Renal interstitial hyaluronan: functional aspects during normal and pathological conditions

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Stridh S, Palm F, Hansell P. Renal interstitial hyaluronan: functional aspects during normal and pathological conditions. Am J Physiol Regul Integr Comp Physiol 302: R1235–R1249, 2012. First published April 18, 2012; doi:10.1152/ajpregu.00332.2011.—The glycosaminoglycan (GAG) hyaluronan (HA) is recognized as an important structural component of the extracellular matrix, but it also interacts with cells during embryonic development, wound healing, inflammation, and cancer; i.e., important features in normal and pathological conditions. The specific physicochemical properties of HA enable a unique hydration capacity, and in the last decade it was revealed that in the interstitium of the renal medulla, where the HA content is very high, it changes rapidly depending on the body hydration status while the HA content of the cortex remains unchanged at very low amounts. The kidney, which regulates fluid balance, uses HA dynamically for the regulation of whole body fluid homeostasis. Renomedullary HA elevation occurs in response to hydration and during dehydration the opposite occurs. The HA-induced alterations in the physicochemical characteristics of the interstitial space affects fluid flux; i.e., reabsorption. Antidiuretic hormone, nitric oxide, angiotensin II, and prostaglandins are classical hormones/compounds involved in renal fluid handling and are important regulators of HA turnover during variations in hydration status. One major producer of HA in the kidney is the renomedullary interstitial cell, which displays receptors and/or synthesis enzymes for the hormones mentioned above. During several kidney disease states, such as ischemia-reperfusion injury, tubulointerstitial inflammation, renal transplant rejection, diabetes, and kidney stone formation, HA is upregulated, which contributes to an abnormal phenotype. In these situations, cytokines and other growth factors are important stimulators. The immunosuppressant agent cyclosporine A is nephrotoxic and induces HA accumulation, which could be involved in graft rejection and edema formation. The use of hyaluronidase to reduce pathologically overexpressed levels of tissue HA is a potential therapeutic tool since diuretics are less efficient in removing water bound to HA in the interstitium. Although the majority of data describing the role of HA originate from animal and cell studies, the available data from humans demonstrate that an upregulation of HA also occurs in diabetic kidneys, in transplant-rejected kidneys, and during acute tubular necrosis. This review summarizes the current knowledge regarding interstitial HA in the role of regulating kidney function during normal and pathological conditions. It encompasses mechanistic insights into the background of the heterogeneous intrarenal distribution of HA; i.e., late nephrogenesis, its regulation during variations in hydration status, and its involvement during several pathological conditions. Changes in hyaluronan synthases, hyaluronidases, and binding receptor expression are discussed in parallel.

kidney; hydration status; diabetes; nephropathy; ischemia-reperfusion; transplantation; kidney stone; cyclosporine A

HYALURONAN (HA) is found in most parts of the body but in higher concentrations in soft connective tissues (45). The average 70 kg human has about 15 g of HA in the body, one-third of which is turned over (degraded and synthesized) every day (139). In the kidney, it is primarily found in the interstitial spaces of the medulla (32, 58, 60, 160), where it participates in fluid balance regulation. HA is a negatively charged molecule of high molecular weight. HA consists of a linear polysaccharide formed of repetitive d-glucuronic acid and N-acetyl-d-glucosamine and is a member of the glycosaminoglycans (GAGs) (Fig. 1). Shared characteristics of GAGs are that they contain sulfate groups (not HA), and their polysaccharide chains are relatively short, <50 kDa and usually 15–20 kDa. However, HA generally consists of long carboxylated chains, usually 200–2,000 kDa. The molecular weight of its polysaccharide chains can be very large. In the synovial...
fluid its average weight is about 7 MDa, which equals a more than 15-μm long strand.

The key capabilities of HA are its water-attracting properties (1 g HA attracts one liter of water) and its ability to form gels in higher concentrations (>0.2 mg/ml). The network of HA occupies space in the solution and excludes larger molecules. This phenomenon is known as steric exclusion and can affect water transport and osmotic activity in the extracellular matrix (23). HA also exerts electrostatic exclusion by its negative charges, which will affect the positively charged ion flow (161). The excluded volume is dependent both on macromolecular size and charge, and is additive, so that the excluded volume caused by steric effect and negative charge can match the excluded volume by the larger, uncharged immunoglobulin G. Thus the major excluding moiety of the extracellular matrix might be a charged macromolecule instead of the usual designated molecule collagen. Exclusion effects can be dominated by negatively charged proteoglycans and, furthermore, are related to tissue hydration.

It can be argued that collagen and elastin also could participate in the phenomena of steric exclusion and charge effects. There are, however, no studies that disclose a similar relationship between these matrix components and hydration and kidney function, as will be described in the present review for HA. We have, therefore, focused the entire review on the interactions displayed by HA.

The appearance of interstitial HA has been under some debate, namely, whether it is primarily considered to be a gel or a mesh work with cables. Selbi and colleagues (131) demonstrated that proximal tubular cells, in addition to surrounding themselves with an HA-rich coat (pericellular matrix), also formed cable-like structures. A study by Kultti et al. (75) demonstrated that the glycocalyx on the plasma membrane is created by dense arrays of HA chains, which are tethered to hyaluronan synthases (HASs) during synthesis, thereby producing prominent microvilli.

A new characteristic that has been discovered for HA is its ability to affect the membrane potential of cells which it covers (56). The addition of extracellular HA of high molecular weight depolarized the membrane of human embryonic kidney cells, human fibroblasts, and central nervous system (CNS) neurons. The digestion of HA by hyaluronidase (Hyal) resulted in hyperpolarization, whereas other GAGs did not attain these properties. The effects were suggested to be due to Donnan effects and steric exclusion of molecules from the high-molecular-weight HA. This would represent an additional pathway for signal transduction, linking the extracellular matrix with intracellular activities, without involving the main HA-binding receptor CD44.

SYNTHESIS

HA is synthesized in the plasma membrane (Fig. 2) (88, 117). There are three vertebrate synthases identified so far: HAS 1–3 (31, 134, 158). The chromosomal localization of the synthases has been determined, and they are located on different chromosomes in both humans and mice (136). The existence of three HAS isoforms in vertebrates and the location on different chromosomes supports the notion that HA is an important regulator of cell behavior besides being a component of tissue structure (135, 136, 158). The different HAS produce different HA chain lengths (67, 120). HAS1 and HAS2 produce HA polymers (up to 2 MDa), which are larger than the polymers produced by HAS3 (up to 1 MDa) (67). Alteration of a single amino acid residue is enough to alternate the chain length produced by the enzyme (120). In the normal kidney, all three HAS are expressed in larger amounts in the medulla than in the cortex. Furthermore, the relative expressions are HAS2 > HAS1 > HAS3 (126, 140).

The major HA-producing cells in the kidney are the renomedullary interstitial cells (RMIC) (51, 61, 112). Renocortical interstitial cells also produce HA, but at a much lower pace (110), and this provides an explanation for the heterogeneous intrarenal HA distribution in the adult kidney. It is also plausible that cortical fibroblasts can be induced to produce more HA by immune mediators, considering the HA accumulation that occurs in the cortex during inflammation (58, 69, 140). HA is also synthesized and secreted from the apical membrane of tubular cells when they regenerate due to inflammation (9) or during hyperglycemic conditions (70). Mesangial cells and vascular smooth muscle cells are also capable of forming HA, which is induced by hyperglycemia (37, 155).

