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New insights into the pathophysiology of oedema in nephrotic syndrome

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ABSTRACT

Oedema is a common clinical manifestation of nephrotic syndrome. However, the pathophysiological mechanism of sodium retention in nephrotic syndrome has been intensely debated for decades. Several clinical and experimental observations argue against the classic or "underfill" hypothesis of oedema formation in nephrotic syndrome. In many patients, oedema formation in nephrotic syndrome is due to the kidney being intrinsically unable to excrete salt and is unrelated to systemic factors (i.e. hypoalbuminaemia, decreased "effective" arterial blood volume, and secondary hyperaldosteronism). The cortical collecting duct is the nephron site of sodium retention in nephrotic syndrome. Activation of the epithelial sodium channel in the cortical collecting duct is responsible for sodium retention in nephrotic syndrome. In nephrotic syndrome, a defective glomerular filtration barrier allows the passage of proteolytic enzymes or their precursors, which have the ability to activate the epithelial sodium channel, thereby causing the the subsequent sodium retention and oedema.

Keywords: Oedema. Nephrotic syndrome. Hypoalbuminemia. Epithelial sodium channel. Plasmin

Avances en la fisiopatología del edema en el síndrome nefrótico

RESUMEN

El edema es una manifestación clínica frecuente del síndrome nefrótico (SN); sin embargo, el mecanismo fisiopatológico responsable de la retención de sodio ha sido un tema de intenso debate durante décadas. Muchas observaciones clínicas y experimentales no apoyan a la hipótesis clásica o del underfill en la formación del edema nefrótico. En numerosos pacientes, el edema propio del SN se produce por un defecto renal intrínseco en la excreción de sodio y es independiente de factores sistémicos (p. ej., hipoalbuminemia, disminución del volumen arterial efectivo o hiperaldosteronismo secundario). El punto de la nefrona donde se produce la retención de sodio en el SN es el túbulo colector cortical. La activación del canal de sodio epitelial a ese nivel es responsable de la retención de sodio en la patología que nos ocupa. Una barrera glomerular defectuosa propia del SN permitiría el paso de enzimas proteolíticas o sus precursores que a su vez activarían el canal de sodio epitelial causando de esa manera su retención y consiguiente edema.

Palabras clave: *Edema. Síndrome nefrótico. Hipoalbuminemia. Canal epitelial de sodio. Plasmina.*

INTRODUCTION

Oedema is defined as the accumulation of fluid in the interstitial space and is a frequent clinical manifestation of nephrotic syndrome (NS). However, its pathophysiology has been under considerable debate for decades. The classic hypothesis, also called the *underfill* hypothesis, postulates that sodium retention in NS is secondary to decreased effective arterial blood volume, hence the term *underfill*. The hypothesis suggests the following sequence of events (Figure 1): urinary loss of proteins in NS, especially albumin, causing hypoalbuminaemia, which in turn causes a decrease in plasma oncotic pressure. This decrease in plasma oncotic pressure would then cause an imbalance in Starling forces, resulting in the

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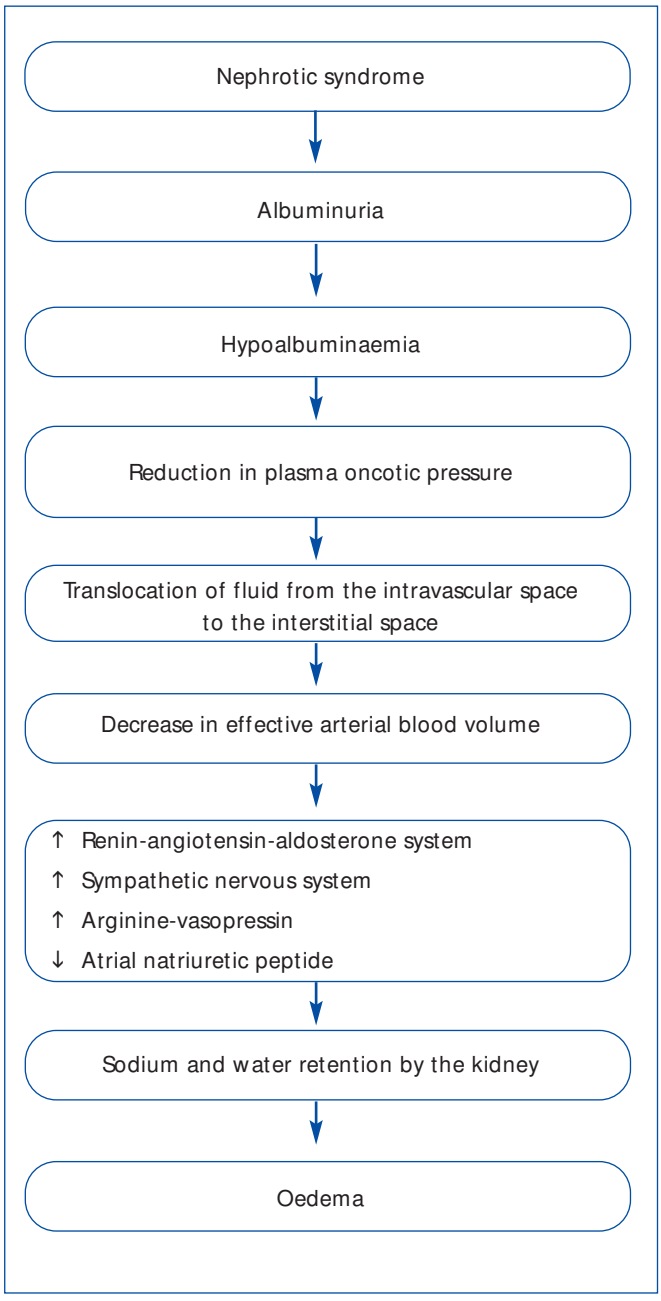

