Chapter

Proteasuria: The Link between Physiopathogenesis and Edema Management in Nephrotic Syndrome?

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Abstract

Nephrotic syndrome is a pathology characterized by severe proteinuria, hypoalbuminemia, dyslipidemia, and edema. Edema has classically been associated with an alteration of the forces that govern Starling's principle. However, some proteins eliminated in excess in the urine (proteasuria) can activate the epithelial sodium channel (ENaC), favoring sodium retention and edema. The α- and γ-ENaC subunits are activated by releasing inhibitory segments that favor the probability of channel opening. Some proteases that have been investigated include plasmin, prostasin, transmembrane protease serine 4, cathepsin, and neutrophil elastases. Therapeutic strategies include water and sodium restriction in the diet, appropriate dosing of diuretics (loop, thiazides, or acetazolamide), and in severe cases, mechanical ultrafiltration. Due to the continuous activation of ENaC in nephrotic syndrome, amiloride is an attractive diuretic strategy that has been shown to be effective in some patients with an acceptable safety profile.

Keywords: nephrotic syndrome, proteasuria, epithelial sodium channel, edema, amiloride

1. Introduction

Peripheral edema and extracellular volume expansion are features commonly observed in patients with nephrotic syndrome (NS), this is consequential from sodium retention. Two different theories have been suggested putting forward the links between protein loss in NS and enhanced sodium reabsorption by the epithelial sodium channel (ENaC). Since 1917 the underfill theory was proposed to explain that hypoalbuminemia due to proteinuria leads to intravascular volume depletion through

the loss of oncotic pressure and this led the way to fluid accumulation in the interstitial space but an underfilled intravascular compartment. This in turn would activate the renin-angiotensin-aldosterone system (RASS). On the other hand, the overfill hypothesis, formulated in 1979 suggest that proteinuria-induced sodium retention is an intrinsic renal event that is independent of the RASS activation. Despite these theories mechanisms leading to primary sodium retention in nephrotic kidneys are not yet well understood. But recently intraluminal activation of the ENaC by abnormally filtered proteases has been proposed as a mechanism that could explain avid sodium reabsorption, which would be consistent with the overfill theory. In 2018 the term "proteasuria" was first introduced to indicate the increased excretion of active plasma proteases found in the urine of patients with NS.

2. Underfill theory in nephrotic syndrome

This theory was first proposed more than 100 years ago by Epstein. In the original article published in 1917 by the American Journal of Medicine he proposed that the loss of protein incurred by the blood serum through the continuous albuminuria causes a decrease in the osmotic pressure of the blood, which favors the absorption and retention of fluid by the tissues.

To understand the exact nature of the processes which leads to the production of edema we must consider for a moment the mechanisms in which the exchange of fluid between the blood and the tissues is regulated. In normal conditions the loss of fluid from the blood is rapidly restored by the passage of fluid from the tissue spaces back into the blood capillaries.

Under normal conditions the hydrostatic pressure within capillaries and the oncotic pressure determine the movement of fluid from the vascular compartment to the interstitium. The oncotic pressure is determined by the proteins in the blood. The Starling equation was created in 1896 by Ernest Starling, he expressed the flux of water across capillaries. Oncotic pressure moves fluid out, while hydrostatic pressure moves fluid in [1]. According to Starling, during the production and absorption of tissue fluid, there is normally a balance between the filtration pressure in the capillaries and the osmotic pressure of the colloids of the blood and tissue fluid. Plasma and tissue fluids are practically identical in everything except protein. Plasma exceeds tissue protein concentrations.

Thus, Epstein suggested that in patients with NS this loss of proteins represented a pressure equal to 20–24 mmHg, a factor strong enough to disturb the equilibrium in the exchange of fluid between the blood and the tissues. Leading to a fall in the osmotic pressure of the blood and giving to the tissues the controlling power to absorb and retain fluid.

The imbalance of Starling's forces leads to interstitial leakage of fluid and decreased circulating volume that in the end will decrease circulating volume and renal perfusion, activation of the RAAS will occur trying to compensate this imparity [2]. The activation of the sympathetic system and the renin angiotensin axis can be observed both in renal parenchymal involvement and in hypovolemia, but it is the increase in plasma vasopressin of non-osmotic origin what supports that the main trigger is hypovolemia [3].

Extravasation of fluid decreases intravascular volume and increases neurohormonal markers of intravascular depletion, such as vasopressin and aldosterone, resulting in a highly concentrated urine with very low sodium content. Therefore,

renal sodium retention in this scenario is a secondary phenomenon, a physiological consequence of intravascular underfilling [4].

2.1 Clinical evidence

Through studies where capillary permeability with albumin marked with TC99m is made, it was shown that the permeability percentage was significantly increased in glomerular diseases compared to healthy controls. The percentage of permeability did not vary from a glomerular disease to another nor was it correlated with the degree of albuminemia or proteinuria [5].

Vande Walleet et al., compared children with NS secondary to minimum change disease versus those without minimal changes and subsequently divided them by the presence or absence of signs and symptoms of hypovolemia. Patients with hypovolemia symptoms in both groups had significantly high levels of norepinephrine, renin, and aldosterone unlike their non -symptomatic counterpart [6].

Usberti et al., studied 16 pediatric and adult patients with NS but normal renal function. They found a decreased plasma sodium concentration, increased plasma vasopressin (AVP), impaired excretion of an acute water load, and elevation of plasma renin activity (PRA) and urinary norepinephrine levels as compared to controls. When they were treated with isotonic albumin infusion they decreased plasma AVP concentration and increased water diuresis [7].

Based on the above, a volume load would decrease the activation of all mechanisms that influence sodium and water reabsorption, however the mechanisms of compensation of the edema at the capillary level are found exhausted so that when administering fluids these are quickly spread to the interstitium, making almost impossible the restoring of intravascular volume.

2.2 Critique to the underfill hypothesis in nephrotic syndrome

Several clinical and laboratory observations have disputed about the accuracy of underfill hypothesis. Historically, the description of edema formation in NS was simple: an unknown trigger leads to proteinuria, as the plasma protein level falls, the intravascular oncotic pressure decreases with consequent leakage of plasma water into the interstice, thus generating edema. Owing to the extravasation of the fluid, the intravascular volume is decreased and neurohormonal markers of intravascular depletion are increased, such as vasopressin and aldosterone, resulting in highly concentrated urine with very low sodium concentrations [8].

The therapeutic consequences of this hypothesis for the treatment of edema are clear, expansion of intravascular volume and restoration of plasma oncotic pressure by administration of albumin [4].

2.3 Treatments based on the underfill hypothesis

Albumin infusion is used to correct hypoalbuminemia since it is the principal cause for edema [3, 4]. Treatment by albumin alone reduces plasma renin activity, but do not reflect adequate diuresis response [9]. Some of these patients require high doses of oral or intravenous diuretics for edema management. Patients with diuretic resistance edema might benefit from coadministration of albumin and furosemide. Several mechanisms are proposed to explain the improved efficacy of combined therapy, the most accepted being that intravenous albumin enhances

the secretion of loop diuretics in proximal tubules, with increased delivery at its primary site of action [10, 11].