DEGRADATION

The turnover of HA is tissue dependent (45), and large amounts of HA are transported by the lymphatic vessels to the

![Fig. 1. Structure of hyaluronan (HA) demonstrating the repeated D-glucuronic acid and N-acetyl-D-glucosamine moieties.](Image)

![Fig. 2. Synthesis and turnover of HA. The HA synthases (HAS) 1–3 produce HA in the plasma membrane of different sizes and at different rates. The hyaluronidases (Hyal) hydrolyze HA. This may begin already on the plasma membrane by Hyal2, followed by 2) binding to CD44, which is a scavenger receptor for HA. After 3) internalization and degradation in endosomes by Hyal2, HA is 4) further degraded by Hyal1 in lysosomes. For more details see Degradation.](Image)
bloodstream (79). In dense structures, HA is more likely to be degraded in situ rather than drained by the lymphatic system, since there are no lymph vessels. It can be speculated that the same is true for remodelling HA, since the renal medulla, as opposed to the cortex, has no lymphatic drainage. It has been established that HA in the lymphatic system enters and is degraded by the lymphatic tissue (44). However, other studies suggest that only a small fraction of HA is removed by lymph drainage (<1%), and that lysosomal Hyal are far more important for the rapid turnover of HA (5). Lymph drainage would thus not normally be significant in HA catabolism.

The half-life of HA in tissue varies from 12 to 72 h (45). When entering the bloodstream, 85–90% of HA is eliminated in the liver and only ~1–2% is excreted by the kidneys. HA that enters the bloodstream is taken up by hepatic endothelial cells and degraded into low molecular-sized products (36). Lymph nodes also have the capacity to extract and degrade HA (44, 45).

It took substantial time before a general concept of Hyal degradation of HA was established in most mammalian tissues (15). The Hyals are still difficult to study, because they are not easily purified, reside in low concentrations, and exert high specific activities, which are unstable without detergents and protease inhibitors (25, 47). There are six Hyals with different topicalities. The sequences for the six Hyal-like genes were found by expression analysis by Csoka et al. (26), which positioned the two clusters of Hyal genes in loci connected to tumor suppression (26). The homology of pairs of genes from mouse and human orthologs is much greater than that of the six human homologues, which suggests that a divergence of the genes took place long before mouse and human diverged over 80 million years ago.

Hyal1 mRNA is present in the heart, kidney, liver, lung, placenta, and skeletal muscle but not in the brain (47). Hyal1 is the predominant form and only Hyal found in human plasma. Hyal2, an acid-active Hyal, is also expressed in various tissues, and its gene is positioned close to Hyal1 (25). It has been suggested that Hyal2 also has important nonenzymatic functions (33). Less is known about Hyal3, which is found in mammalian testis, bone marrow (25), and the neonatal kidney (140). The PH-20/SPAM1, which is important during egg fertilization by the sperm, has Hyal activity (50, 140) and is known as testicular Hyal. Hyal 4 is primarily expressed in the placenta and skeletal muscle (25), and it is suspected that Hyal 4 has no HA-degrading activity. The final sixth Hyal, i.e., Hyal P1, is a human pseudogene caused by premature termination codons (26).

In Fig. 2, the cellular degradation pathway for HA is depicted. It has been suggested that the breakdown is initiated on the plasma membrane by Hyal2 before internalization (caveolin-enriched invaginations) by the HA receptor CD44 (19). Hyal2 reduces high-molecular-weight HA into low molecular HA but does not display enzymatic activity on low molecular fragments (82). The fragments of ~20 kDa are then delivered to low pH lysosomes, where they are further digested by Hyal1 (43). It seems that the CD44-mediated catabolism of HA in keratinocytes is independent of caveoli (143), thereby representing a novel endocytic route for catabolism in this specific cell type.

**RECEPTORS**

The dominating HA cell surface receptor is CD44 (6). It is a polymorphic type I transmembrane glycoprotein with a diversity determined by differential splicing at 10 variable exons coding for a part of the extracellular domain. It also exhibits cell type-specific glycosylation and participates in matrix-to-cell signaling, cell migration, cell-to-cell aggregation, and receptor-mediated internalization/degradation of HA (27, 64).

CD44 provides a signal response to HA and participated in HA endocytosis as a scavenger receptor (80). The cell surface signaling by HA is commonly associated with cell migration or proliferation, and HA-CD44 signaling activates several pathways. Fragments of HA can activate the nuclear transcription factor NF-κB (41), and CD44 is critical for the NF-κB response. Proinflammatory genes induced by HA (92, 93, 121) are regulated by NF-κB and have NF-κB sites at their 5’ flanking region. High molecular HA fails to activate NF-κB, which is consistent with the inflammatory response to low molecular HA.

In the kidney, CD44 resides mostly in the inner stripe of the outer medulla on the basolateral membranes of the collecting ducts, on the basolateral membranes of the descending limb of the loop of Henle, and on the macula densa cells (32). In cultured rat RMIC (51), CD44 is downregulated during hypoxic conditions and upregulated during hyperosmotic conditions. This can provide an important regulatory step for determination of the interstitial content of HA by changing cellular uptake and degradation depending on the osmotic milieu. During renal disease/damage, CD44 is upregulated concomitantly with HA (32, 52, 94).

HA turnover increases during inflammation (93), and low-molecular-weight HA fragments accumulate, which induces inducible nitric oxide (NO) synthase (iNOS) in macrophages via synergy with interferon-γ. The iNOS induction requires the activation of NF-κB. HA could possibly be a regulator of macrophage activation in chronic tissue inflammation, where increased extracellular matrix turnover is a characteristic feature. As previously mentioned, low molecular HA has been proposed to participate in T-cell activation (116).

Another HA receptor is the receptor for HA-mediated motility (RHAMM) described by Hardwick et al. (62). It is a HA-binding protein located intracellularly and on the cell surface. RHAMM is involved in tumor cell migration, which has relevance for oncogenesis and morphogenesis (150).

A third HA receptor is the Ca2+-independent endocytic HA receptor of hepatic endothelial cells identified by Yanniello-Brown et al. (163). This receptor mediates internalization of HA by hepatic (liver) endothelial cells.

Other HA receptors have been discovered such as hyaluronan receptor for endocytosis (HARE) (168), layilin (16), and lymphatic vessel endothelial receptor 1 (LYVE-1) (11, 118). ICAM-1 (CD54) has also been suggested as an HA receptor (90), but in a study by Weigel et al. (157), it was established that HARE is the endocytic HA receptor in liver endothelial cells and not ICAM-1 or CD44. HARE is expressed on hepatic sinusoidal endothelial cells (168).

Layilin is an integral membrane protein that binds HA and is implicated in cell adhesion and motility as it, like CD44, can...
bind cytoskeleton membrane linker proteins with its cytoplasmic domain and HA with its extracellular domain (16).

LYVE-1 is a HA receptor located on the lymph vessel wall and is believed to be of importance for HA removal from both wounds and normal tissue to the lymphatic system for later degradation and transportation to the circulation and finally to the liver (11, 118). In contrast to CD44, which focuses on mobility (144), LYVE-1 seems to keep HA inside the lymph vessel (11). LYVE-1 internalizes HA into the lymphatic endothelial cell and is an endocytic HA receptor (118).