Figure 1. Classic or underfill hypothesis of oedema formation in nephrotic syndrome.

movement of fluid from the intravascular space to the interstitial space, causing a decrease in effective arterial blood volume and consequently, relative hypovolaemia. This would then result in activation of the renin-angiotensin-aldosterone and sympathetic nervous systems, increased antidiuretic hormone release and inhibition of atrial natriuretic peptide release. Activation of these systems would cause sodium and water retention in the kidneys, with subsequent oedema. However, several experimental and clinical observations made over the years do not support this hypothesis.

EXPERIMENTAL AND CLINICAL OBSERVATIONS AGAINST THE UNDERFILL HYPOTHESIS[1,2] (Table 1)

Patients and laboratory rats with low serum albumin levels do not develop oedema or sodium retention

Joles et al[3] measured the plasma and interstitial oncotic pressure of Nagase rats, which are mutant rats characterised by analbuminaemia. The researchers found no signs of sodium retention in those animals. Furthermore, Lecomte et al[4] carried out observations on patients with congenital analbuminaemia and found that most had no oedema. Many other published series of patients with congenital analbuminaemia do not report the appearance of oedema as the main symptom.[5] Steyl et al[6] studied 50 patients hospitalised in a general medical ward in South Africa and noted that 24 patients had a serum albumin level lower than 3.5g/dl, mostly associated with chronic inflammation (tuberculosis). Of these 24 patients,

Table 1. Arguments against the underfill hypothesis ofoedema formation in nephrotic syndrome

1. Patients and laboratory rats with low serum albumin levels do not develop oedema or sodium retention.
2. Natriuresis in the recovery phase of nephrotic syndrome begins when proteinuria disappears but before serum albumin returns to normal levels.
3. The absolute decrease in plasma oncotic pressure does not affect the volume of the intravascular space in nephrotic syndrome.
4. Plasma and blood volumes are normal or increased in nephrotic syndrome.
5. Intravascular space expansion with albumin does not increase natriuresis in patients with nephrotic syndrome.
6. The activation of the renin-angiotensin-aldosterone system is not involved in the development of oedema in nephrotic syndrome.
7. Bilateral adrenalectomy does not prevent sodium retention in nephrotic syndrome in laboratory rats.

only six had oedema. These six patients with oedema had an alternative diagnosis that clearly explained the presence of oedema (*cor pulmonale*). During the study, they found some patients with serum albumin levels below 1.5g/dl, but none of them had oedema.

Natriuresis in the recovery phase of nephrotic syndrome begins when proteinuria disappears but before serum albumin returns to normal levels[7]

The absolute decrease in plasma oncotic pressure does not affect the volume of the intravascular space in nephrotic syndrome

Studies performed on dogs suggest that the absolute decrease in plasma oncotic pressure would not affect plasma or blood volume.[8] Patients with NS caused by glomerulonephritis were studied by measuring their plasma and interstitial oncotic pressure: 12 patients in the active phase, 3 in complete remission and 3 in partial remission.[9] The researchers found that plasma and interstitial oncotic pressure were decreased in the active phase of NS but slowly returned to normal values during remission. During this time, the oncotic pressure gradient between plasma and interstitium was constant.[9] These studies show that it is the change in the oncotic pressure gradient between plasma and interstitium and not just the absolute decrease in plasma oncotic pressure that causes the movement of fluid from the intravascular to the interstitial space.

