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Sodium Retention and Volume Expansion in Nephrotic Syndrome: Implications for Hypertension

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Abstract

Sodium retention is a major clinical feature of nephrotic syndrome. The mechanisms responsible for sodium retention in this setting have been a subject of debate for years. Excessive sodium retention occurs in some individuals with nephrotic syndrome in the absence of activation of the renin-angiotensin-aldosterone system, suggesting an intrinsic defect in sodium excretion by the kidney. Recent studies have provided new insights regarding mechanisms by which sodium transporters are activated by factors present in nephrotic urine. These mechanisms likely have a role in the development of hypertension in nephrotic syndrome, where hypertension may be difficult to control, and provide new therapeutic options for the management of blood pressure and edema in the setting of nephrotic syndrome.

Keywords

nephrotic syndrome; proteinuria; epithelial sodium channel; serine proteases; potassium sparing diuretics

Introduction

Two main hypotheses have been posited to explain the development of sodium retention in nephrotic syndrome: the underfill hypothesis and the overfill hypothesis. The premise of the underfill hypothesis is that sodium retention in nephrotic syndrome is primarily due to decreased effective circulating volume caused by fluid shifts from the intravascular to the interstitial compartment as a direct consequence of a decrease in plasma oncotic pressure by hypoalbuminemia. These changes activate sodium and water retention in the kidney. The

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overfill hypothesis postulates that sodium retention reflects an intrinsic defect in kidney sodium handling, which in turn causes volume expansion. After a brief review of evidence in support of these general hypotheses, we address basic mechanisms that may contribute to sodium retention and hypertension in nephrotic syndrome.

Evidence in support of the underfill and overfill hypotheses

It is clear that some individuals with nephrotic syndrome exhibit renal sodium retention in the setting of an activated renin-angiotensin-aldosterone system, consistent with the central tenet of the underfill hypothesis [1–3]. Capillary oncotic pressure is reduced in patients with nephrotic syndrome. According to Starling's law, a decrease in capillary oncotic pressure favors net movement of fluid across the capillary wall. This is particularly true in patients with severe hypoalbuminemia [3]. However, a parallel decrease in interstitial oncotic pressure may blunt this effect. There is evidence that the latter phenomenon occurs in some patients with nephrotic syndrome, potentially secondary to increased lymphatic drainage [4,5]. Regardless, there is sufficient underfilling of regions of the vascular bed in some nephrotic individuals to activate pathways that stimulate renal sodium retention [1–3]. However, there is heterogeneity in intravascular volume status in patients with nephrotic syndrome. Meltzer *et al.* studied a group of 16 consecutive adults with untreated idiopathic nephrotic syndrome and observed that, at presentation, some individuals had a renin and aldosterone profile suggestive of an underfilled intravascular space [6]. However, other individuals exhibited suppressed plasma renin activity and urinary aldosterone levels, suggesting intravascular volume expansion. In these individuals with nephrotic syndrome, intravascular volume depletion was not a prerequisite for sodium retention.

Likewise, low serum albumin does not appear to be sufficient to induce renal sodium retention in some individuals with nephrotic syndrome. It is well known that individuals lacking plasma albumin do not usually develop significant sodium retention. A large pediatric European case series of congenital analbuminemia showed that edema occurred only in a minority of these individuals. When it occurred, it was not a prominent feature of the syndrome [7]. Furthermore, Oliver *et al.* observed that natriuresis occurs in the recovery phase of nephrotic syndrome, even in the presence of hypoalbuminemia [8]. They studied 14 children with nephrotic syndrome on a low sodium diet, and after a variable period of observation, initiated corticosteroid therapy. The onset of natriuresis occurred before serum albumin normalized. Moreover, volume expansion with intravenous albumin does not enhance natriuresis in selected individuals with nephrotic syndrome. Koomans and colleagues studied the effect of albumin infusion in 10 individuals with nephrotic syndrome [9]. Although blood volume increased up to 120% following a single infusion of 75 g of albumin, effectively suppressing renin and aldosterone, there was no change in urine sodium excretion. Finally, measured plasma and blood volumes are mostly normal or increased in nephrotic syndrome. Geers *et al*. measured plasma volume by administration of radioactive albumin in 88 individuals with nephrotic syndrome and 51 controls [10]. Plasma and blood volumes were normal or increased in the majority of individuals and low in only 2% of cases. These findings suggest that low serum albumin and intravascular volume are not the primary mediators of sodium retention in some individuals with nephrotic syndrome.