**GENERAL FUNCTIONS**

A few of the functions of HA are to compose part of the extracellular space with the highest concentrations in soft connective tissues, to take part in the structure of cartilage bound to the proteoglycans aggrecan, to exert roles in the homeostasis of the extracellular space, and to regulate the distribution and transport of plasma proteins in tissues. HA also regulates cell functions such as cell proliferation, recognition functions, cell locomotion, inflammation regulation, and cell protection (77).

HA modulates the transvascular fluid balance, and thus tissue hydration, and binds hyaladherins and thereby stabilizes the extracellular matrix (5). In the case of tissue damage or tissue/organ development, HA binds surface receptors and activates signal pathways and thus regulates cell motility, invasion, and proliferation (105).

After Schmidt-Nielsen (130) described the specificities of the renal HA matrix and its potential for concentrating functions, Knepper et al. (74) suggested a fluid-concentrating model for the action of HA. This model is based on the concept of the interstitium as a gel rather than as an aqueous compartment. The HA matrix can store mechanical energy from pelvic contractions and utilizes this energy to lower interstitial pressure after the completion of each contraction of the pelvic wall, thus driving water efflux from the descending limb of Henle to the blood. The osmolality in the lumen will then surpass that of the interstitium by a net flux of fluid, resulting in concentration of the urine, but the validity of this model is unknown. The contractions occur in hamster papillae, which are long and thin (119). However, these effects should, if at all, be smaller in the rat, which have wider and thicker papillae. The human kidney in contrast to rodents kidney, is multipapillary, and is most likely not exposed to this effect. It may be that the concentration gradient is maintained due to a segmental compartmentalization in the medulla where HA is important for maintenance of the gradient.

Although it is well accepted that HA acts to preserve tissue hydration, it has been suggested that GAGs like HA can be involved in tissue sodium storage without accompanying water retention (146). This could be an important contributor to the regulation of extracellular fluid volume during situations of positive sodium balance, by binding of sodium to negatively charged interstitial matrix components with polyanionic characteristics.

Levick and co-workers (83, 91, 127) have made a series of studies of the role of fluid flux through the synovium and its effect on the synthesis of HA. They show that besides providing lubrication, HA greatly attenuates transsynovial fluid loss, a phenomenon called outflow buffering. This ability to retain fluid in the joint by HA depends on the amount of HA present and declines if the HA chain length is reduced.

The action of HA is size dependent (105). In tissue injury, the HA fragments of low molecular weight will accumulate. These fragments stimulate cell proliferation and migration, whereas larger polymers signal inhibition and promote dormancy. Low molecular HA is a result of degradation of high molecular HA (80). Low molecular HA has been speculated to act as an endogenous signal to activate T-cells (116). HA is, similarly to other GAGs degraded by reactive oxygen species (ROS) but is more susceptible to it than sulfated GAGs (97).

**HYALURONAN AND NORMAL KIDNEY FUNCTION**

**Hyaluronan During Different States of Body Hydration**

In the healthy kidney, HA is mainly found in the interstitium of the inner medulla (papilla) and in much smaller amounts in the cortex and the outer zone of the outer medulla (32, 58, 60, 160). The amount of HA in the inner medulla and the papilla is 50–100 times higher than that of the cortex and outer medulla during normal physiological conditions in the rat kidney (Fig. 3). The papilla thus has the highest tissue hydration and HA content. The HA content is in the order of 0.6 mg/ml (60), which is enough to form a gel-like structure. The heterogeneous distribution manifests during the completion of nephrogenesis (140) and is described in a section below (Nephrogenesis). The heterogeneous HA distribution in the kidney suggests that HA in the extratubular stroma not only exerts structural support but also has a physiological function.
in fluid reabsorption. In this section of the review, findings are summarized which suggest that HA participates in the regulation of medullary water reabsorption by dynamical changes in interstitial HA content in relation to body fluid homeostasis, resulting in altered fluid reabsorption.

In 1958, Ginetzinsky (49) suggested that the action of vasopressin (ADH, antidiuretic hormone) in the renal medulla is exerted through the activation of Hyals, which changes the interstitial properties of fluid transportation. The urinary-concentration mechanism and the action of ADH were subjects of great controversy in the 1950s. Since then, progress has been made in our understanding of the cellular actions of ADH acting on the V1- and V2-receptors. The cyclic process involves vesicles carrying aquaporins from cytoplasm to the apical membrane and deposits the aquaporins, which are then recovered by endocytosis (102). A complete description of the structure of the channels themselves is at hand, and the discovery of the channels was awarded the Nobel Prize in 2003 (1). ADH has several other effects but is beyond the scope of the present review. It would thus seem that the hypothesis proposed by Ginetzinsky is incorrect. However, data will be summarized showing that Ginetzinsky most likely was correct regarding HA; i.e., ADH not only regulates aquaporin trafficking in the renal medulla but also takes part in regulating interstitial HA turnover, which thereby effectively enhances the fluid flux.

Hansell et al. (60) found that 2 h of hydration increased papillary and outer medullary HA by \( \sim 50\% \), whereas cortical HA remained unchanged. Furthermore, 24-h dehydration decreased papillary HA content by 17%. Thus the amount of papillary HA changed in accordance to body hydration status, and papillary HA peaked at the maximum diuretic response and minimum urine osmolality. It was concluded that HA is involved in renal water handling by changing the physicochemical characteristics of the interstitial matrix of the papilla and possibly by affecting the interstitial hydrostatic pressure (60, 76, 167). A possible mechanistic view of the effects is the following: the repulsion between the negatively charged carboxylate groups of the sugar moieties in HA (glucuronic acid, GlcUA) protrudes outward at regular intervals and contributes to the structure and size of the HA molecule. The negatively charged gel attracts positive ions and increases osmosis, which will attract water. The properties of the water-containing gel antagonize water reabsorption. Furthermore, the medullary thick ascending limb of the loop of Henle (mTAL), which generates the medullary osmotic gradient, may be functionally compromised when interstitial diffusion characteristics are changed due to elevated HA. Also, the efficiency of the vasa recta ("counter-current exchanger"), which helps to maintain the osmotic gradient by recirculating fluid-electrolytes in the medulla, may also be affected when diffusion characteristics are changed. Finally, the interstitial swelling ("functional edema"), which occurs in response to elevations in HA, increases the diffusion distances between the tubules and blood vessels, which also will affect fluid flux (A graphical presentation is given in Fig. 4).

The RMIC is a major HA producer in the medullary interstitium. Previous in vitro studies have demonstrated that this cell produces HA in a manner dependent on the media osmolality (51, 61). During hyperosmolar conditions (dehydration), HA is reduced in the media. Conversely, during hyposmotic conditions (hydration), HA production increases. CD44 is the main HA-scavenging receptor, and its expression on the surface of RMICs in culture is elevated during hyperosmotic conditions and reduced during hyposmotic conditions (51). This would suggest that part of the changes in medullary interstitial HA during variations in hydration status are achieved by increased CD44-mediated intracellular HA degradation. Furthermore, RMIC has angiotensin II (AT1) (169) and ADH (V1) receptors (132, 170). ADH infusion reduces renal medullary interstitial HA in vivo, and both ADH and angiotensin II reduce HA production by RMICs (125). The low cortical levels of interstitial HA do not change in response to hydration or ADH infusion (60, 125). This might be explained by the very low baseline production of HA by cortical interstitial cells (110).