It's recognized that two groups of patients with NS benefit form albumin infusions: patients with intravascular hypovolemia, and those with severe refractory edema. Patients with severe hypoalbuminemia, especially those with refractory and resistant steroid illness, require repeated courses of albumin infusion [10]. Once the patient goes into remission, the first symptom (besides the disappearance of proteinuria) is a large diuresis, before plasma protein levels (and thus oncotic pressure) have normalized [12, 13].

Generally, loop diuretics were administered as bolus dose during or at end of the albumin infusion. The rate of albumin infusion must be monitored to avoid risk of overload and pulmonary edema, as it has associated in some patients [14]. The effect of albumin is transient, and during relapse most patients would excrete the amount infused over next 24–48 hours [10]. It is recommended that adequate urine output be measured before initiating albumin infusion, and being careful in patients with hypertension, pulmonary edema, respiratory distress, or congestive heart failure.

Several clinical studies have sought the improvement of refractory edema with albumin infusion in the treatment of NS with partial efficacy and opposite results. A recent Cochrane review failed to draw any conclusion due lack of studies as they excluded cross-over studies [15]. Only one study met their inclusion criteria (26 children with minimal change disease) and compared albumin infusion plus furosemide with and equal volume of dextrose. The authors reported clinical improvement as a greater weight loss difference overall at 10 days; however, they also identified changes in serum sodium and blood pressure but data in text are inconsistent and reflects low certainty evidence.

Meena J, et al. [10], performed a meta-analysis that included six studies, involving 69 patients, only one study included children, and most of them had small sample size and high risk of bias. This meta-analysis showed that combination therapy was more effective in increasing diuresis than furosemide therapy alone.

2.4 Analbuminemia and edema

Inherited albumin abnormalities include bisalbuminemia and analbuminemia. Analbuminemia, also called idiopathic hypoalbuminemia, is a rare congenital disorder of albumin characterized by very low levels of this protein inherited as an autosomal recessive trait [16]. The diagnosis is realized with electrophoresis who shown a markedly decreased levels of albumin and elevation of other proteins.

An exponential increase in "non-albumin" plasma proteins (globulins and proteins of higher molecular weight) compensates intravascular colloid osmotic pressure [17]. With the increase of other plasma proteins, a decrease in hydrostatic pressure has been found, which explains the absence of edema in these patients, or slight edema. Also, patients with analbuminemia had slightly decreased renal plasma flow, slightly diminished glomerular filtration and markedly augmented filtration fraction compared to healthy subjects [16].

There is very little correlation among the markedly decreased levels of serum albumin and the reported signs and symptoms of the patients. Clinically, 50% of patients are completely asymptomatic and the rest have fatigue, asthenia, and orthostatism. One third of the patients presents without edema [4], while the others have slight edema of legs and ankles. Lipid metabolism abnormalities are observed in some patients [16, 17].

Finally, other observations that do not fit well with underfill hypothesis includes that reducing the activity of the renin-aldosterone axis by mineralocorticoid receptor antagonists, such as spironolactone, or angiotensin-converting enzyme inhibitors does not result in a marked increase in sodium excretion in most patients with NS [9, 18]. Also, attempts at trying to measure blood volume and/or neurohumoral markers of volume depletion, such as renin/aldosterone, do not show a consistent observation, but suggest volume depletion in some subjects with NS, and normal or excess volume in others [4, 9, 13]. Not all nephrotic states are equal.

3. Overfill theory in nephrotic syndrome

There are two paradigms of edema formation in nephrosis: the so-called underfill and overfill models; it is thought that these can be present in the same individual at different times over the course of their disease. Both result in sodium and water retention and increased interstitial fluid volume presenting as oedema.

The overfill hypothesis postulates that sodium retention is a primary renal phenomenon and would be produced by an intrinsic renal defect in sodium excretion, which in turn would produce expansion of plasma volume (hence the term overfill) [19]. The overfill theory, first formulated by Meltzer et al., in 1979, states that sodium retention is mainly caused by the diseased kidney due to a tubular defect leading to sodium avidity without any signs of volume depletion or a stimulated RAAS [20].

The molecular mechanisms of sodium retention in the NS have derived from the use of the animal model of NS induced by the action of the aminonucleoside puromycin (PAN), which, when administered to rats, produces proteinuria mass and sodium retention [21, 22].

Regulation of ENaC occurs through two main mechanisms: regulation of channel density at the apical membrane and regulation of channel opening probability [23]. ENaC receptor density is regulated by both aldosterone and vasopressin. The open channel probability is regulated by proteolytic processing and by anionic phospholipids present in the inner cell membrane [24, 25]. In the rat PAN nephrosis model, they showed that amiloride increases urine sodium excretion and reduces ascites volume. This effect was attributed both to the ability of amiloride to inhibit ENaC and the ability of amiloride to inhibit urokinase-type plasminogen activator and thus reduce the amount of active plasmin present [1]. Subsequently, plasmin would activate ENaC, and sodium retention would occur with the consequent appearance of edema. In many patients with NS, the pathophysiology of edema would be caused by an intrinsic renal defect in sodium excretion, due to retention in the cortical collector duct (CCD) [26].

In the clinical assessment it should be noted that because Na + retention occurs both in underfill and overfill states, it is not possible to use the FeNa+ to clinically differentiate primary from secondary Na + retention in NS. An increase in RAAS and circulating aldosterone effect can be inferred based on more readily measured values such as an increased transtubular potassium gradient (TTKG) index, uK+/uCr, uNa+, which is observed in hypovolemic patients and not when blood volume is preserved [3]. This also suggests a primary role of aldosterone in the intrinsic activation of Na+/K+ ATPase in CCD. Such patients may benefit from diuretic use. By contrast, diuretic use in patients with NS and secondary Na + retention triggered by hypovolemia or circulatory insufficiency may have serious deleterious consequences.

4. Epithelial sodium channel (ENaC)

The epithelial sodium channel (ENaC) was first cloned in 1993 and its subunits were detected in 1994 by Canessa and Rossier's research group [27, 28]. At the renal level, it is found in the distal portion of the distal convoluted tubule, connecting duct, and collecting duct (aldosterone-sensitive distal nephron-ASDIN). It promotes an electrogenic gradient by the diffusion of sodium (Na+) from the tubular lumen, which facilitates the renal secretion of potassium (K+).

4.1 Biophysical properties and chemical structure

ENaC channels (also called SCNN1 and amiloride-sensitive epithelial sodium channel) are Na^+ and Li^+ permeable channels with very little conductance for K^+ (4 pS) and no conductance for divalent cations; it can have a long opening time, up to 10 seconds, depending on the action of proteases on the channel [29–31]. They are members of the ENaC/degenerin family of cation-selective channels, related to acid-sensitive ion channels (ASICs) [32].

ENaC has 4 subunits (α, β, γ, δ, encoded as SCNN1 A, B, G, and D, respectively) and requires the assembly of 3 subunits (α- β- γ- or δ- β- γ-) to get maximum capacity, although there is partial activity with αENaC, αβENaC or αγENaC [33].

The αβγENaC channel is sensitive to low concentrations of amiloride (IC_{50} 0.1 μM) or benzamil (IC_{50} 0.01 μM), a Li^+/Na^+ permeability ratio of 1.6, and has low conductance at physiological Na^+ concentrations (5 pS); while δβγENaC has higher Na^+ conductance (12 pS), lower Li^+/Na^+ permeability ratio (0.6) and requires higher inhibitory concentrations of amiloride and benzamil (IC_{50} 2.6 μM and 0.27 μM, respectively) [34, 35], in addition, the δ subunit reduces sensitivity to protease-mediated activation [36, 37]. ENaC has a long extracellular region connected to two transmembrane domains (TM1 and TM2) that form the pore and where the channel gate is, as well as a short cytoplasmic portion of the NH2- and COOH- termini.