While aldosterone plays a role in sodium retention in some individuals with nephrotic syndrome [11], in other individuals, the role of aldosterone appears minimal. For example, in a study examining individuals with nephrotic syndrome, blockade of the renin-angiotensin-aldosterone axis with captopril failed to change urine sodium excretion, despite suppression of plasma aldosterone [12]. Usberti *et al.* reported similar findings with spironolactone [13]. Studies in animals support these findings. de Seigneux *et al.* studied adrenalectomized rats with experimentally-induced nephrotic syndrome [14]. To prevent adrenal insufficiency, they administered dexamethasone, which has potent glucocorticoid activity but does not stimulate the mineralocorticoid receptor. The animals developed sodium retention despite lacking aldosterone, suggesting that mineralocorticoid receptor activation has a minimal role in sodium retention in this rat model of nephrotic syndrome.

Several experimental observations provide compelling evidence for an intrinsic defect in sodium excretion in some individuals with nephrotic syndrome. Ichikawa and *et al.* studied rats subjected to unilateral renal artery infusion of puromycin aminonucleoside (PAN), where proteinuria developed exclusively in the PAN-treated kidney [15]. Only the proteinuric kidney exhibited enhanced sodium retention, which was due to enhanced distal tubular sodium absorption. This observation confirmed that systemic factors, such as hypoalbuminemia or activation of neurohormonal systems are not required for enhanced renal sodium retention in the setting of nephrotic syndrome. Other work showed that PAN-induced volume expansion is responsive to amiloride, suggesting that epithelial sodium channel (ENaC) activation has a role in sodium retention in this setting [16,17]. Subsequent studies, discussed below, have provided new insights regarding factors in the tubular fluid in nephrotic kidneys that can activate ENaC.

ENaC activation in nephrotic syndrome

ENaC mediates the absorption of sodium from the ultrafiltrate in the late distal convoluted tubule, connecting tubule, and collecting duct. These channels have a key role in the regulation of extracellular fluid volume and blood pressure, and are activated by volume regulatory hormones such as aldosterone and vasopressin [18]. Other factors, including specific proteases, regulate ENaC [19]. The first hint that proteases could regulate ENaC was from Orce *et al.*, who showed that protease inhibitors decreased short-circuit current, a measure of ENaC activity, in toad urinary bladders [20]. Subsequent work has shown that many proteases are capable of activating ENaC [21–30]. In nephrotic syndrome, proteases in the tubular ultrafiltrate appear to have an important role in activating ENaC [31,32].

The mechanism by which proteases activate ENaC has been elucidated over the past decade. Individual ENaC channels are composed of three structurally related subunits, termed α, β, and γ [33]. Each subunit has two transmembrane segments that contribute to the channel pore and are connected by a large extracellular region. Proteases activate ENaC by cleaving the α and γ subunits at multiple specific sites within their extracellular regions (Figure 1). As nascent channels pass through the cell's biosynthetic pathway, furin, a serine protease residing in the trans-Golgi network, cleaves the α subunit twice [23]. This dual cleavage event releases a small intrinsic inhibitory tract of 26 amino acids, allowing the channel to transition from a low activity state to a moderately active state [23,34,35]. Furin cleaves the γ

subunit only once [23]. Subsequent cleavage of the γ subunit by a second protease distal to the furin cleavage site releases an inhibitory tract imbedded within the γ subunit, transitioning channels to a very high activity state [36]. The importance of release of the inhibitory tract is shown in studies of an engineered γ subunit where the inhibitory tract has been deleted, which produces highly active channels [36,37]. Furthermore, perfusion of channels with a soluble peptide corresponding to a released inhibitory tract suppresses channel activity [36,38].

In laboratory animals, increases in aldosterone levels, either due to dietary sodium restriction or aldosterone infusion, are associated with an increase in γ subunit cleavage [39–42]. However, this observed increase in γ subunit cleavage does not necessarily denote that a γ subunit has been cleaved twice, which is a key requirement for release of the intervening γ subunit inhibitory tract. Recent studies have provided strong evidence for release of the γ subunit inhibitory tract in the settings of aldosterone administration or volume depletion. Proteolytic cleavage of the rat ENaC γ subunit by furin results in a 75 kDa C-terminal cleavage product [23]. Infusion of rats with aldosterone led to a reduction in the size of this cleavage product to 70 kDa [43], consistent with a second cleavage event distal to the furin site. When aldosterone-treated rats were treated with an extracellular serine protease inhibitor, there was a shift in the size of the γ subunit from 70 kDa to 75 kDa, consistent with the protease inhibitor preventing γ subunit cleavage at a site distal to the furin cleavage site, while allowing furin cleavage.