NO and prostaglandins are involved in the mechanisms resulting in elevated medullary HA levels during hydration (124, 125). These hormones are well-known regulators of renal fluid handling. Both iNOS and COX 2 are present in RMICs (22), and Nitro-L-arginine methyl ester (L-NAME) (unselective NOS inhibitor) and indomethacin (unselective inhibitor of cyclooxygenase, COX, 1 and 2) both inhibit the elevation of papillary HA upon hydration. Although the exact mechanism is yet to be determined, prostaglandin E2 increases HA production in glomerular cells (86), and NO stimulates COX 2 expression and prostaglandin E2 release in renal medullary cells through mitogen-activated protein kinases (162).

The concept of an involvement of ADH in medullary HA turnover during changes in hydration status emanates from the pioneering work of Ginetzinsky in 1958 (49). Ginetzinsky found that urinary Hyal activity increased during dehydration and virtually dropped to zero during hydration. Furthermore, the infusion of ADH resulted in increased urinary Hyal activity, which was independent of the reduced urinary volume. After the discovery of the aquaporins and the in detail unraveling of the mechanisms by which ADH regulates their insertion in the apical membrane, the work by Ginetzinsky was forgotten (49). Repetition and extension of some of the exper-
iments by Hansell et al. (60, 125) and Rügheimer et al. (60, 125) (in vivo and in vitro) reveal that the reduction in medullary HA during ADH infusion is mediated by V₁ but not V₂ receptors (60, 125). Furthermore, during hydration, plasma ADH is reduced concomitantly with increased medullary HA and reduced urinary Hyal activity (126). Finally, in vitro studies using RMIC demonstrate that the HA content of the media is reduced when ADH is present (125). Thus it seems that Ginetzinsky (49) was correct regarding HA: ADH not only inserts aquaporin 2 water channels into the apical membrane of the collecting system of the medulla but also reduces the amount of interstitial HA. The V₂ receptor-mediated increase in membrane permeability (increased apical insertion of aquaporin 2) together with the V₁ receptor-mediated increase in interstitial diffusion characteristics (reduced interstitial HA) will maximize fluid reabsorption during dehydration, whereas the opposite occurs during hydration. Both receptor subtypes are G protein coupled, but the V₁ receptor activates phospholipase C, which mobilizes Ca²⁺ and stimulates protein kinase C (PKC), whereas the V₂ receptor signals via cAMP to activate protein kinase A (107).

The rapid elevation of medullary interstitial HA during hydration does not primarily involve increased gene expression of the proteins involved in HA turnover (126). However, the reduction of HA after 24 h of dehydration is associated with a reduced HAS2 expression (126). Presumably, the rapid HA increase during hydration is accomplished by reduced Hyal activity or reduced transport of HA to cellular catabolic sites. Modulation of catabolic turnover would thus provide a rapid response mechanism for changing the HA levels rather than creating alterations in the synthetic reactions (25).

In the Brattleboro rat, a model of diabetes insipidus caused by absence of ADH, the outer medullary HA levels are elevated, although the papillary levels are similar to those in normal rats (60). The elevated HA levels may counteract the ability for water diffusion in this region. Furthermore, the mTAL located in the outer medulla is an important structure for generation of the hyperosmotic gradient along the renal medulla (“counter-current multiplier”). By changing diffusion characteristics of the outer medulla, the concentration gradient might be compromised leading to reduced concentrating ability and diuresis, which are typical features of the Brattleboro rat. There are also indications that the cortical HA levels are higher in the Brattleboro rat, which might contribute to the higher diuresis.

Another animal species showing the involvement of renomedullary HA in the regulation of fluid handling is the desert rodent (gerbil). Gerbils demonstrate a unique ability to concentrate urine during water deprivation. If the hypothesis is correct concerning HA levels and renomedullary reabsorptive capacity, low HA levels are anticipated to facilitate water reabsorption. This is also the case: the medullary HA levels in the desert rodent are only approximately one-fourth to one-third of those in the rat during normal physiological conditions (53, 60), which would infer different permeability characteristics in the medullary interstitium. A mechanism underlying these relatively low HA levels can be the desert rodent’s significantly higher plasma ADH levels (approximately four times higher) compared with the rat (10, 65), which may keep the desert rodent’s Hyal activity high. The low medullary interstitial HA content, together with highly expressed ADH-regulated aquaporins, result in the capacity of gerbils to produce highly concentrated urine and therefore preserve body water. Göransson et al. (53) compared the HA levels and diuretic response to hydration in rats and desert rodents (gerbils) with HA levels and diuresis (53). Hydration in the normal rat resulted in an increase in papillary HA content with a maximum at 2 h and reversion to normal after 6 h. Hydration in the desert rodent, however, caused a decrease in papillary HA with a minimum occurring after 2 h and remained reduced throughout the 6 h of the experiment. Urine flow rate increased rapidly in the rat with a peak after 2 h but increased only slowly and slightly in the desert rodent. The ability of the rat to rapidly elevate papillary interstitial HA can counteract the acute hydration, minimizing reabsorption by changing the physicochemical characteristics of the interstitial extracellular matrix to promote diuresis. Thus the action of HA would be a complement to the downregulation of aquaporins on reduced ADH. However, as stated above, reduced ADH in plasma such as during hydration not only reduces aquaporins but may also reduce Hyal activity, which might be an important underlying mechanism of the elevated HA levels found in the medullary interstitial space. The desert rodent, genetically adapted to a diurnally different ecological niche, has very different papillary HA turnover and regulation. Decreased papillary HA during hydration and the moderate diuretic response might reflect a genetic difference in adaptation to the desert rodent habitat in an arid environment to maximize water conservation ability. There are, unfortunately, no published studies on HA production from RMIC from desert rodents in culture, which could shed light on the HA turnover during variations in media osmolality and make comparison with that of the rat. Regarding the RMICs, there are also species-related differences. This cell type is found in the medulla, and its most characteristic feature is the abundance of lipid droplets (14). RMICs are believed to provide structural support to the renal tubules and blood vessels but also to be involved in the regulation of renal blood flow and urine concentration, possibly involving the lipid found in the lipid droplets. In hydrated rats, which excreted urine of relatively low osmolality, the number of lipid droplets in the RMICs was two times larger than in untreated rats. When similar studies were conducted in gerbils, it was found that the number of lipid droplets decreased after water loading (14). Differences in ADH handling have been studied: during long periods of water deprivation, the ADH depletion is smaller in the gerbil, thereby suggesting a greater synthesis ability (35).

In a review by Wiig et al. (161), it is discussed that hydration might determine the relative importance of electrostatic forces of the extracellular matrix components so that tissue hydration is correlated to the excluded volume. The expected outcome of dehydration would be that the macromolecules would be crowded in the narrowing space and that electrostatic events in the tissue would increase. However, the opposite was observed. In dehydration, the electrochemical charge would not exert a large effect, although the charge contributes considerably in hydration. These results suggest that there might be a change in matrix geometry causing the network to collapse. The exact consequence of the latter in relation to diuretic response is presently unknown.

The changes described of interstitial HA during variations in hydration status should influence hydraulic conductivity, al-
though no such measurements have been performed in this tissue. However, in the peritoneum, increased amounts of HA reduces hydraulic conductivity, while the opposite has been shown for reduced HA in both the peritoneum and mesentery (42, 109, 122, 156, 166). It is thus plausible that the elevation in renomedullary HA observed after hydration reduces hydraulic conductivity, whereas the opposite occurs during dehydration.