The topology of each individual ENaC subunit resembles a hand: "central palm" linking TM1 and TM2 by "wrist" and squeezes a "ball" together with domains: "finger", "thumb" and "knuckle" [38]. Among these domains is GRIP (gating relief inhibition by proteolysis) that harbors inhibitory peptides. The GRIP domain has been found in αENaC or γENaC. βENaC also has GRIP domains, but they are not cleaved by proteases [39]. The removal of GRIP causes a conformational change in the molecule that alters the wrist domain, affecting the conductance of the channel (**Figure 1**).

The extracellular portion has regions of high relevance for ENaC function: Na + binding site in the α subunit, with inhibition capacity in response to extracellular concentrations of Na^+ [40]; protease binding sites that favor the removal of inhibitory sites on the α or γ subunit, which activates the channel [38]; glycosylation sites relevant to channel maturation; and cysteine-rich domains (CRDs), involved in the interaction between the "finger" and "thumb" domains within the same α or γ subunit [41].

ENaC expression is increased especially with stimulation of aldosterone, arginine vasopressin, and cortisol. In the ASDIN region, the presence of 11βHSD2 converts cortisol to 11-dehydrocorticosterone, allowing aldosterone to stimulate the mineralocorticoid receptor (MR) [42].

ENaC represents the only member of the ENaC/Degenerin ion channel family that constitutively opens in the absence of an activating stimulus, that is, it has

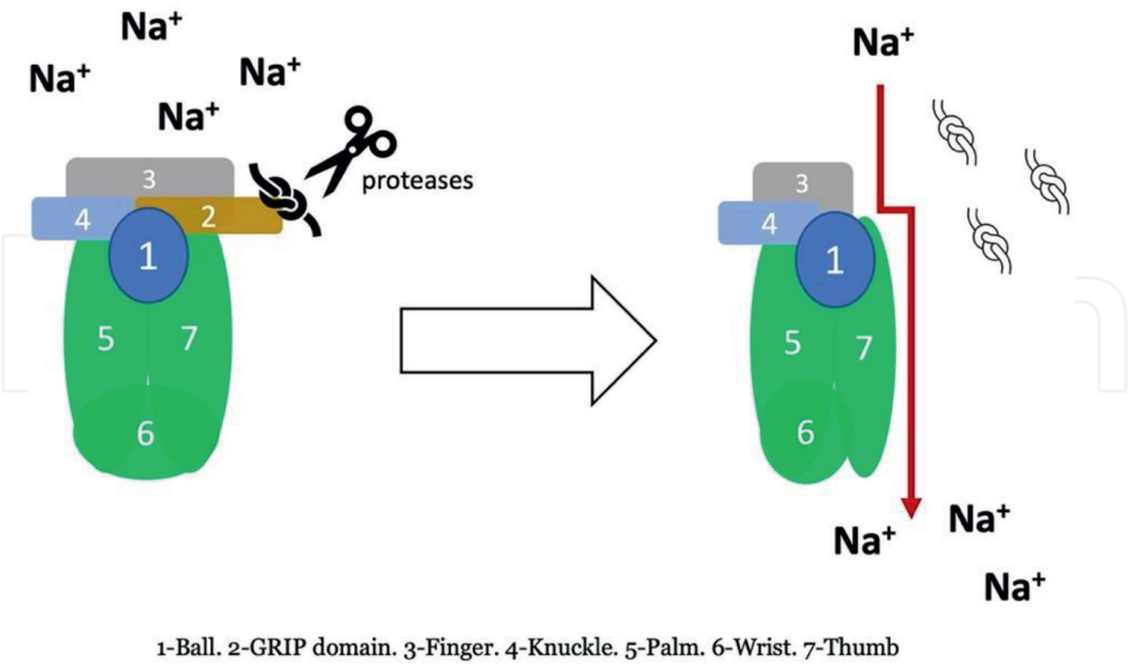

Figure 1.
Function and regulation of the epithelial Na^+ channel (α–/γ-subunit).

a high probability of spontaneous opening (Po) [43]. Therefore, detailed control over the expression of ENaC in the cell membrane, as well as the regulation of Po, is essential. Defects in specific sequences that reduce Po led to a risk of developing Pseudohypoaldosteronism type 1 (PHA1) [44], the opposite effect leading to an increase in Po produces Liddle syndrome [45]. Factors that can modify Po include intra- and extracellular pH, Na + ion concentration, temperature, laminar shear stress, and GRIP domain cleavage by proteases [43].

4.2 Nephrotic syndrome and regulation of ENaC by urinary proteases

The first observations that suggested the regulation of ENaC by proteases were published by Orce et al. [46], who showed that aprotinin (serine protease inhibitor) reduced Na + transport in toad bladder epithelium. It was confirmed by the studies by Bohnert et al., using aprotinin in mice with NS and showing improvement in natriuresis [47]. For their part, Vallet et al. demonstrated that ENaC could be activated by the protease trypsin [31], and subsequently identified prostasin as a serine protease capable of activating ENaC. Furin, another serine protease, can activate the α- and γ-subunits [48]. Cleavage of the α-subunit requires the binding of furin to two specific sites, releasing an inhibitory tract of 26 residues [49]. Furin, on the other hand, binds only once to the γ subunit, releasing a 40-residue inhibitory tract [49]. Other proteases that can activate ENaC include transmembrane protease serine 4 (TMPRSS 4), urokinase, plasmin, pancreatic elastase, cathepsin B, neutrophil elastase, kallikrein, and bacterial proteases [50–54].

In NS there is clearance of many proteins, some of which retain their enzymatic function. Several studies have detected plasminogen and plasmin in urine and their urinary concentrations have been correlated with albuminuria [55–58]. In the long term (25 years of follow-up), the urinary plasminogen/plasmin ratio correlated with the incidence of arterial hypertension and cardiovascular mortality, although

independently of albuminuria [58]. A case of membranous nephropathy associated with a Liddle syndrome phenotype with ENaC hyperactivation without direct evidence of mutation of any subunit was recently reported [45].

Experiments in Xenopus laevis oocytes have shown the activation of γENaC by plasminogen and urokinase-type plasminogen activator (uPA); however, in knockout models (uPA −/−) with NS, sodium retention was not different compared to uPA +/+ models. However, amiloride prevented sodium retention in uPA −/− nephrotic syndrome mice; this suggests that uPA has an important but not essential role for γENaC-mediated sodium retention [59].

In a recent doxorubicin-induced nephrotic syndrome model, two types of genetically modified mice were produced, one group of knock-in mice with inactivating mutations of the prostasin protein and another group with activating mutations of the prostasin protein. Cleavage of αENaC and γENaC was observed in the same proportion, demonstrating that ENaC activation in nephrotic syndrome occurred independently of prostasin activity [60].

In addition, Artunc et al. evaluated Factor VII activating protease (FASP) in a study involving murine models and humans with nephrotic syndrome. In humans, high levels of FSAP in both active and zymogen forms were detected in urine. Mutation of the prostasin activation site at γENaC in mice prevented stimulation of the channel by FSAP. However, the absence of FSAP did not prevent cleavage of the α- and γ-ENaC subunits [61].

