasminogen activator (uPA) is the most prevalent protease in nephrotic urine in rats and thus, an attractive candidate (10). In the present studies in *Acta Physiologica*, we (2) and Bohnert et al. (1) independently tested the hypothesis that uPA is important for ENaC activation and sodium retention in murine models of NS. There is considerable agreement in the data between the two studies, but the conclusions as to the role of uPA-plasmin in the two studies are different. Both conclusions are justifiable based on the reported data and the difference, likely reflects the fact that the two groups have not performed the same experiments. Thus, the findings are stimulating and suggest further experiments to clarify the differences.

There are many similarities among the findings: The murine NS models based on either administration of the nephrotoxic doxorubicin or conditional deletion of the podocin gene in adult mice both demonstrate glomerular proteinuria, oedema, weight gain and hyperlipidemia (8). Bohnert et al. (1) documents hypoalbuminemia, which has been established previously in the conditional podocin knockout mice (11). This suggest, that irrespective of the underlying mechanisms (toxicity or podocyte gene knockout), glomerular proteinuria caused NS. Importantly, both studies showed that enhanced sodium reabsorption was blocked by amiloride demonstrating a crucial role for ENaC in these murine NS models. This is compatible with the finding in both studies, that nephrotic urine activated ENaC current *in vitro*, leading to apical sodium entry through ENaC. Thus, ENaC appears to be the rate-limiting step in the increased sodium retention and both studies support the “overfill” hypotheses in which the kidneys drive sodium retention. Bohnert et al. (1) identified an increased urinary uPA activity in nephrotic mice confirming our previous studies in humans and rats. This is also in line with our observation of increased urine protease activity in podocin knockout mice. Thus, uPA and plasminogen are aberrantly filtered across the damaged glomerular filtration barrier and plasminogen is activated to plasmin in the filtered urine. The proteolytic activation of ENaC depends on a cleavage site in the γ-subunit of ENaC. Both we and Bohnert et al. identified increased γENaC cleavage at a site where plasmin has been shown to cleave in the nephrotic mice, this has also been demonstrated previously in PAN nephrotic rats by a shift in the molecular weight of γENaC (12). Both Bohnert et al. and a previous paper by Svenningsen et al. (10) failed to show cleavage of ENaC by urokinase by itself, and thus the interesting and remaining question is, to which extent the different proteases contribute to ENaC activation. The role of urinary plasmin for the activation of ENaC was addressed in the two studies (1, 2). Bohnert et al. showed that urine excretion of
plasmin was blocked in homozygous uPA knockout mice when inducing NS by doxorubicin; however, body weight gain and urinary sodium excretion were not different between nephrotic wildtype and uPA knockout mice. This led to the conclusion that uPA-plasmin is not essential for sodium retention in NS.

We, on the other hand, demonstrated that the administration of a uPA-neutralizing antibody lead to the inhibition of urinary plasmin formation in podocin deficient, nephrotic mice, and this was associated with a significantly reduced sodium accumulation at day 8 after the initiation of proteinuria when calculated as total sodium balance. We did not observe any difference in daily urine sodium excretion or daily sodium balance during the first 3-4 days after the initiation of proteinuria, indicating that the effect could only be detected after the NS became fulminant. Thus, we conclude that uPA-activated plasmin is a significant contributor to sodium accumulation; however, it is not the sole explanation for this. The plasminogen system is quite redundant and other activators than uPA, e.g. tPA, could have led to local plasmin formation despite inactivation or deletion of uPA. We think that the discrepancies are likely to be explained by the different methodologies to determine sodium handling. Bohnert et al. used urine sodium/creatinine ratios in spontaneously voided morning urine samples, whereas we calculated total sodium balance that included intake as well as urinary and faeces collection using 24-hour collections in metabolic cages. We believe this provides for a more accurate and sensitive estimate of sodium balance, which allowed us to detect minor differences in sodium retention. In addition, it is possible that the constitutive inactivation of uPA will lead to a compensatory activation of other tubular proteases with the ability to activate ENaC. This may blunt the effect of uPA knockout in NS. It is interesting, that both studies find an identical weight gain between groups. It has previously been shown, that the non-selective serine protease inhibitor aprotinin significantly attenuated body weight gain from day 7 after induction of nephrotic syndrome compared to placebo -treated nephrotic mice. The selective uPA neutralizing antibody treatment showed a minor and late difference in accumulated Na-balance which is likely why weight change was borderline significant. Irrespective of the observed differences and possible explanations both studies suggest that blocking ENaC function is an appropriate target for the treatment of sodium and fluid retention in NS, which is supported by recent case reports (5-7). An ongoing, randomized clinical trial is currently exploring the effect of amiloride in paediatric patients with NS (13).

There are still questions that remain to be answered with respect to the mechanism of sodium retention during NS, as both studies suggest that uPA is not the sole mediator, and ENaC activation may not explain some aspects of potassium balance in NS (14). This emphasizes the need for further experimental studies.

Corresponding author: Gitte Hinrichs; e-mail: ghinrichs@health.sdu.dk
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