It is not known whether it is the quantity or concentration of HA that is controlled in the renal interstitium during normal physiological conditions. It can be speculated that it is the quantity that is controlled. This would also be in accordance with the in vitro data, since it seems that the osmolality of the extracellular fluid and hormones (vasopressin released in response to osmolarity) affect the turnover of HA by the RMIC.

As to the origin of urine HA, an early study in 1987 by Laurent et al. (78) showed that the main fraction of urinary HA was in the range of 4,000 to 12,000 Da, which suggests that it originates from blood and arises by glomerular filtration. A small fraction was of higher molecular weight and could have been produced in the urinary tract.

To summarize, renomedullary interstitial HA changes in relation to body hydration, which enables modification of interstitial diffusion characteristics, thereby affecting renal fluid reabsorption. RMICs are major contributors to interstitial HA and are involved in its turnover during variations in hydration. ADH, NO, angiotensin II, and prostaglandins are classical hormones/compounds involved in renal fluid handling and are important mediators for the changes occurring in the interstitial HA content.

Nephrogenesis

During the development of the embryo, each of the HAS isoforms are uniquely expressed, both spatially and temporally (99). HAS2 is probably the most important synthase during embryonic development, since HAS2 
\(-/-\) mice die at midgestation (20), whereas HAS1 
\(-/-\) or HAS3 
\(-/-\) mice are viable and without lethal defects (20).

HA has been implicated in nephrogenesis. The extracellular matrix surrounding migrating and proliferating cells in embryonic development contains high levels of HA that decrease as differentiation progresses (148). The development of the embryonic kidney is different from the genesis of other viscera (137). The kidney formation follows its phylogenetic history. During development, three different organs form: the pronephros, the mesonephros, and the metanephros (72). However, only the metanephros remain to become the adult mammalian kidney (137). The metanephros, the early structures emerging in the fifth week of gestation, have as main purpose to induce differentiation of the definitive kidneys. The successive development of the kidneys by a series of events creates the presuppositions for malformation in the case of faulty interaction. The results of the faulty interactions will vary depending on the stage of development where it occurs.

The extracellular matrix is a mediator of morphogenetic branching (115). HA, being the largest GAG, is implicated in several studies to play a role in embryogenesis (114). HA accumulates in the immature metanephros but decreases as branching begins and Hyals increase (12). The changes are different in different parts of the developing kidney, and a change in HA amount cannot be assigned to a morphological change in the kidney, although HA tends to accumulate as the mesenchyme converts to an epithelium.

Pohl et al. (114) reported that the formation of branching tubules in vitro is sensitive to the matrix environment. HA stimulates early branching phenomena such as cellular process formation and the development of multicellular cords and functions as a cell survival factor. HA absence, by inhibition of binding or degradation, decreased cell survival and morphogenesis. It was proposed that HA, CD44, and HAS, which are all expressed in a spatiotemporal pattern, together form a pathway for morphoregulation.

In a study by Platt et al. (113), the development of nephrons in fetal mouse kidneys was investigated. For the in vitro part, the fetal kidneys were excised at gestation day 12 and cultured whole. They were then subjected to media containing heparan sulfate, heparin, chondroitin sulfate, or HA. Kidneys incubated in heparin or heparan sulfate had as little as 10% of the nephrons compared with kidneys incubated in control or in chondroitin sulfate or HA. However, the nephrons matured equally in all media.

As previously highlighted, HA is heterogeneously distributed in the mature human and rodent kidney, a prerequisite for normal kidney function (53, 60, 69, 160). The heterogeneous distribution of HA is established postnatally in the rat; i.e., during the first 3 wk after birth, which corresponds to late nephrogenesis. The sequence of events leading to this heterogeneous distribution has been described, in which an increased Hyal activity has been suggested (12, 103). Stridh et al. (140) demonstrated that the cortical expression of HAS2 decreased, whereas the medullary expression of Hyal increased during late nephrogenesis, which can explain the intrarenal dissipation of HA during the completion of nephrogenesis. Thus the rat kidney content of HA (of both cortex and medulla) is high during the first weeks after birth and is then rapidly reduced during the following 2 wk, reaching adult low levels in the cortex after day 21 (Fig. 5). The reduction is particularly conspicuous in the cortex, which is virtually void of HA 3 wk after birth, at which time HA is primarily found in the adventitial layer of vessels but is also associated with the glycocalyx.

Fig. 5. Changes in cortical interstitial HA during the final stages of nephrogenesis, which in the rat occurs during the first 3 wk after partus. The normal reduction of HA is inhibited if the animals are neonatally treated (before day 14) with angiotensin-converting enzyme (ACE) inhibitor. See Nephrogenesis for further details. Modified from Stridh et al. (140) with permission from the publisher.
along endothelial cells (39). At day 21 after birth, the HA content of the medulla is 12 times higher than that of the cortex. During adulthood, the HA levels in the medulla increase so that the medullary levels exceed the cortical stagnant level by a factor of about 50 (103, 140). It is worthwhile mentioning that HA is also expressed by renal tubular cells in human fetal kidneys but not in adult kidneys (151). Induction can occur, however, in adult kidneys in response to hyperglycemia or cytokines (70).

The rate of fluid intake and excretion in the newborn infant is in the order of seven times greater in relation to body weight compared with that of the adult (55). The low capacity of the neonate to concentrate urine has several different probable causes. Among them are few vasopressin V2-receptors in the collecting duct, low capacity to reabsorb sodium in the thick ascending limb, low amount of urea in the medullary interstitium (2), and high cortical and medullary interstitial HA levels (103). The high HA levels that are present during kidney development are certainly a prerequisite for normal organ development but have also been suggested to play a role in antagonizing water reabsorption and limiting urine concentration performance before the completion of nephrogenesis (141).

ACE inhibitors, which are used in the treatment of hypertension and diabetes complications, have also been found to affect nephrogenesis and HA turnover (46, 54, 103, 140). When testing vascular responses to ACE inhibition, Friberg et al. (46) discovered that ACE inhibition also produced kidney damage. There were persistent irreversible histopathological abnormalities even after withdrawing ACE inhibition manifested as inflammation in cortical tubular interstitium, papillary atrophy, and pelvic dilation. The abnormalities resulted in impaired urine concentrating ability in the rats. The same abnormalities were observed after angiotensin II AT1 receptor blockade, which highlights the role of AT1 receptor signaling as a crucial component of normal nephrogenesis. The “window of vulnerability” in the rat is open to up to day 14 after birth. Treatment with ACE inhibitor after day 14 in the rat does not result in any of these changes. The expression and activity of ACE in the rat kidney increases after birth and peaks around day 14 (24, 71). Both angiotensinogen (29) and AT1-receptor expression (106, 149) peak in the newborn period, after which they decline, possibly explaining the vulnerability window. The structural abnormalities found in rats and pigs after neonatal inhibition of RAS resemble the renal histological changes observed in human fetuses and newborns with ACE inhibitor fetopathy (133).

The mechanisms of the kidney damage by ACE inhibition were later studied (54, 103). The urinary-concentrating defect is caused by impaired tubular water reabsorption in the medullary collecting duct. This impairment is likely due to papillary atrophy and elevated interstitial HA, leading to interstitial edema and inflammation. There is also a decline in medullary tissue hyperosmolality. This together with reduced expression of aquaporin-2 resulted in decreased tubular reabsorption of water. It was also reported that rats challenged with water deprivation had impaired renal urinary-concentrating ability. There were no changes in renal hemodynamics, glomerular filtration rate (GFR), or electrolyte handling in the tubuli.