These data suggest that cleavage of ENaC subunits probably requires the action of multiple proteases rather than the action of a single protease [62]. Likewise, the incomplete activation of ENaC by proteases in the zymogen state can produce intolerance to treatment with distal blockers such as Triamterene, and potentially cause a state of kidney injury due to severe salt loss, due to the absolute blockade of the channel [63].

5. Edema

Edema is one of the defining characteristics of nephrotic syndrome, as well as being the symptom that most frequently requires medical intervention. This can be severe, representing an increase in body weight of up to 30%. Likewise, it is associated with complications such as movement restriction, increased skin tension with consequent skin denudation, increased risk of soft tissue infection and pulmonary edema [2].

There is currently no consensus regarding the treatment of edema in nephrotic syndrome. In general, we can divide the treatment of edema into non-pharmacological and pharmacological management.

5.1 Conservative management

An essential part of treatment is to determine intravascular volume status since the initiation of high doses of diuretics and fluid restriction in a patient with effective volume depletion can precipitate renal function deterioration. Some authors recommend an initial goal of weight loss of 0.5–1 kg/day, to prevent complications such as acute kidney injury, hydroelectrolytic imbalance and thromboembolism secondary to hemoconcentration [11].

Another key aspect of treatment is achieving a negative sodium balance. Strict sodium restriction of less than 100 mEq/day or 2.3 g/day has been shown to have an additive effect to pharmacological therapies. While fluid restriction can be reserved for those patients with hyponatremia, it is generally suggested to limit fluid intake accord to fluid overload [3, 11].

Regarding hygienic-dietary recommendations, in adults it is recommended to restrict protein intake to 0.8–1 g/kg/day and maintain blood pressure goals <125/75 mmHg if the patient has proteinuria >1 g/day and < 130/80 mmHg with proteinuria >1 g/day [3].

About 20% of patients have infectious complications. There are no current recommendations for prophylactic antibiotics, although immunization against *Streptococcus pneumoniae* is recommended, according to age and previous immunizations [11].

5.2 Diuretics

Loop diuretics are usually the first line of treatment. They act by inhibiting the NKCC2 cotransporter on the apical surface of the thick ascending limb of the loop of Henle, which is responsible for approximately 25% of total sodium reabsorption. They bind strongly to proteins and are secreted in the proximal tubule [2].

Furosemide has variable intra- and inter-individual bioavailability; this varies between 20 and 60% orally and can be reduced to 30% in edematous states. The presence of intestinal edema can limit its absorption and hypoalbuminemia decrease delivery to its site of action, requiring higher doses, with a greater probability of presenting adverse effects or its parenteral administration.

On the other hand, its chronic use causes hypertrophy and hyperplasia of the epithelial cells of the distal tubule, with increased expression of the NCC cotransporter, limiting the natriuretic effect. This phenomenon is usually counteracted by adding diuretics from different classes. For example, the combination with thiazides increases diuresis, however, it requires close monitoring to avoid severe hypokalemia and metabolic alkalosis.

A randomized clinical trial in patients with nephrotic syndrome and refractory edema comparing the use of acetazolamide + hydrochlorothiazide vs. furosemide + hydrochlorothiazide, both followed by the administration of furosemide; demonstrated greater weight loss and increased diuresis with the combination of acetazolamide + hydrochlorothiazide [64].

Although the administration of amiloride or mineralocorticoid receptor antagonists (MRA) reduce the risk of loop diuretic-induced hypokalemia, they have a minimal diuretic effect on their own; except for spironolactone at high doses (400 mg daily), which was associated with weight loss and increased urinary excretion of sodium in patients with nephrotic syndrome, compared to healthy controls [65].

5.3 Albumin

The combined use of 25% albumin with loop diuretics is controversial. Although it is proposed that its administration improves the efficacy of these drugs by increasing their arrival at their site of action, their therapeutic effect is short, and their use is expensive.

The first reports of the concomitant use of albumin infusion with furosemide were promising, however, subsequent reports failed to demonstrate a significant increase in natriuresis or in a decrease in edema [15].

A recent meta-analysis, which included 13 studies with 422 patients, showed an increase of 31.45 ml/hour in urine output (95% CI, 19.30–43.59) and an increase in sodium excretion of 1.76 mEq/hour (95% CI, 0.83–2.69) with the coadministration of albumin and Furosemide, compared with the use of Furosemide alone; although with a high heterogeneity between the studies (I^2 = 87%, p < 0.01) and (I^2 = 92%, p < 0.01), respectively. It was also observed that the diuretic and natriuretic effect were more significant in those patients with serum albumin <2.5 g/dL, serum creatinine >1.2 mg/dL, estimated glomerular filtration rates (eGFR) < 60 ml/min/1.73 m2 and in those who received doses of albumin greater than 30 gr. In addition, these increases occurred mainly in the first 12 hours after the administration of the albumin infusion [66].

Therefore, we can conclude that, although this combination could increase diuresis and natriuresis in selected patients, the response to treatment is variable. In addition to this, care must be taken with the use of albumin, since there is a risk of increasing overload, precipitating hypertensive uncontrol and the development of acute pulmonary edema, particularly in oliguric patients [11].

5.4 Vasopressin receptor antagonists (AVPr)

These drugs, also called aquaretics, act by reducing the luminal expression of aquaporins, increasing the urinary excretion of sodium-free water. There are case reports that describe the successful reduction of refractory edema in nephrotic syndrome with the use of Tolvaptan, without being associated with significant adverse effects. However, randomized controlled trials are required to standardize its dosage, duration of treatment and evaluate its safety profile in nephrotic syndrome [67, 68].

6. Amiloride

Amiloride is a pyrazine-carbonyl-guanidine derivative [69]. It consists of a pyrazine ring substituted with an acylguanidine group attached to position 2 of the ring, where amino groups are attached to positions 3 and 5 of the ring, and a Cl group attached to position 6 (**Figure 2**). Amiloride is a weak base with a pKa of 8.7 in aqueous solution. The protonation of amiloride occurs at the guanidine group. Due to these acid-base properties, amiloride can effectively penetrate biological and artificial membranes [70]. Amiloride is rarely soluble in water.

6.1 Pharmacodynamics

Amiloride reaches the nephron largely by glomerular filtration and acts on the luminal membrane of the distal convoluted tubule and collecting ducts [69]. In the distal tubules and cortical collecting ducts, there are principal cells that have epithelial sodium channels (ENaC) in their luminal membranes. ENaC is a heterotrimer consisting of three subunits: alpha, beta, and gamma, such that maximum sodium permeability is induced when all three subunits are co-expressed in the same cell. ENaC are a highly regulated site for sodium reabsorption, entering the cell by passive diffusion down the electrochemical gradient created by the basolateral Na^+/K^+ pump [71]. High sodium permeability depolarizes the luminal membrane but not the basolateral membrane. The transepithelial potential difference provides a driving force for potassium secretion into the lumen through apical potassium channels.