Dual γ subunit cleavage by furin and a second protease releases the intervening peptide tract. Evidence for this comes from recent studies that employed an antibody raised against the γ subunit inhibitory tract. Sodium restriction lead to loss of the inhibitory tract from the C-terminal fragment, consistent with proteolytic removal of the inhibitory tract and channel activation [44].

Clues as to how these imbedded inhibitory tracts suppress channel activity were elucidated from a structural model of the α subunit [45]. The extracellular region of each subunit is formed by multiple subdomains. Those in proximity to the membrane are comprised of β sheets, whereas those in the periphery are formed by α helices connected by loops. The inhibitory tracts lie within the periphery of the extracellular region, where they would be expected to be easily accessible to extracellular proteases [45]. Kashlan *et al*. suggested that the inhibitory tracts are located at an interface between two extracellular subdomains, which tethers these domains and favors a low activity state [46]. Consistent with this hypothesis, chemically cross-linking these extracellular subdomains stabilized the channel in a low activity state [46].

While furin cleaves the γ subunit at a site proximal to its inhibitory tract, a growing number of proteases has been identified that can cleave at sites distal to the inhibitory tract, releasing the inihibitory tract. This group of channel activating proteases includes prostasin [21,24,47], transmembrane protease serine 4 (TMPRSS4) [24,48,49], matriptase [24,50], cathepsin B [28,51], elastase [25,26,52], kallikrein [27,53] and plasmin [31,32].

Several lines of evidence suggest that activation of ENaC by proteases aberrantly filtered through damaged glomeruli contributes to urinary sodium retention in nephrotic syndrome.

Increased γ subunit cleavage has been observed in the setting of nephrotic syndrome[54]. Urinary plasminogen has been found in a growing number of disease processes associated with glomerular proteinuria [31,32,55–58]. Plasminogen can be converted to its active form, plasmin, by urokinase in the tubular lumen [31,32,59–61]. Resolution of nephrotic syndrome is associated with reductions in levels of urinary plasminogen and plasmin [62]. ENaC's γ subunit has a plasmin cleavage site distal to its inhibitory tract, and plasmin can directly cleave and activate the channel [31,32,57], releasing the γ subunit inhibitory tract [32,63]. Plasmin may also influence ENaC activity by activating other proteases in a proteolytic cascade, analogous to the blood clotting and complement protein cascades. Svenningsen and colleagues showed that both plasmin and nephrotic urine were capable of activating ENaC currents in cultured cells, but that activation of ENaC was reduced following a knock-down of the serine protease prostasin [63]. While plasmin directly cleaves and activates ENaC, they suggested plasmin, at low concentrations, may activate the channel via prostasin, a serine protease expressed in the kidney tubule. Other proteases that are activated by plasmin could be participating in a cascade leading to ENaC activation.

While prostasin-dependent cleavage of the γ subunit and channel activation have been observed *in vitro* [21,36], prostasin may activate ENaC by other mechanisms. Interestingly, prostasin does not require its proteolytic activity to activate ENaC [44,64]. Prostasin is present within a protein complex that contains ENaC, and recent work suggests that prostasin can recruit other proteases to this complex, resulting in channel cleavage and activation [44]. Svenningsen *et al.* have suggested that prostasin facilitates the targeting of plasmin to ENaC at the plasma membrane [63]. Alternatively, plasmin could facilitate the conversion of prostasin from its inactive form to an active form.

Is there a minimum, or threshold level of urinary plasmin that is needed to activate ENaC and stimulate sodium retention by the kidney? Buhl and co-workers found that in diabetic patients with resistant hypertension, microalbuminuria is associated with sufficient plasmin to activate ENaC in cultured cells [58]. Thus, even at low levels, proteases filtered by leaky glomeruli may contribute to hypertension.