As noted above, it has been demonstrated that the kidneys neonatally treated with ACE inhibitor displayed elevated HA content both in the cortex and medulla (103, 140), interstitial inflammation with infiltrating cells and fibrosis, impaired urinary concentrating ability, and papillary atrophy (103). HA accumulated in a focal fashion and colocalized with immune-competent cells. It cannot be determined whether HA appears first and through its chemoattractive properties attracts immune cells to the area, or vice versa. It is, furthermore, not known when or how the remaining HA in the cortex attains inflammatory properties, as this is not evident during the first 2 wk after birth in neonatally ACE-treated animals. Whether this occurs in parallel with the development of the immune system in the rat or is due to synthesis of low molecular HA in the kidney is unknown. The elevated interstitial levels of HA will antagonize water reabsorption and contribute to interstitial inflammation, fibrosis, and inability to concentrate urine.

Renal lymphatics are only present in the cortex and not in the medulla. It could be hypothesized that at least the cortical removal of HA during neonatal kidney maturation is due to the development of renal lymphatics. To test this hypothesis, a lymphatic endothelial cell marker podoplanin was used in normal and neonatally ACE-inhibited rats (140). The ACE-treated rats show higher expression of the lymphatic endothelial cell marker podoplanin, suggesting an upregulation of lymphatic vessels instead of the opposite. Thus angiotensin II mediates HA reduction during kidney maturation via a pathway not involving lymph vessels.

To summarize, HA contribution to the kidney development is mostly accomplished by inducing morphogenic branching and differentiation. The mechanism by which HA is reduced in the kidney during maturation involves reduced HAS2 and increased Hyal1 expression through a process sensitive to angiotensin II, whereas lymphatic vessels are not involved. The low capacity of the immature kidney to concentrate urine and respond to a hyperosmolar challenge is at least partially due to the high HA levels, and this inability is accentuated if the normal removal of cortical HA is abrogated.

HYALURONAN AND RENAL DISEASE

Diabetic Nephropathy

Insulinopenic diabetes mellitus is a chronic, autoimmune disease characterized by the absence of insulin-producing β-cells. Diabetic patients often acquire secondary complications due to sustained hyperglycemia and concomitantly elevated oxidative stress (32a, 81). Eventually, both type 1 and type 2 diabetics will develop nephropathy, neuropathy, and retinopathy. Approximately every fourth patient with type-1 diabetes develops diabetic nephropathy (3), which today is the leading cause of end-stage renal failure.

Both functional and structural abnormalities are evident in this disease. Among the functional abnormalities in the chronic stages of renal disease are reduced GFR and urinary leakage of proteins. In the early stages, increased GFR is often observed (89), and the glomerular hyperfiltration is suggested to be involved in the progression of diabetic nephropathy (164). Although virtually every renal structural component is affected in diabetic nephropathy, there are some traditional definitions that are highlighted among the observed glomerular, vascular, and tubulointerstitial lesions (reviewed by Najafian and Mauer, 98). Among these lesions are thickening of the glomerular and tubular basement membranes, glomerulosclerosis, expansion...
of the mesangium, and hyalinosis of afferent and efferent arterioles. Extracellular matrix accumulation is a central abnormality in diabetic nephropathy (138). Cortical tubulointerstitial fibrosis has been widely accepted as the best histological correlation of renal function in glomerular diseases (101, 128). Normal extracellular matrix components such as collagen types IV and VI, laminin, fibronectin, and HA are present in excess as a result of increased synthesis and/or reduced removal (40, 73, 123).

During hyperglycemia, several kidney cells (proximal tubular cells, renal fibroblasts, mesangial cells, glomeruli) in culture produce HA at an increased rate (70, 86, 142, 155), implying that diabetes promotes induction of HA in the kidney, which may be involved in the development of diabetic nephropathy due to changes in matrix composition and properties that affect function.

In interstitial fibroblasts and mesangial cells, elevated glucose concentration has been shown to stimulate HA production through the PKC/transforming growth factor β1 (TGF-β1) cascade, whereas, in proximal tubular cells, HA elevation was associated with NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells)-activated transcription of HAS2 (86, 142, 155). Other studies demonstrate increased HA content in diabetic kidneys. Malathy and Kurup (87) reported a marginally increased HA in kidneys from diabetic rats, whereas Lewis et al. (85) and Berenson et al. (13) found increased HA content in human kidneys. Wang and Hascall (155) demonstrated accumulation of HA in the glomeruli of diabetic kidneys. The expression of the enzymes responsible for prostaglandin formation (cyclooxygenase -1 and -2) is elevated in the renal medulla during diabetes, and prostaglandin E2 formation is increased (100). Prostaglandin E2 is a known stimulator of HA production (86, 147). In line with the proposal by Young et al. (165), Wang and Hascall (155) found that in response to hyperglycemia, mesangial cells increase HA production and thus cellular matrix HA content, which in turn increases monocyte adhesion. Monocyte and macrophage infiltration of the glomerulus, together with activation and proliferation of glomerular mesangial cells, is followed by mesangial expansion, the dominating glomerular abnormality in diabetic nephropathy.

It is proposed by Hascall et al. (63) that cells that are exposed to pathogens, noxious agents, or metabolic imbalance activate intracellular HAS. These cells will produce an HA-based extracellular matrix that can be recognized by inflammatory cells, which then adhere and respond. It has, however, recently been suggested that intracellular HA synthesis only occurs in cells dividing in hyperglycemic conditions (154). A link has been suggested between the initial effects of hyperglycemia on the biochemistry of the region and later pathological changes in the glomerular structure of diabetes and a role for HA in pathogenesis of diabetic nephropathy (86). Mahadevan et al. (86) report that glomeruli from diabetic rats produce more HA compared with nondiabetic controls both during hyperglycemic and normoglycemic conditions. During low-glucose conditions, prostaglandin exposure increased HA production even more from the diabetic glomeruli. HA was reduced by cyclooxygenase inhibition. Both exogenous HA and prostaglandin inhibition reduced sulfated GAGs from both control and diabetic glomeruli. Summarized, these findings show that increased prostaglandin production in a high-glucose environment results in increased HA production by the glomerulus, forming a link between hyperglycemia and later structural changes of the glomerulus.

Jones et al. (70) found that proximal tubular cells could, via generation of cytokines, alter the extracellular matrix and thus the renal interstitium. Among these changes is the generation of HA production, which is regulated by the proximal tubular cells. The addition of interleukin (IL)-1β or β-glucose to a primary cell culture of human proximal tubular cells resulted in increases in HA production. In immortalized human proximal tubular cells (HK-2 cells), the addition of IL-1β or β-glucose also increased HA and induced HAS2 mRNA. HAS3 mRNA was expressed constitutively by these cells, whereas HAS1 mRNA was not detectable. The inhibition of NF-κB revoked HA increase of both IL1-β and β-glucose. Increased HA synthesis in response to IL1-β or β-glucose is due to NF-κB-activated HAS2 transcription.