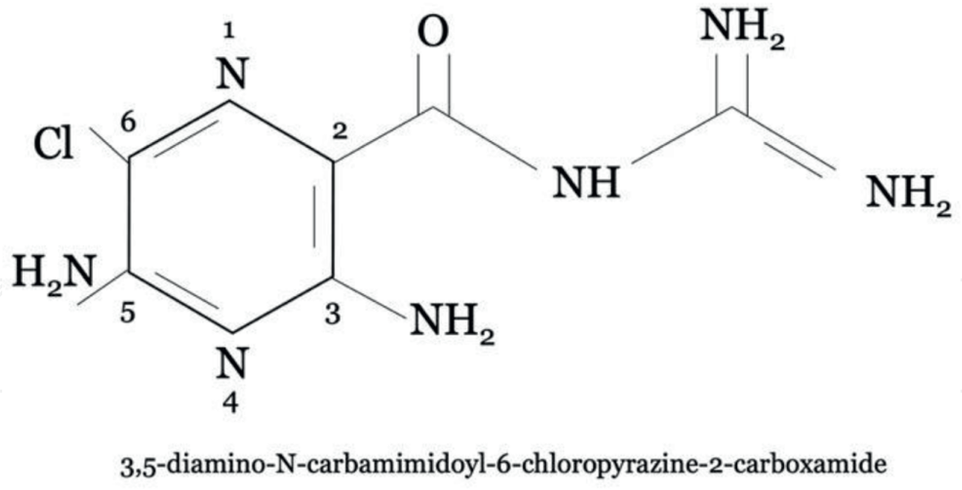

Figure 2.
Amiloride chemical structure.

Amiloride reversibly blocks principal cell luminal ENaC from binding to the channel pore, increasing Na + and Cl- excretion rates. Inhibition of ENaC hyperpolarizes the luminal membrane, reducing the negative transepithelial lumen voltage. Normally, the negative potential difference of the lumen facilitates cation secretion, their attenuation then decreases the excretion rates of K^+, H^+, Ca^{2+}, and Mg^{2+} [69].

6.2 Pharmacokinetics

One study suggested that a single oral dose of 20 mg amiloride had a biological half-life of 9.6 ± 1.8 hours in humans. Its effects began approximately 2 hours after drug administration, and peak activity was reached within 4 to 8 hours [72].

Amiloride is absorbed from the gastrointestinal tract and has an oral bioavailability of about 50%. Co-administration with food may decrease absorption by up to 30%, but the absorption rate does not change. After administration, the onset of diuretic activity usually occurs within 2 hours. Maximum diuresis occurs within 6–10 hours. Diuretic effects persist for approximately 24 hours after administration. Amiloride is not metabolized in the liver. About 50% is excreted unchanged by the kidneys in the urine, about 40% is excreted in the feces. In patients with normal renal function, amiloride has a serum half-life of approximately 6–9 hours [73].

6.3 Clinical evidence in nephrotic syndrome

Nephrotic syndrome is associated with aberrant glomerular filtration of plasminogen and conversion to plasmin in the urine proteolytically activates ENaC and thus contributes to sodium retention and edema. Amiloride inhibits urokinase-type plasminogen activator (uPA) activity in urine, which attenuates plasminogen activation and protease activity in urine in vivo. Urinary uPA is a relevant target for amiloride in vivo [74].

Several lines of evidence support proteolytic activation of ENaC in nephrotic syndrome. In the experimental model of nephrotic syndrome (nephrotic syndrome induced by puromycin aminonucleoside (PAN) and adriamycin), amiloride blocks sodium retention. Three simultaneous patient case reports showed the therapeutic

effect of amiloride and triamterene [45, 75, 76] in patients with different morbidities but severe and resistant nephrotic edema.

Urinary plasmin has been shown to be the dominant serine protease in nephrotic urine that activates ENaC [26, 77]. Plasmin is generated from the proteolytic activation of plasminogen by urokinase-type plasminogen activator. Urinary plasmin excretion in serial samples from nephrotic patients was identified as an independent factor for edema remission [78]. In pediatric patients, volume-expanded acute nephrotic syndrome was associated with significantly higher urine plasmin compared with the remission phase [55]. In the urinary system, uPA is the dominant plasminogen activator [79]. Urinary urokinase is inhibited by amiloride in nephrotic rats [74], mice [59, 80], and diabetic patients [81] by preventing activation of plasmin and ENaC in proteinuria. Since amiloride is a uPA and ENaC blocker, these findings cannot be used to conclude that urinary plasmin is the causal link between proteinuria and ENaC activation.

Two studies have directly addressed the hypothesis that uPA-activated plasmin is involved in sodium retention during nephrotic syndrome [59], one of them using uPA-deficient mice rendered nephrotic using doxorubicin injection. With this approach, excretion of urinary plasmin, but not plasminogen, was blocked in uPA knockout mice with nephrotic syndrome. On the other hand, another study found that inhibition of uPA activity during nephrotic syndrome reduced sodium accumulation [80]. Nephrotic syndrome was induced using inducible inactivation of the podocyte slit diaphragm protein podocin, resulting in massive proteinuria and sodium retention. Amiloride blocked sodium retention and urinary plasmin excretion.

Although plasmin appears to be the dominant active serine protease in nephrotic urine [26], other urinary proteases such as cathepsin B, identified by mass spectrometry, are also present [82]. Thus, at present, several serine proteases might redundantly contribute to ENaC activation and sodium retention during nephrotic syndrome, and direct inhibition of specific proteases only partially attenuates proteinuria-induced sodium retention.

7. Conclusion

The pathophysiology of edema in nephrotic syndrome is probably related to several factors, essentially the alteration of the forces that govern Starling's principle and sodium retention due to hyperactivation of ENaC in the aldosterone-sensitive distal nephron. Therapeutic strategies that include knowledge of the basic mechanisms that generate edema may be useful to the clinician to make better decisions during decongestion treatment. The use of amiloride has a very intuitive approach on one of the pathways that promote sodium retention. Its efficacy has been proven in some case reports, but it is necessary to carry out controlled studies that can thoroughly evaluate its theoretical efficacy.

Conflict of interest

"The authors declare no conflict of interest."

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References

[1] Siddall EC, Radhakrishnan J. The pathophysiology of edema formation in the nephrotic syndrome. Kidney International. 2012;**82**(6):635-642. DOI: 10.1038/ki.2012.180

[2] Gupta S, Pepper RJ, Ashman N, Walsh SB. Nephrotic syndrome: Oedema formation and its treatment with diuretics. Frontiers in Physiology. 2018;**9**:1868. DOI: 10.3389/fphys.2018.01868

[3] Cadnapaphornchai MA, Tkachenko O, Shchekochikhin D, Schrier RW. The nephrotic syndrome: Pathogenesis and treatment of edema formation and secondary complications. Pediatric Nephrology. 2014;**29**(7):1159-1167. DOI: 10.1007/s00467-013-2567-8

[4] Bockenhauer D. Over- or underfill: Not all nephrotic states are created equal. Pediatric Nephrology. 2013;**28**(8):1153-1156. DOI: 10.1007/s00467-013-2435-6

[5] Rostoker G, Behar A, Lagrue G. Vascular hyperpermeability in nephrotic edema. Nephron. 2000;**85**(3):194-200. DOI: 10.1159/000045661

[6] Vande Walle JG, Donckerwolcke RA, Koomans HA. Pathophysiology of edema formation in children with nephrotic syndrome not due to minimal change disease. Journal of the American Society of Nephrology. 1999;**10**(2):323-331. DOI: 10.1681/ASN.V102323