Plasma and blood volumes are normal or increased in nephrotic syndrome

Geers et al[10] measured plasma volumes in 88 patients with NS and in 51 controls. Plasma volume was measured by administration of radioactive albumin I.[131] Blood volume was calculated based on plasma volume and haematocrit. The plasma and blood volume of NS patients was found to be high in 14%, normal in 84% and low in only 2% of cases.

Intravascular space expansion with albumin does not increase natriuresis in patients with nephrotic syndrome

The effect of an intravenous infusion of hyperoncotic albumin (75g) was observed in patients with NS.[11] After the infusion, blood volume increased up to 120% of baseline. Plasma renin activity and serum aldosterone concentration decreased to the point of being suppressed. Urinary sodium excretion did not change significantly.

The activation of the renin-angiotensin-aldosterone system is not involved in the development of oedema in nephrotic syndrome

Brown et al[12] administered captopril to a group of NS patients and observed no change in sodium excretion despite suppressing serum aldosterone concentrations. In another study, Usberti et al[13] reported similar findings when using spironolactone.

Adrenalectomy does not prevent sodium retention and the development of ascites in nephrotic syndrome in laboratory rats

De Seigneux et al studied a group of rats from which they had removed both adrenal glands. The rats were administered dexamethasone to prevent adrenal failure.[14] The researchers induced NS in the rats by administering puromycin. The rats developed oedema and sodium retention despite having been adrenalectomised. These findings suggest that aldosterone does not play a major role in sodium retention that is characteristic of NS.

ALTERNATIVE HYPOTHESIS OR *OVERFILL* HYPOTHESIS OF OEDEMA FORMATION IN NEPHROTIC SYNDROME

Contrary to the classic hypothesis, the alternative hypothesis (also called the *overfill* hypothesis) postulates that sodium retention in many NS patients is a primary renal phenomenon and may be caused by an intrinsic renal defect in sodium excretion, which in turn causes an expansion in plasma volume (hence the term *overfill*). Although the molecular mechanism of sodium retention in the kidneys has not been clearly explained, there are a number of studies on this topic, which we describe below.

Molecular mechanisms of sodium retention in nephrotic syndrome

The first observations supporting the *overfill* hypothesis were made by Chandra[15] and Ichikawa.[16] Most of our understanding of the molecular mechanisms of sodium retention in NS has come from the use of animal models that induced NS by puromycin aminonucleoside (PAN). When PAN is administered to rats, it causes massive proteinuria and sodium retention. The renal histopathology induced by PAN resembles minimal change disease.[17-19] Using the technique of selective unilateral perfusion through the left renal artery with PAN first described by Bricker in dogs[20] and later by Hoyer in rats,[21] Chandra[15] and Ichikawa[16] showed that proteinuria and sodium retention were confined to the kidney perfused with PAN. The unilateral NS model allows

the study of a proteinuric kidney and a control kidney in the same animal. It must be emphasised that the sodium retention by the kidney perfused with PAN occurred without a reduction in plasma protein concentration, suggesting that the sodium retention observed in NS was due to an intrinsic renal defect in sodium excretion rather than due to extrinsic or systemic factors such as hypoalbuminaemia.

The cortical collecting tubule is the reabsorption point for sodium in nephrotic syndrome

Ichikawa[16] also performed micropuncture studies of superficial nephron tubular segments in the unilateral NS model in rats, and showed that the amount of sodium at the end of the distal convoluted tubule is the same in the proteinuric kidney as in the normal kidney. The final urine of the nephrotic kidney, however, contained three times less sodium than the urine from the normal kidney, suggesting that stimulation of sodium reabsorption in the NS occurs in the cortical collecting tubule.

The role of NHE3 in sodium retention in the NS

Despite the findings of Ichikawa et al, other studies have postulated that sodium retention in NS may occur in other nephron segments. Sixty-six percent of sodium filtered by the glomerulus is reabsorbed in the proximal tubule by the action of the Na-H cotransporter (NHE3). It would be reasonable then to assume that this segment would, at least in part, contribute to the sodium retention observed in NS. Besse-Eschmann et al[22] found that NHE3 activity (normalised to the amount of protein) was increased by 88% in rats treated with PAN, compared to control rats. NHE3 is present at two locations of the proximal tubular brush border, forming oligomers: *1)* in the intervillous space, where it is associated with the megalin receptor (a protein responsible for the reabsorption of albumin and other substances filtered by the glomerulus), representing the inactive form of NHE3, and *2)* in the microvillous space, where it is free and represents the active form of the transporter.[23] The researchers also found that in rats treated with PAN there was NHE3 movement from the intervillous space to the microvillus space.[22] They suggested that albumin filtered by the NS's defective glomerular barrier could dissociate NHE3 from megalin and increase movement of NHE3 to the microvilli so that it might perform its sodium retention function from there.[22]