This raises the question if the increased HA during hyperglycemia is directly involved in the development of diabetic nephropathy? Diabetic patients and animal models of diabetes often have osmotic diuresis due to hyperglycemia, as high diuresis can lead to volume depletion. HA levels are elevated during hyperglycemia, and the medullary HA content also varies with hydration status. Rügheimer et al. (125) reported that diabetic animals primarily have elevated medullary HA levels, which correlated with increased HAS2 mRNA expression. Hydration in diabetic animals does not elevate medullary HA further, and these animals do not display the normal diuretic response. It was suggested that the renomedullary HA levels are maximized in the diabetic rat and cannot be increased further in response to hydration, in contrast to what occurs in the normal kidney. The inability to regulate medullary HA may thus have a direct functional consequence on defective volume regulation. It is plausible that this model of diabetic nephropathy will also involve the cortical tissue as the severity of the disease progress.

Investigation of HA production during high-glucose conditions was furthered by Takeda et al. (142). Normal rat kidney cells increase HA production in response to elevated glucose levels, but neither L-glucose nor mannitol had effect. Furthermore, high glucose enhanced PKC activity, and exposure to an activator of PKC or TGF-β1 increase HA production. The effects of high glucose on HA production were abrogated by incubation with PKC inhibitors or anti-TGF-β-neutralizing antibody. Finally, incubation with high HA levels promoted kidney cell proliferation. Thus HA production is stimulated by high glucose through a PKC/TGF-β pathway, suggesting that HA might be involved in the pathogenesis of interstitial fibrosis in diabetic kidney disease.

HA might also be a marker of kidney status in diabetics. In the previously mentioned investigation of streptozotocin (STZ)-induced diabetic rat kidneys by Ikegami-Kawai et al. (66), Hyal activity increased from day 3 of diabetes. Whole kidney Hyal activity increased until the third week, resulting in a more than doubled amount that of the corresponding controls. Cortical Hyal activity increased significantly more compared with the medullary activity. The heterogeneous alteration of enzyme activity suggests different roles of HA in diabetic nephropathy for the different kidney regions. Additionally, Hyal activity increased only in the STZ-induced diabetic rats and not in spontaneously diabetic Goto-Kakizaki
rats, yet without progressed nephropathy. Hyal may thus potentially be used as a marker of diabetic nephropathy. This may provide the possibilities for the diagnosis of patients and a new clinical role for HA. The elevated Hyal activity in the diabetic kidney may also contribute to the inability to gain sufficient elevation in interstitial HA during hydration (123), making HA levels clinically significant in more ways than merely as a marker.

Of interest are also the studies by Campo et al. (21), showing that treatment of diabetic mice with high molecular HA reduces diabetic nephropathy. The authors suggest that high-molecular-weight HA decreases CD44 and PKC gene expression, which reduces inflammation and secondary pathologies in diabetes. This would imply an important pathological pathway involving CD44 and PKC in this disease but may also infer that some of the pathological phenotypes include a response mediated by low molecular HA.

Obesity is often found in conjunction with diabetes. It is of interest in this context that Dwyer and colleagues (34) also found an elevation of HA selectively in the renal medulla of obese rabbits. The authors suggested that a possible distension could occur in the renal medulla with consequences for interstitial hydrostatic pressure.

To summarize, the renal content of HA is elevated during diabetes. Both HAS2 mRNA and Hyal activity are elevated and coincide with proteinuria, overt diuresis, and depressed kidney function. The pro-inflammatory and water-attracting properties of HA may be involved in the progression of diabetic nephropathy.

Ischemia Reperfusion Injury

After the demonstration in 1990 that a local accumulation of HA occurs in the renal cortex after renal transplant rejection, it was speculated that ischemia per se could induce increased renal HA content (58, 160). Ischemia-reperfusion injury is characterized by cellular hypoxia, injuries to the tubuli and endothelium, sustained vasoconstriction, and leukocyte infiltration (17) and results in acute renal failure associated with high mortality (32).

In studies applying unilateral warm renal ischemia (32, 52, 69, 84, 94), primarily the cortex of the kidney with induced ischemia-reperfusion injury displayed highly elevated levels of HA. The amount of accumulated HA increases with increasing duration of ischemia, and the gene expression of HAS2 is elevated, thereby providing a possible origin of the HA accumulation (52). Such an induction could be a result of elevated levels of cytokines and growth factors. The water content is consequently elevated in the injured kidney and correlated to the amount of HA, suggesting interstitial edema. In one study (69), treatment with intravascular Hyal prevented HA and water accumulation after the ischemia, providing a plausible treatment modality.

The localization of HA, as well as that of its principal receptor CD44 after renal ischemia, has been determined (32, 52, 94). Declèves and colleagues (32) compared that further to the localization and expression of neutrophils, macrophages, and proliferating cell nuclear antigen (PCNA: marker of S-phase DNA synthesis)-positive cells, which are known to reside in ischemic areas. Ischemia-reperfusion injury did not alter the amount of HA in the inner medulla, but it should be noted that the inner medulla was shown to be insensitive to ischemia and did not develop ischemic lesions or infiltration of inflammatory cells. In its high molecular form, HA is proposed to be involved as a protective agent against ischemic lesions by restraining proinflammatory signaling. In contrast to the medullary regions, HA and CD44 appeared in the cortex and the outer stripe of the outer medulla following induced ischemia-reperfusion injury, where they persisted during the repair period. CD44 is expressed in all infiltrating neutrophils and in 30% of all macrophages found in inflammatory granulomas with high HA levels.

HA expression on endothelium and blood leukocytes is a prerequisite for neutrophil recruitment in the inflamed kidney, whereas the vast amount of leukocytes even before HA accumulation indicates that it is not a prerequisite for early leukocyte infiltration (32). Between days 7 and 14 following ischemia, HA is accumulated in the cortex and outer stripe of the outer medulla. Thereafter it slowly decreases but still remains at day 28 in association with inflammatory cells, such as macrophages, in areas of regeneration.

Tubular cells are able to synthesize and secrete HA from their apical membrane when they regenerate due to inflammation (9). The HA formed by the tubular cells is deposited on the apical side and is not responsible for the HA accumulation in the cortical interstitium after injury. The synthesis is activated during tubular cell proliferation by mechanical injury. Expression of HA is always accompanied by expression of CD44. After completed healing, neither HA nor CD44 are expressed on the apical surface, but CD44 continues to be expressed on the basal membrane. The HA production by the apical region of tubular cells is suggested to support their proliferation.

To summarize, renal ischemia induces the accumulation of HA primarily in the renal cortex that may increase the risk for the development of interstitial edema, a situation that may be circumvented by Hyal treatment but not by traditional diuretic therapy. The elevation seems primarily to be occurring through the induction of HAS2 expression in response to inflammatory mediators.

Transplantation and Rejection

The transplantation of renal allograft increases the risk of ischemia-reperfusion injury, which affects the survival of the graft (145). In transplant rejection, there is an autoimmune inflammation (48) that usually continues until the organ is destroyed. Early in the rejection process, alloantigens are recognized, and there is an immune activation and inflammatory reaction with a large number of cytokines being neosynthesized. Some cytokines such as IL-1 and TNF-α directly influence mesenchymal cells and will thus be able to alter the connective tissue matrix.