[7] Usberti M, Federico S, Meccariello S, Cianciaruso B, Balletta M, Pecoraro C, et al. Role of plasma vasopressin in the impairment of water excretion in nephrotic syndrome. Kidney International. 1984;**25**(2):422-429. DOI: 10.1038/ki.1984.34

[8] Bockenhauer D, Aitkenhead H. The kidney speaks: Interpreting urinary sodium and osmolality. Archives of Disease in Childhood. Education and Practice Edition. 2011;**96**(6):223-227. DOI: 10.1136/archdischild-2011-300115

[9] Usberti M, Gazzotti RM, Poiesi C, D'Avanzo L, Ghielmi S. Considerations on the sodium retention in nephrotic syndrome. American Journal of Nephrology. 1995;**15**(1):38-47. DOI: 10.1159/000168800

[10] Meena J, Bagga A. Current perspectives in management of edema in nephrotic syndrome. Indian Journal of Pediatrics. 2020;**87**(8):633-640. DOI: 10.1007/s12098-020-03252-9

[11] Hull RP, Goldsmith DJ. Nephrotic syndrome in adults. BMJ. 2008;**336**(7654):1185-1189. DOI: 10.1136/bmj.39576.709711.80

[12] Oliver WJ. Physiologic responses associated with steroid-induced diuresis in the nephrotic syndrome. The Journal of Laboratory and Clinical Medicine. 1963;**62**:449-464. DOI: 10.5555/uri:pii:0022214363900441

[13] Brown EA, Markandu N, Sagnella GA, Jones BE, MacGregor GA. Sodium retention in nephrotic syndrome is due to an intrarenal defect: Evidence from steroid-induced remission. Nephron. 1985;**39**(4):290-295. DOI: 10.1159/000183392

[14] Reid CJ, Marsh MJ, Murdoch IM, Clark G. Nephrotic syndrome in childhood complicated by life threatening pulmonary oedema. BMJ. 1996;**312**(7022):36-38. DOI: 10.1136/bmj.312.7022.36

[15] Ho JJ, Adnan AS, Kueh YC, Ambak NJ, Van Rostenberghe H, Jummaat F. Human albumin infusion for treating oedema in people with nephrotic syndrome. Cochrane Database of Systematic Reviews. 2019;**7**:CD009692. DOI: 10.1002/14651858.CD009692.pub2

[16] Russi E, Weigand K. Analbuminemia. Klinische Wochenschrift. 1983;**61**(11):541-545. DOI: 10.1007/BF01486843

[17] Dammacco F, Miglietta A, D'Addabbo A, Fratello A, Moschetta R, Bonomo L. Analbuminemia: Report of a case and review of the literature. Vox Sanguinis. 1980;**39**(3):153-161. DOI: 10.1111/j.1423-0410.1980.tb01851.x

[18] Brown EA, Markandu ND, Sagnella GA, Jones BE, MacGregor GA. Lack of effect of captopril on the sodium retention of the nephrotic syndrome. Nephron. 1984;**37**(1):43-48. DOI: 10.1159/000183206

[19] Rondon-Berrios H. New insights into the pathophysiology of oedema in nephrotic syndrome. Nefrología. 2011;**31**(2):148-154. DOI: 10.3265/Nefrologia.pre2010.Nov.10724

[20] Meltzer JI, Keim HJ, Laragh JH, Sealey JE, Jan KM, Chien S. Nephrotic syndrome: Vasoconstriction and hypervolemic types indicated by renin-sodium profiling. Annals of Internal Medicine. 1979;**91**(5):688-696. DOI: 10.7326/0003-4819-91-5-688

[21] Caulfield JP, Reid JJ, Farquhar MG. Alterations of the glomerular epithelium in acute aminonucleoside nephrosis. Evidence for formation of occluding junctions and epithelial cell detachment. Laboratory Investigation 1976;34(1):43-59. PMID: 1246124

[22] Ryan GB, Karnovsky MJ. An ultrastructural study of the mechanisms of proteinuria in aminonucleoside nephrosis. Kidney International. 1975;**8**(4):219-232. DOI: 10.1038/ki.1975.105

[23] Snyder PM. Minireview: Regulation of epithelial Na+ channel trafficking. Endocrinology. 2005;**146**(12):5079-5085. DOI: 10.1210/en.2005-0894

[24] Hamm LL, Feng Z, Hering-Smith KS. Regulation of sodium transport by ENaC in the kidney. Current Opinion in Nephrology and Hypertension. 2010;**19**(1):98-105. DOI: 10.1097/MNH.0b013e328332bda4

[25] Ma HP, Eaton DC. Acute regulation of epithelial sodium channel by anionic phospholipids. Journal of the American Society of Nephrology. 2005;**16**(11):3182-3187. DOI: 10.1681/ASN.2005040434

[26] Svenningsen P, Bistrup C, Friis UG, Bertog M, Haerteis S, Krueger B, et al. Plasmin in nephrotic urine activates the epithelial sodium channel. Journal of the American Society of Nephrology. 2009;**20**(2):299-310. DOI: 10.1681/ASN.2008040364

[27] Canessa CM, Horisberger JD, Rossier BC. Epithelial sodium channel related to proteins involved in neurodegeneration. Nature. 1993;**361**(6411):467-470. DOI: 10.1038/361467a0

[28] Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, et al. Amiloride-sensitive epithelial Na+ channel is made of three homologous subunits. Nature. 1994;**367**(6462):463-467. DOI: 10.1038/367463a0

[29] Palmer LG, Frindt G. Gating of Na channels in the rat cortical collecting tubule: Effects of voltage and membrane

stretch. The Journal of General Physiology. 1996;**107**(1):35-45. DOI: 10.1085/jgp.107.1.35

[30] Kemendy AE, Kleyman TR, Eaton DC. Aldosterone alters the open probability of amiloride-blockable sodium channels in A6 epithelia. The American Journal of Physiology. 1992;**263**(4 Pt 1):C825-C837. DOI: 10.1152/ajpcell.1992.263.4.C825

[31] Vallet V, Chraibi A, Gaeggeler HP, Horisberger JD, Rossier BC. An epithelial serine protease activates the amiloride-sensitive sodium channel. Nature. 1997;**389**(6651):607-610. DOI: 10.1038/39329

[32] Noreng S, Posert R, Bharadwaj A, Houser A, Baconguis I. Molecular principles of assembly, activation, and inhibition in epithelial sodium channel. eLife. 2020;**9**. DOI: 10.7554/eLife.59038

[33] Bonny O, Chraibi A, Loffing J, Jaeger NF, Grunder S, Horisberger JD, et al. Functional expression of a pseudohypoaldosteronism type I mutated epithelial Na+ channel lacking the pore-forming region of its alpha subunit. The Journal of Clinical Investigation. 1999;**104**(7):967-974. DOI: 10.1172/JCI6821

[34] Ayasse N, Berg P, Andersen JF, Svendsen SL, Sorensen MV, Fedosova NU, et al. Benzamil-mediated urine alkalization is caused by the inhibition of H(+)-K(+)-ATPases. American Journal of Physiology. Renal Physiology. 2021;**320**(4):F596-F607. DOI: 10.1152/ajprenal.00444.2020

[35] Vallee C, Howlin B, Lewis R. Ion selectivity in the ENaC/DEG family: A systematic review with supporting analysis. International Journal of Molecular Sciences. 2021;**22**(20). DOI: 10.3390/ijms222010998