If proteolytic activation of ENaC is a key contributor to sodium retention and extracellular volume expansion in the setting of proteinuria, should pharmacologic targeting of ENaC, or of specific channel activating proteases, be used as a therapeutic approach in this setting? Clinical studies are needed to address these questions. The use of ENaC inhibitors (potassium-sparing diuretics, such as amiloride) may be effective [65], but the proclivity of these agents for inducing hyperkalemia may be compounded by impaired potassium excretion in nephrotic syndrome where individuals who also have a decreased glomerular filtration rate, low aldosterone levels, or are receiving inhibitors of the renin-angiotensin-aldosterone system.

Other factors influence renal sodium handling in nephrotic syndrome

Extracellular fluid volume expansion associated with nephrotic syndrome should be opposed by the actions of atrial natriuretic peptide (ANP). Volume expansion induces release of pro-ANP from atrial myocytes [66,67]. Active ANP is generated from pro-ANP by proteolytic

cleavage. ANP acts on the vasculature of the kidney to increase glomerular filtration while reducing sodium reabsorption in the proximal tubule and inner medullary collecting duct [68–73]. Serum ANP levels are frequently elevated in nephrotic syndrome [74–76]. However, ANP-dependent natriuresis is blunted in nephrotic syndrome, reflecting a dampening of ANP-dependent signaling mechanisms [74,77,78], and contributing to the failure of ANP to correct extracellular volume overload.

An additional mechanism that may contribute to decreased ANP signaling in nephrotic syndrome is decreased conversion of pro-ANP to active ANP. Cleavage of pro-ANP is mediated by the protease corin [79], which is expressed in the kidney as well as in cardiac tissue. Corin is down-regulated in nephrotic kidneys, with an associated increase in pro-ANP and reduction in ANP [80]. Corin-null mice exhibit impaired sodium excretion and salt-sensitive hypertension that is responsive to amiloride but not calcium channel blockers [81]. Thus, decreased ANP activation by corin may contribute to the failure of ANP to correct hypervolemia in nephrotic syndrome.

Alterations in nitric oxide signaling may also contribute to enhanced renal sodium retention in nephrotic syndrome. Rats with PAN-induced proteinuria exhibited a decrease in nitric oxide synthase expression in the kidney [82]. Reduced nitric oxide signaling in nephrotic syndrome may contribute to increased sodium retention by increasing tubulo-glomerular feedback and collecting duct ENaC-mediated sodium reabsorption [83–87].

Other factors may have roles in enhancing tubular sodium absorption in nephrotic syndrome. For example, the Rho family GTPase Rac1 has been reported to activate the mineralocorticoid receptor in an aldosterone-independent manner [88]. Dahl salt-sensitive rats exhibit proteinuria [89], and salt-loading of Dahl salt-sensitive rats stimulates Rac1, which activates the mineralocorticoid receptor [90] and increases ENaC expression [91]. Whether Rac1 activity is up regulated in other proteinuric states remains to be seen. This mechanism could contribute to aldosterone-independent activation of mineralocorticoid receptor-dependent signaling pathways in nephrotic syndrome.

Conclusion

Edema is one of the clinical hallmarks of nephrotic syndrome. In some individuals, activation of the renin-angiotensin-aldosterone system leads to excessive sodium retention with expansion of the extracellular fluid volume and edema. However, other individuals exhibit sodium retention in spite of a suppressed renin-angiotensin-aldosterone system. Evidence suggests that mechanisms intrinsic to the kidney contribute to sodium retention in nephrotic syndrome, including activation of ENaC by aberrantly filtered proteases. One such protease, plasminogen, is found in nephrotic urine and can be activated by tubular urokinase, producing plasmin. Plasmin, either directly or as part of a protease cascade, cleaves and activates ENaC. Future studies are needed to address whether ENaC inhibitors, such as amiloride, are efficacious in treating extracellular volume expansion and edema in nephrotic syndrome. However, use of these agents may be limited by the risk of hyperkalemia.

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Clinical Summary

- A number of mechanisms contribute to renal sodium retention in nephrotic syndrome. A subset of nephrotic individuals retains sodium in the setting of an activated renin-angiotensin-aldosterone system. Others exhibit sodium retention without activation of this system.
- Filtered serum proteases activate the epithelial sodium channel (ENaC), providing an alternative mechanism for sodium retention in the setting of proteinuria.
- Plasminogen is filtered by damaged glomeruli, and can be converted to its active form (plasmin) by tubular urokinase. Plasmin directly activates ENaC, and may also act in concert with other proteases, such as prostasin, to activate ENaC.
- Homeostatic mechanisms that would normally oppose volume overload, such as atrial natriuretic peptide signaling, appear to be blunted in some individuals with nephrotic syndrome.
- Inhibitors of ENaC may be beneficial in managing volume overload in the setting of nephrotic syndrome, but their use may be limited by hyperkalemia.