HA is present early in the injured transplant (108). Wells et al. and Hällgren et al. investigated rejected human kidneys for HA localization (58, 159, 160). In the normal kidneys, HA is present almost exclusively in the medulla. However, both chronically and acute rejecting kidneys displayed increased HA levels, especially in cortex and sclerotic vessels, and was accompanied by edema. The increased amount of HA is most prominent in areas of tubular atrophy (160). It was proposed that cortical HA may be used as a marker for acute rejection (159). Work on the hypothesis that edema due to transplant...
rejection is caused by the accumulation of HA has established that there is an accumulation of HA as early as 2 days from transplantation (57, 58). The levels become increasingly higher as the rejection progresses to necrosis. Increased levels of HA induce steric exclusion and interstitial edema. The increased HA content of the cortex during rejection can be seen as a wide separation of individual tubules from each other (48), suggesting a possible steric exclusion and reduced oxygen delivery to the excluded areas due to the increased diffusion distances. The findings by Häglgren et al. (57, 58) suggest a possible advantage in therapy to decrease HA synthesis in transplant grafts or to enhance HA degradation to prohibit accumulation.

To improve the success of kidney transplantation, cyclosporine A has been used as an effective immunosuppressant. However, long-term application of cyclosporine A induces nephrotoxic effects, which can lead to graft failure (30). In a study by He Han and colleagues (59), it was demonstrated that chronic cyclosporine A treatment in normal kidneys results in HA accumulation and increased CD44 expression, and treatment length correlated with the amount of HA and CD44 expression. It was suggested that upregulation of HA and its binding receptors is involved in interstitial fibrosis in chronic cyclosporine A-induced renal injury.

To summarize, transplantation edema, which is a key feature of graft rejection, is dependent on the accumulation of HA not only under experimental conditions but also in the clinical setting. Furthermore, cyclosporine A, which is used to suppress the immunological response, stimulates HA accumulation through its nephrotoxic effects during chronic use.

Kidney Stone Formation

Kidney stone formation occurs commonly in numerous species (95). Most kidney stones, nephroliths, are calcareous and account for more than 80% of stones. Uric acid stones account for 5–10%. The more uncommon kinds are cystine, struvite, and ammonium acid urate stones. The term nephroliths is not a diagnosis per se but a manifestation of another underlying problem. Generally, stone formation is associated with reduced kidney function. It is interesting that HA is an important constituent of the organic matrix of kidney stones. Furthermore, HA is present in kidney stones in fractions that are in disproportion to HA urinary levels (96, 104). The role of HA in renal stone disease has previously been reviewed by Verkoelen (152) and was the theme of the PhD thesis by Asselman (7).

The mechanisms involved in renal stone disease are not fully understood. Reduced urinary volume increases the risk of stone formation due to the increased concentration of the solutes (18, 28). The low urine volume can be due to insufficient water intake or excessive water loss (95). Curhan et al. (28) studied 24-h urine collection and proved that 24-h urine chemistries are important for kidney stone prediction, but that the severity and importance of the association vary with age and gender. Patients forming calcium oxalate stones often have low urine volumes (18). This might, as is often assumed, be due to environmental factors but could also be due to dysfunctional water handling (153). Randall’s plaque formation originates from calcium oxalate crystals, which results in nephroliths (95). Randall’s plaques form in the papillae and are commonly, but not always, associated with stone formation. The plaques start expanding on the basement membrane of the thin loop of Henle and expand through the interstitium, eventually protruding into the uroepithelium of the papillae.

The main pathophysiological factor mediating calcium nephrolithiasis is hypercalciuria, which might be caused by defects in the kidney, bone, or gut and thus be due to either renal leakage or reabsorption malfunction (95). High dietary salt increases the risk of nephrolithiasis, whereas dietary salt restriction reduces the risk. High sodium intake increases the risk in two ways: by promoting crystallization of calcium salts by reducing the concentration of citrate and by inducing hypercalciuria, since urinary excretion of sodium and calcium are linearly correlated. Hypercalciuria is caused by systemic acidosis and protein load (95). In chronic acidosis due to distal renal tubular acidosis, the high urinary pH allows calcium phosphate stones to form. Acidosis produces calcium leakage from the kidneys and bone calcium release.

As speculated by Verkoelen (152), HA should be an antagonist to crystallization when calcium salts are in solution due to the physicochemical properties of HA (numerous carboxyl groups can bind calcium). Since HA is normally found in the interstitial space of the renal medulla, it should thereby prevent calcification in this area. However, in situations with kidney disease, elevated HA levels (both cortex and medulla), especially when associated with the luminal surface of renal tubular cells (8, 151), may serve as a binder for precipitated calcium salts. Crystal binding to HA may lead to the retention of crystals in the renal tubules (nephrocalcinosis) and to calcified plaques in the interstitial space (Randall’s plaques). Intratubular HA increases the risk of crystal retention leading to stone formation due to its binding capabilities of calcium oxalate crystals (9). Crystals have been found associated with cell surface HA and CD44 in renal tubules of preterm infants and renal transplant patients (151), both groups known for tubular nephrocalcinosis (111, 129).

In a study of intratubular crystals in idiopathic stone formers and intestinally by-passed obesity patients, only the by-passed patients showed signs of stone formation (38). It is not known why the by-passed patients become susceptible to kidney stones. However, the same patients tested positive for HA, whereas patients with idiopathic stones had no intramedullary collecting duct crystals and no HA staining. This supports the notion of HA being a binding molecule for crystals and suggests a role for HA in the pathogenesis of stone formation. To summarize, HA is likely to be involved in kidney stone formation when kidney function is distorted. By crystal binding, HA can increase the risk of stone formation. During normal physiological conditions, when calcium salts are in solution, HA can inhibit crystal formation by binding calcium that might otherwise precipitate.

Conclusion

HA has unique physicochemical properties for attracting water. In the kidney, which regulates water homeostasis, renomedullary interstitial HA levels increase during acute hydration and decrease during dehydration. It is plausible to suggest that the changes in interstitial HA in response to changes in hydration will affect hydraulic conductivity. An elevation in interstitial HA and a reduction in vasopressin-regulated aquaporins occurring simultaneously during hydration, will in-
crease diuresis, whereas the opposite occurs during dehydration. The relative contribution of HA to the changes occurring in diuresis during variations in diabetic nephritis is not known and warrants further investigation.

The water-attracting properties of HA have implications for kidney function during renal disease. Most diseases where the kidney is affected are associated with elevated levels of interstitial HA in the cortex or medulla in conjunction with disturbed fluid and electrolyte balance. Again, the relative contribution of HA to the disturbed function is unclear. However, in renal-ischemia reperfusion studies using Hyl treatment, renal HA and water content was reduced, thereby suggesting a therapeutical potential.

**Perspectives and Significance**

The importance of HA at the cellular level has been well known, whereas that for the organ and organ system level has been less studied. The knowledge of how HA affects fluid balance by changing renomedullary interstitial fluid and solute transport depending on hydration status has emerged during the past decade. The mechanisms underlying the intrarenal heterogeneous distribution of HA have been elucidated by following HA syntheses and Hyals and HA in diuresis is not known and also about ACE inhibition fethopathy.

HA is also implicated in several renal pathological conditions such as diabetic nephropathy, ischemia-reperfusion injury, transplant rejection, kidney stone formation, and during conditions such as diabetic nephropathy, ischemia-reperfusion in renal disease. The mechanisms underlying the intrarenal heterogeneous distribution of HA have been elucidated by following HA syntheses and Hyals and HA in diuresis is not known and also about ACE inhibition fethopathy.

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