[36] Haerteis S, Krueger B, Korbmacher C, Rauh R. The delta-subunit of the epithelial sodium channel (ENaC) enhances channel activity and alters proteolytic ENaC activation. The Journal of Biological Chemistry. 2009;**284**(42):29024-29040. DOI: 10.1074/jbc.M109.018945

[37] Wichmann L, Vowinkel KS, Perniss A, Manzini I, Althaus M. Incorporation of the delta-subunit into the epithelial sodium channel (ENaC) generates protease-resistant ENaCs in Xenopus laevis. The Journal of Biological Chemistry. 2018;**293**(18):6647-6658. DOI: 10.1074/jbc.RA118.002543

[38] Kleyman TR, Kashlan OB, Hughey RP. Epithelial Na(+) channel regulation by extracellular and intracellular factors. Annual Review of Physiology. 2018;**80**:263-281. DOI: 10.1146/annurev-physiol-021317-121143

[39] Kleyman TR, Carattino MD, Hughey RP. ENaC at the cutting edge: Regulation of epithelial sodium channels by proteases. The Journal of Biological Chemistry. 2009;**284**(31):20447-20451. DOI: 10.1074/jbc.R800083200

[40] Kashlan OB, Blobner BM, Zuzek Z, Tolino M, Kleyman TR. Na+ inhibits the epithelial Na+ channel by binding to a site in an extracellular acidic cleft. The Journal of Biological Chemistry. 2015;**290**(1):568-576. DOI: 10.1074/jbc.M114.606152

[41] Blobner BM, Wang XP, Kashlan OB. Conserved cysteines in the finger domain of the epithelial Na(+) channel alpha and gamma subunits are proximal to the dynamic finger-thumb domain interface. The Journal of Biological Chemistry. 2018;**293**(13):4928-4939. DOI: 10.1074/jbc.M117.819367

[42] Nesterov V, Bertog M, Canonica J, Hummler E, Coleman R, Welling PA, et al. Critical role of the mineralocorticoid receptor in aldosterone-dependent and aldosterone-independent regulation of ENaC in the distal nephron. American Journal of Physiology. Renal Physiology. 2021;**321**(3):F257-FF68. DOI: 10.1152/ajprenal.00139.2021

[43] Wichmann L, Althaus M. Evolution of epithelial sodium channels: Current concepts and hypotheses. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2020;**319**(4):R387-R400. DOI: 10.1152/ajpregu.00144.2020

[44] Hanukoglu I, Hanukoglu A. Epithelial sodium channel (ENaC) family: Phylogeny, structure-function, tissue distribution, and associated inherited diseases. Gene. 2016;**579**(2):95-132. DOI: 10.1016/j.gene.2015.12.061

[45] Yamaguchi E, Yoshikawa K, Nakaya I, Kato K, Miyasato Y, Nakagawa T, et al. Liddle's-like syndrome associated with nephrotic syndrome secondary to membranous nephropathy: The first case report. BMC Nephrology. 2018;**19**(1):122. DOI: 10.1186/s12882-018-0916-3

[46] Orce GG, Castillo GA, Margolius HS. Inhibition of short-circuit current in toad urinary bladder by inhibitors of glandular kallikrein. The American Journal of Physiology. 1980;**239**(5):F459-F465. DOI: 10.1152/ajprenal.1980.239.5.F459

[47] Bohnert BN, Menacher M, Janessa A, Worn M, Schork A, Daiminger S, et al. Aprotinin prevents proteolytic epithelial sodium channel (ENaC) activation and volume retention in nephrotic syndrome. Kidney International. 2018;**93**(1):159-172. DOI: 10.1016/j.kint.2017.07.023

[48] Hughey RP, Bruns JB, Kinlough CL, Harkleroad KL, Tong Q, Carattino MD, et al. Epithelial sodium channels are activated by furin-dependent proteolysis. The Journal of Biological Chemistry. 2004;**279**(18):18111-18114. DOI: 10.1074/jbc.C400080200

[49] Kleyman TR, Eaton DC. Regulating ENaC's gate. American Journal of Physiology. Cell Physiology. 2020;**318**(1):C150-CC62. DOI: 10.1152/ajpcell.00418.2019

[50] Caldwell RA, Boucher RC, Stutts MJ. Neutrophil elastase activates near-silent epithelial Na+ channels and increases airway epithelial Na+ transport. American Journal of Physiology. Lung Cellular and Molecular Physiology. 2005;**288**(5):L813-L819. DOI: 10.1152/ajplung.00435.2004

[51] Alli AA, Song JZ, Al-Khalili O, Bao HF, Ma HP, Alli AA, et al. Cathepsin B is secreted apically from Xenopus 2F3 cells and cleaves the epithelial sodium channel (ENaC) to increase its activity. The Journal of Biological Chemistry. 2012;**287**(36):30073-30083. DOI: 10.1074/jbc.M111.338574

[52] Buhl KB, Friis UG, Svenningsen P, Gulaveerasingam A, Ovesen P, Frederiksen-Moller B, et al. Urinary plasmin activates collecting duct ENaC current in preeclampsia. Hypertension. 2012;**60**(5):1346-1351. DOI: 10.1161/HYPERTENSIONAHA.112.198879

[53] Butterworth MB, Zhang L, Liu X, Shanks RM, Thibodeau PH. Modulation of the epithelial sodium channel (ENaC) by bacterial metalloproteases and protease inhibitors. PLoS One. 2014;**9**(6):e100313

[54] Passero CJ, Mueller GM, Myerburg MM, Carattino MD, Hughey RP, Kleyman TR. TMPRSS4-dependent activation of the epithelial sodium channel requires cleavage of the

gamma-subunit distal to the furin cleavage site. American Journal of Physiology. Renal Physiology. 2012;**302**(1):F1-F8. DOI: 10.1152/ajprenal.00330.2011

[55] Andersen RF, Buhl KB, Jensen BL, Svenningsen P, Friis UG, Jespersen B, et al. Remission of nephrotic syndrome diminishes urinary plasmin content and abolishes activation of ENaC. Pediatric Nephrology. 2013;**28**(8):1227-1234. DOI: 10.1007/s00467-013-2439-2

[56] Hinrichs GR, Michelsen JS, Zachar R, Friis UG, Svenningsen P, Birn H, et al. Albuminuria in kidney transplant recipients is associated with increased urinary serine proteases and activation of the epithelial sodium channel. American Journal of Physiology. Renal Physiology. 2018;**315**(1):F151-FF60. DOI: 10.1152/ajprenal.00545.2017

[57] Buhl KB, Oxlund CS, Friis UG, Svenningsen P, Bistrup C, Jacobsen IA, et al. Plasmin in urine from patients with type 2 diabetes and treatment-resistant hypertension activates ENaC in vitro. Journal of Hypertension. 2014;**32**(8):1672-1677. DOI: 10.1097/HJH.0000000000000216

[58] Ray EC, Miller RG, Demko JE, Costacou T, Kinlough CL, Demko CL, et al. Urinary Plasmin(ogen) as a prognostic factor for hypertension. Kidney International Reports. 2018;**3**(6):1434-1442. DOI: 10.1016/j.ekir.2018.06.007

[59] Bohnert BN, Daiminger S, Worn M, Sure F, Staudner T, Ilyaskin AV, et al. Urokinase-type plasminogen activator (uPA) is not essential for epithelial sodium channel (ENaC)-mediated sodium retention in experimental nephrotic syndrome. Acta Physiologica (Oxford, England). 2019;**227**(4):e13286. DOI: 10.1111/apha.13286