The role of the Na^+/K^+-ATPase pump in sodium retention in nephrotic syndrome

Another sodium transporter that has been reported to be involved in sodium retention in NS is the Na^+/K^+-ATPase pump. Deschenes et al[24] found that the activity of this pump was increased in rats treated with PAN when compared to control rats. These authors also observed that the increase in pump activity was confined to the cortical collecting tubule.[24] However, many subsequent studies have shown that, in rats treated with PAN, the activity of this pump and other sodium transporters (such as NHE3) is decreased when compared to control rats.[25]

The role of ENaC in sodium retention in nephrotic syndrome

Another sodium transporter that has been reported to be strongly involved in sodium retention in NS is the epithelial sodium channel or amiloride-sensitive sodium channel (ENaC). ENaC is composed of three subunits: α, β, and γ. The first studies conducted on the role of ENaC in sodium retention in NS showed that there was no increase in protein expression (nor mRNA) of any of the three subunits of ENaC in rats treated with PAN when compared to control rats.[26] However, subsequent studies have shown an increase in protein expression of the three subunits of ENaC,[14,25] as well as an increase in the movement of these subunits from the cytosol to the apical plasma membrane.

ENaC is regulated by several factors, one of which is the enzyme 11-β-hydroxysteroid dehydrogenase type 2 (11βHSD2). Mineralocorticoid receptor activation causes an increase in ENaC activity by increasing expression of the gene that encodes the ENaC α subunit and a decrease in its intracellular recycling system mediated by the ubiquitin ligase Nedd4-2.[27] Cortisol has the same affinity as aldosterone to the mineralocorticoid receptor. However, aldosterone acts as the sole agonist of this receptor even though the concentration of cortisol in plasma is 100 times the concentration of aldosterone. The 11βHSD2 enzyme usually protects the mineralocorticoid receptor from cortisol activation by locally transforming it into cortisone, which is inactive on this receptor. Nevertheless, in pathological states such as in the syndrome of apparent mineralocorticoid excess, the activity of the 11βHSD2 enzyme is reduced, allowing the cortisol to activate the mineralocorticoid receptor and cause sodium retention.[28] A study by Kim et al[29] showed that the activity of the 11βHSD2 enzyme is reduced in rats with NS caused by mercuric chloride-induced membranous nephropathy when compared to control rats, which may explain the sodium retention in these animals. However, other studies have not confirmed these findings.[14,30]

Another important factor in the regulation of ENaC is the group of serine proteases. These are a group of proteolytic enzymes that cleave the α and γ ENaC subunits in specific sites and thereby increase sodium conductance through the channel.[31,32] Under experimental conditions, sodium conductance is low in ENaC that has not been exposed to

proteolysis by serine proteases. The first step in ENaC activation by serine proteases occurs in the Golgi complex, where a protease called furin cleaves the α subunit at the R205 and R231 sites (thus releasing an inhibitory peptide of 26 amino acids) and the γ subunit at the R143 site.[33] If this ENaC conductance were measured under experimental conditions, it would be intermediate. After this enzymatic process, the channel is assembled in the apical plasma membrane. For ENaC to be completely active and have high sodium conductance, it must be activated by a second protease (such as prostasin, neutrophil elastase or pancreatic elastase).[34]

The first observations on ENaC activation by serine proteases in proteinuric states were made by Kastner et al.[35] Passero et al[36] found that ENaC currents increased when ENaC was exposed to plasmin, suggesting that plasmin acts as a second protease and is capable of activating ENaC. Passero[36] also discovered that plasmin activates ENaC by cleaving the γ subunit at the K194 site.