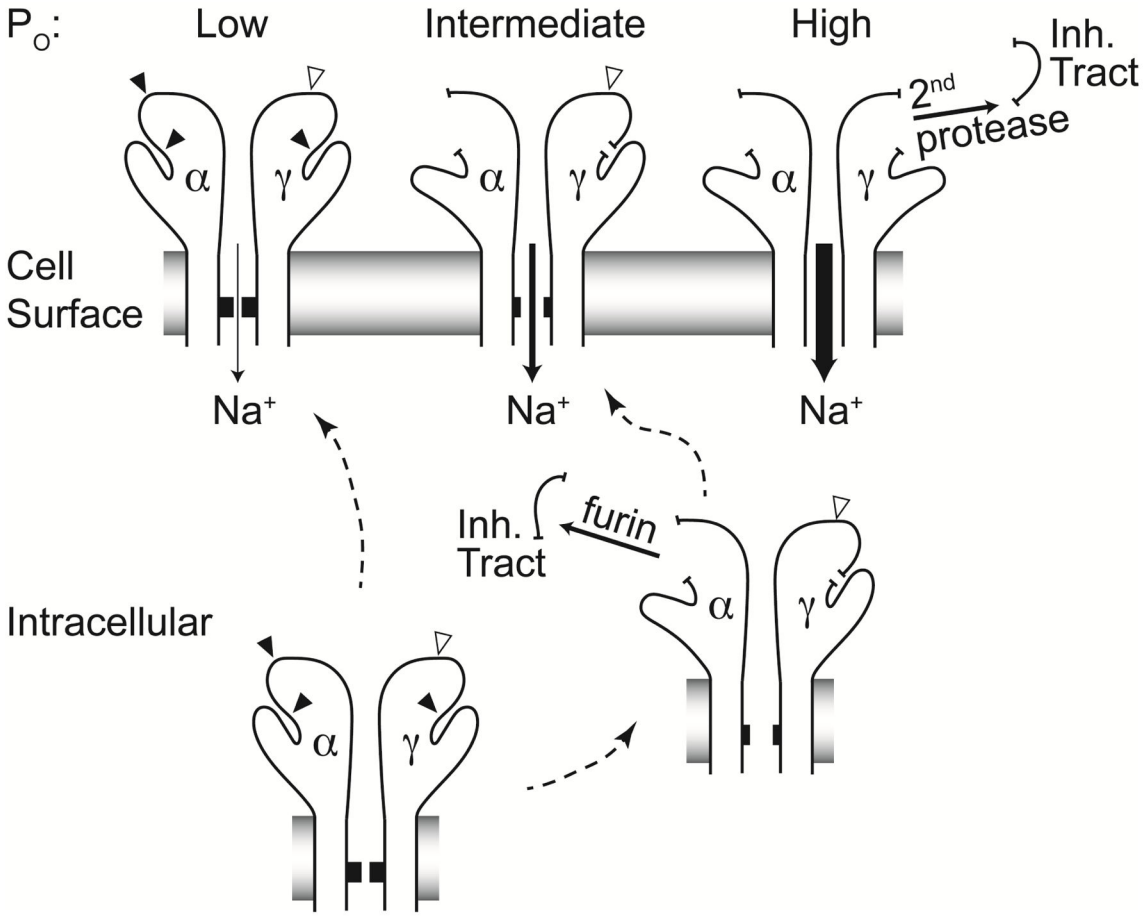

Figure 1.

Two of ENaC's three subunits (α and γ) undergo proteolytic cleavage. Furin cleaves the channel before it reaches the cell surface (closed arrowheads). Some channels escape furin cleavage and arrive at the cell surface intact. These channels exhibit very low activity, or open probability (P_o). Furin cleaves the α subunit twice and the γ subunit once. Dual cleavage of the α subunit by furin releases that subunit's inhibitory tract, resulting in channels with intermediate activity. The γ subunit harbors one furin cleavage site proximal to its inhibitory tract, and sites for various other extracellular proteases distal to the inhibitory tract (open arrowhead). Release of the γ subunit inhibitory tract occurs when the subunit has been cleaved by both furin and a second protease, resulting in a maximally activated channel.