[60] Essigke D, Bohnert BN, Janessa A, Worn M, Omage K, Kalbacher H, et al. Sodium retention in nephrotic syndrome is independent of the activation of the membrane-anchored serine protease prostasin (CAP1/PRSS8) and its enzymatic activity. Pflügers Archiv: European Journal of Physiology. 2022;**474**(6):613-624. DOI: 10.1007/s00424-022-02682-y

[61] Artunc F, Bohnert BN, Schneider JC, Staudner T, Sure F, Ilyaskin AV, et al. Proteolytic activation of the epithelial sodium channel (ENaC) by factor VII activating protease (FSAP) and its relevance for sodium retention in nephrotic mice. Pflügers Archiv: European Journal of Physiology. 2022;**474**(2):217-229. DOI: 10.1007/s00424-021-02639-7

[62] Carattino MD, Mueller GM, Palmer LG, Frindt G, Rued AC, Hughey RP, et al. Prostasin interacts with the epithelial Na+ channel and facilitates cleavage of the gamma-subunit by a second protease. American Journal of Physiology. Renal Physiology. 2014;**307**(9):F1080-F1087. DOI: 10.1152/ajprenal.00157.2014

[63] Essigke D, Ilyaskin AV, Worn M, Bohnert BN, Xiao M, Daniel C, et al. Zymogen-locked mutant prostasin (Prss8) leads to incomplete proteolytic activation of the epithelial sodium channel (ENaC) and severely compromises triamterene tolerance in mice. Acta Physiologica (Oxford, England). 2021;**232**(1):e13640. DOI: 10.1111/apha.13640

[64] Fallahzadeh MA, Dormanesh B, Fallahzadeh MK, Roozbeh J, Fallahzadeh MH, Sagheb MM. Acetazolamide and hydrochlorothiazide followed by furosemide versus furosemide and hydrochlorothiazide followed by furosemide for the treatment

of adults with nephrotic edema: A randomized trial. American Journal of Kidney Diseases. 2017;**69**(3):420-427. DOI: 10.1053/j.ajkd.2016.10.022

[65] Shapiro MD, Hasbargen J, Hensen J, Schrier RW. Role of aldosterone in the sodium retention of patients with nephrotic syndrome. American Journal of Nephrology. 1990;**10**(1):44-48. DOI: 10.1159/000168052

[66] Lee TH, Kuo G, Chang CH, Huang YT, Yen CL, Lee CC, et al. Diuretic effect of co-administration of furosemide and albumin in comparison to furosemide therapy alone: An updated systematic review and meta-analysis. PLoS One. 2021;**16**(12):e0260312. DOI: 10.1371/journal.pone.0260312

[67] Park ES, Huh YS, Kim GH. Is tolvaptan indicated for refractory oedema in nephrotic syndrome? Nephrology (Carlton, Vic.). 2015;**20**(2):103-106. DOI: 10.1111/nep.12348

[68] Tanaka A, Nakamura T, Sato E, Ueda Y, Node K. Different effects of tolvaptan in patients with idiopathic membranous nephropathy with nephrotic syndrome. Internal Medicine. 2017;**56**(2):191-196. DOI: 10.2169/internalmedicine.56.7539

[69] Vidt DG. Mechanism of action, pharmacokinetics, adverse effects, and therapeutic uses of amiloride hydrochloride, a new potassium-sparing diuretic. Pharmacotherapy. 1981;**1**(3):179-187. DOI: 10.1002/j.1875-9114.1981.tb02539.x

[70] Garty H, Benos DJ. Characteristics and regulatory mechanisms of the amiloride-blockable Na+ channel. Physiological Reviews. 1988;**68**(2):309-373. DOI: 10.1152/physrev.1988.68.2.309

[71] Kleyman TR, Cragoe EJ Jr. Amiloride and its analogs as tools in the study of ion transport. The Journal of Membrane Biology. 1988;**105**(1):1-21. DOI: 10.1007/BF01871102

[72] Benos DJ. Amiloride: A molecular probe of sodium transport in tissues and cells. The American Journal of Physiology. 1982;**242**(3):C131-C145. DOI: 10.1152/ajpcell.1982.242.3.C131

[73] Sun Q, Sever P. Amiloride: A review. Journal of the Renin-Angiotensin-Aldosterone System. 2020;**21**(4):1470320320975893. DOI: 10.1177/1470320320975893

[74] Staehr M, Buhl KB, Andersen RF, Svenningsen P, Nielsen F, Hinrichs GR, et al. Aberrant glomerular filtration of urokinase-type plasminogen activator in nephrotic syndrome leads to amiloride-sensitive plasminogen activation in urine. American Journal of Physiology. Renal Physiology. 2015;**309**(3):F235-F241. DOI: 10.1152/ajprenal.00138.2015

[75] Hinrichs GR, Mortensen LA, Jensen BL, Bistrup C. Amiloride resolves resistant edema and hypertension in a patient with nephrotic syndrome; a case report. Physiological Reports. 2018;**6**(12):e13743. DOI: 10.14814/phy2.13743

[76] Hoorn EJ, Ellison DH. Diuretic resistance. American Journal of Kidney Diseases. 2017;**69**(1):136-142. DOI: 10.1053/j.ajkd.2016.08.027

[77] Passero CJ, Mueller GM, Rondon-Berrios H, Tofovic SP, Hughey RP, Kleyman TR. Plasmin activates epithelial Na+ channels by cleaving the gamma subunit. The Journal of Biological Chemistry. 2008;**283**(52):36586-36591. DOI: 10.1074/jbc.M805676200

[78] Chen JL, Wang L, Yao XM, Zang YJ, Wang Y, Li ZJ, et al. Association of

urinary plasminogen-plasmin with edema and epithelial sodium channel activation in patients with nephrotic syndrome. American Journal of Nephrology. 2019;**50**(2):92-104. DOI: 10.1159/000501059

[79] Svenningsen P, Hinrichs GR, Zachar R, Ydegaard R, Jensen BL. Physiology and pathophysiology of the plasminogen system in the kidney. Pflügers Archiv: European Journal of Physiology. 2017;**469**(11):1415-1423. DOI: 10.1007/s00424-017-2014-y

[80] Hinrichs GR, Weyer K, Friis UG, Svenningsen P, Lund IK, Nielsen R, et al. Urokinase-type plasminogen activator contributes to amiloride-sensitive sodium retention in nephrotic range glomerular proteinuria in mice. Acta Physiologica (Oxford, England). 2019;**227**(4):e13362. DOI: 10.1111/apha.13362

[81] Oxlund CS, Buhl KB, Jacobsen IA, Hansen MR, Gram J, Henriksen JE, et al. Amiloride lowers blood pressure and attenuates urine plasminogen activation in patients with treatment-resistant hypertension. Journal of the American Society of Hypertension. 2014;**8**(12):872-881. DOI: 10.1016/j.jash.2014.09.019

[82] Larionov A, Dahlke E, Kunke M, Zanon Rodriguez L, Schiessl IM, Magnin JL, et al. Cathepsin B increases ENaC activity leading to hypertension early in nephrotic syndrome. Journal of Cellular and Molecular Medicine. 2019;**23**(10):6543-6553. DOI: 10.1111/jcmm.14387