Perhaps the most convincing evidence to date about the role of serine proteases in ENaC activation in NS is the recently published report by Svenningsen et al.[37] They found that urine in nephrotic rats treated with PAN increased the ENaC currents and that amiloride abolished them. Svenningsen et al investigated why the urine of these nephrotic rats activated ENaC and found that the ENaC currents were abolished when ENaC was exposed to aprotinin, a known inhibitor of serine proteases. Another important observation was that the urine of nephrotic rats did not increase ENaC currents when subjected to heat. When serine protease activity in this urine was measured, it was found to be high. All these findings suggest that the urine of nephrotic rats contains a serine protease capable of activating ENaC.[37] Several previous studies performed on NS patients have documented the presence of plasminogen in the urine of these patients.[38,39] After several purification steps and mass spectrometry (MALDI-TOF), Svenningsen et al found that plasminogen and/or plasmin were the serine proteases responsible for ENaC activation in the urine of nephrotic rats. The urine of nephrotic rats contained both substances, but the plasma from these animals only contained plasminogen, suggesting that plasmin was formed in the urine *in situ* and was not filtered out of the plasma.[37] Plasmin is known to come from the activation of plasminogen through the enzymatic action of urokinase, which is normally present in the collecting tubule.[40] Svenningsen et al[37] observed that the cortical collecting tubule cells of nephrotic rats had urokinase activity. They also observed that while the combination of plasminogen and urokinase increased ENaC currents in oocytes, plasminogen and urokinase were incapable of doing so in isolation.[37] Another important finding was that amiloride not only blocks ENaC but also blocks the urokinase enzyme responsible for converting plasminogen into plasmin.[37] Significantly, Svenningsen et al[37] were able to reproduce all of the previously described results with urine from NS patients. To summarise, the plasminogen present in plasma is probably filtered through NS's own defective glomerular barrier and is then converted into plasmin by the action of urokinase present in the collecting tubule. Plasmin would then activate ENaC, resulting in sodium retention with the subsequent appearance of oedema (Figure 2).

The use of amiloride to treat nephrotic oedema

Treatment of oedema in NS is traditionally based on a low sodium diet (2.3g of sodium a day or 6g of sodium chloride a day) and the use of loop diuretics. Amiloride is a potassium-sparing diuretic and its use has traditionally been restricted to the prevention of hypopotassaemia associated with the use of loop diuretics. However, according to the findings described above, the use of amiloride may play an important role in the treatment of oedema in NS. In our clinical experience, the use of amiloride enhances diuresis caused by loop diuretics. This has been reproduced experimentally[41] and has also been reported in other clinical studies.[42] We usually start treatment of nephrotic oedema with a 1mg dose of bumetanide orally twice a day and a 5mg dose of amiloride orally once a day. Amiloride should not be used in isolation but rather in combination with loop diuretics, given that although the collecting tubule plays a key role in sodium retention in NS, in absolute terms it only contributes 4% of total filtered sodium reabsorption.

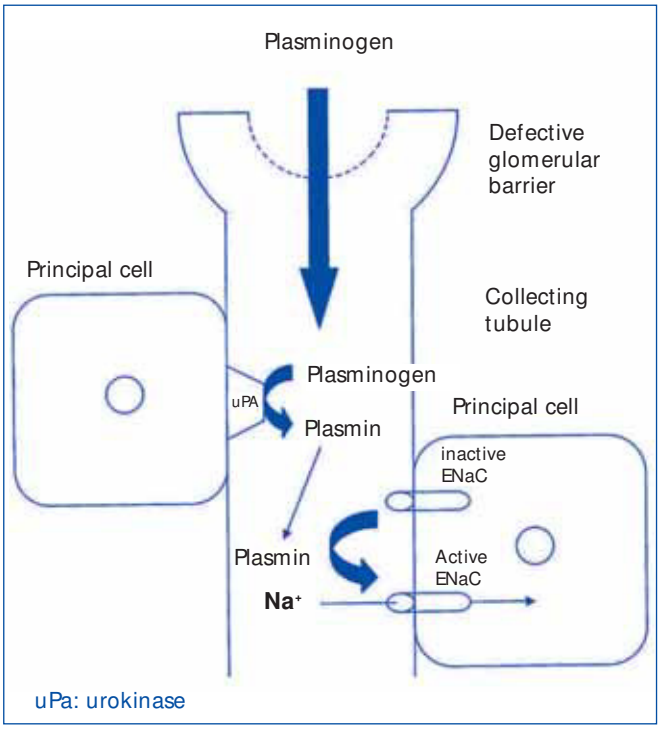

Figure 2. Plasmin in the cortical collecting duct activates ENaC.

KEY CONCEPTS

1. In a large number of NS patients, the pathophysiology of oedema is not related to the presence of hypoalbuminaemia, decreased intravascular space volume or secondary hyperaldosteronism.
2. NS oedema is caused by an intrinsic renal defect in sodium excretion.
3. Sodium retention in NS occurs in the cortical collecting tubule.
4. ENaC, one of the sodium transporters present in the cortical collecting tubule, is involved in sodium retention in NS.
5. The defective glomerular barrier in NS allows the passage of many proteins, among them plasminogen.
6. Urokinase, an enzyme naturally present in the cortical collecting tubule epithelium, is responsible for converting plasminogen into plasmin.
7. Plasmin, formed in situ in the cortical collecting tubule, activates ENaC causing sodium retention and oedema.
8. Amiloride enhances diuresis caused by loop diuretics in NS.

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